The Effect Of Giving Aloe Vera Gel On Collagen Growth In Wistar Rats (Rattus Norvegicus) With Exposure To Ultra Violet-B Light

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Abstract.

Exposure to UV rays can cause photochemical damage to the DNA of cells in the body, triggering cancer formation, especially skin cancer in humans. The skin may lose elasticity. Research is needed to ensure that currently, we are still looking for plants or foods that contain collagen, which can prevent wrinkles. This research is an original experiment carried out in a laboratory. Two types of variables play a role in this research: independent variables and dependent variables. In this experiment, aloe vera gel was used as an independent variable as a substitute for UVB light exposure. Collagen development, measured in mouse skin, is the dependent variable here. This study found that the standard control group had no collagen growth and was stable. This was because the typical control treatment was not exposed to UVB rays; hence, collagen formation was stable. The group administered 15% aloe vera extract gel had a more substantial effect on rat skin tissue collagen development after UVB light exposure than the 5% and 10% groups. This is because a higher dose of aloe vera extract includes more chemical components that promote mouse skin collagen formation.

Keywords: *Aloe Vera, Collagen, Skin Aging, UV Radiation and Anti-Aging Medicine.*

I. INTRODUCTION

The body, organs, and cells age throughout time. Every living thing goes through it throughout adulthood. Aging is a gradual process that increases weariness, sickness, and death risk. Aging is multifaceted. Several hypotheses explain different elements of aging. Physical, psychological, and social changes occur over time in humans as they age. Recent beliefs are related to damage, in which DNA oxidation can cause biological systems to fail, or programmed aging, in which internal processes like DNA methylation cause aging [1], [2].Few studies have examined how UV radiation affects collagen molecules in solution or aggregate fiber form. UV radiation causes cross-linking and breakdown, depending on oxygen, pH, collagen type, and UV wavelength. UV rays cause skin photoaging, which causes thickness, wrinkles, pigmentation, and dryness [3]–[5]. Photoaging occurs when UV-B radiation damages DNA and skin function and structure. Photoaging causes collagen damage from ROS production, causing wrinkles [3].Understanding Anti-Aging Medicine (AAM) is emerging in medical science and practical medicine. The goal is to reduce age-related disorders and treat aging. The goal is to lengthen the healthy lifespan of young people. Medical science has a new discipline, AAM. AAM believes aging can be delayed, slowed, or reversed into a healthier state [6], [7]. Nobody can prevent aging. However, some criteria can be met to look young in old age. However, premature aging is concerning. Several reasons cause aging, including psychological stress, pollution, and smoking [8]–[10].

Draelos says research demonstrates that vehicle traffic soot and particulates cause 20% more pigment scars on the forehead and cheeks. Similar particles promote melanocyte growth. This study was coordinated with prior studies that found PAHs (polycyclic aromatic hydrocarbons) attached to combustion nanoparticles, which cause facial pigmentation. This may explain why industrial or heavily polluted locations have more lentigines and melasma [10]. Another skin-damaging combustion is cigarette smoke. Smokers had greater upper lip creases, solar elastosis, telangiectasias, and facial skin laxity, according to research. Smoking produces ambient nanoparticles that induce accelerated aging, like smog and soot [11], [12].Psychological factors increase aging, according to several research. Stress was positively related to

increasing leukocyte telomere length [13], [14]. A cell's telomere length influences its division and replication rate. Telomeres regulate cell reproduction like clocks. All eukaryotic chromosomes have them at the ends, and they inhibit degradation. Telomeres prevent chromosomal ends from merging, reducing genetic instability. Under stress, telomeres shorten with each cell replication and cannot repair themselves because cells lack telomerase. These cells could be prevented from prem prematurity by introducing telomerase.Indonesia is blessed with its tropical position, which provides year-round sunshine. Sunlight provides life-giving energy. The sun emits visible and invisible light. Sunlight also emits radiation [15]. UVR cannot be seen or felt. Some people are subjected to artificial UVR in medicine, industry, disinfection, and cosmetics, even though many are exposed to solar UVR.

UVR helps produce vitamin D and is healthy in trim levels. UVR is carcinogenic. Therefore, excessive exposure can harm health. UVR can damage skin, eyes, and other bodily parts [3]–[5], [15].Since UV light produces vitamin D, small amounts are necessary for health. Vitamin D strengthens bones and muscles [16], [17]. Oral vitamin D supplementation is recommended for people with low sun exposure, highly pigmented skin who live at high altitudes, or those who cover their entire body when outdoors for religious or cultural reasons.Three layers make up skin: epidermis, dermis, and subcutis [18]. Collagen, elastin, and other fibers support skin structure in the dermis [18], [19]. UV radiation damages these components, giving skin a smooth, youthful appearance. UVA and UVB waves harm the skin. UV rays damage D; thus, cells make epidermal melanin to protect it. Melanin is your skin's attempt to block radiation [16], [17], [20].UVB rays, shorter than UVA, cause most sunburn. UVA radiation with longer wavelengths causes most photoaging damage. UVA rays destroy collagen strands deep in the dermis. This injury increases aberrant elastin production. Extreme elastin production produces metalloproteinase. Many enzymes that rebuild collagen malfunction and destroy collagen, preventing skin reconstruction. Repeated daily UVA exposure wrinkles poorly repaired skin and depletes collagen, resulting in rough skin [3], [15], [17].MMP-1 is the primary collagen degrader in photoaging skin. MMP-1 destroys collagen and elastin fibrils, which give skin strength and suppleness. Even short-term UV exposure increases MMP-1 activity in the skin, generating wrinkles and photoaging. UV light for several hours causes MMPs, especially gelatinase and collagenase, which degrade dermal collagen [3], [10], [21].

UV light breaks down collagen faster than aging. It penetrates the dermis, creating aberrant elastin buildup. When elastin builds up, enzymes accidentally break down collagen, causing “sun scars.” Continuous exposure accelerates wrinkles and sagging. Another primary source of free radicals is UV light. Free radicals are unstable oxygen molecules with one electron. Electrons occur in pairs; thus, molecules must scavenge missing electrons from other molecules, creating chain reactions that destroy cells. Free radicals stimulate collagen-degrading enzymes and modify a cell's genetic material, which can cause cancer [22], [23].Aloe vera is frequently used for treatment and care. Aloe vera gel or cream can cure chronic wounds, including psoriasis lesions (twice daily for 4-8 weeks), pressure sores (1-3 months), veins, diabetes, herpetic ulcers, and chronic anal fissures (2-3 weeks). Hekmatpo et al. (2019) assessed lesion score, depth, size, edema around the wound area, exudate and necrotic tissue, inflammation, pain and bleeding, and infection in addition to healing time [24]. Aloe vera has been shown to reduce the above variables. Hekmatpou observed no difference in pressure sore healing between saline and Aloe vera. Their findings may be due to the small sample size (30 instances). Many studies examined hospital stay, scar treatment expense, and wound redness and irritation as secondary objectives. Aloe vera outperformed other treatments [24]–[27].Traditional beliefs are that wounds should not be closed, allowing them to dry out and fall away, hinder cell migration, and growth factors that cure wounds, according to some studies.

Aloe vera wound dressing damages wet wett and promotes fibroblast and epidermal migration. Aloe vera (1–100 mg/kg) may help wounds heal [25], [26], [28], [29]. The quality of the literature, difficulty of access to all articles, and unpublished reports limit this systematic review. Additionally, only English and Persian literature was reviewed. These factors have drastically altered our sample size for numerous data parameters, preventing us from obtaining statistically meaningful results. Aloe Vera's effects on wound healing are difficult to determine because not all papers are masked trials. Articles with multiple procedures or indications without particular outcome data for Aloe vera's wound healing capabilities were removed. This

study did not have a primary summary or meta-analysis, so data analysis to assess publication bias with specialized tools was not done. However, qualitative survey and survey research suggested approaches to lessen publication bias.The purpose of this study was to investigate and test the impact of Aloe vera gel administration on collagen growth in Wistar rats (Rattus norvegicus) that had been subjected to ultraviolet-B (UVB) light.

II. METHODS

This study represents a genuine experiment conducted in a lab setting. The effect of aloe vera gel administration on collagen growth in Wistar rats (Rattus norvegicus) exposed to ultraviolet-B radiation was studied using a post-test with a control group design. This research employed a pre-and post-test experimental design with a control group [30]. Everything observed constitutes a new data point. Since it is the research variables that need to be observed or quantified, researchers must have a firm grasp of them [31]. There are two types of variables at play in this study: independent variables and dependent variables. In this experiment, aloe vera gel was used as the independent variable instead of exposure to UVB rays. Collagen development, as measured in rats, skin is the dependent variable here. Test animals were acclimated for seven days at Medan State University's Faculty of Mathematics and Natural Sciences Animal House. Aloe Vera extract cream follows.

After seven days of acclimatization, mice were randomly separated into five groups for treatment. The tools used in this research include rat cages, digital scales, masks, gloves, minor surgical tools (stainless still tray, scalpel, blade, scissors, and tweezers), UVB lamp and UVmeter, preparation-making tools, LC camera evolution and Olympus Bx51 microscope, laptop and Adobe PhotoShop Cs2 device. Meanwhile, the ingredients used include aloe vera gel, distilled water, anesthetic (ketamine), xylazine, male white rats, rat food, and drink. To evaluate if phytochemical substances may increase skin moisture in dry mouse skin, skin moisture was observed. Using SPSS, quantifiable data (independent variables) was examined for significance on the treatment group's effect (dependent variable).

III. RESULTS AND DISCUSSION

Result

Table 1. Phytochemical Test Results of Aloe Vera Extract

Compound	Result	Note
Flavonoids	Orange and yellow solution	+
Saponin	The solution forms foam.	+
Phenolic	Blackish green solution	+
Tannin	Green solution	+

The chemicals or chemical substances included in aloe vera extract are listed in Table 1. These include flavonoids, saponins, phenolics, and tannins.

Table 2. Collagen Density Test Results in Rat Skin Tissue

Repetition	Collagen Density (%)				
	KN	P0	P1	P2	P3
1	38,426	28,042	42,707	47,928	**51,032**
2	34,469	26,620	39,968	41,416	**54,965**
3	37,235	30,650	42,198	45,173	**49,305**
4	36,319	27,549	43,414	42,736	**48,365**
5	35,216	27,324	41,560	44,259	**50,325**
Mean (%)	36,333	28,037	41,969	44,302	**50,798**
±SD	±1,408	±1,384	±1,171	±2,222	**±2,271**
Score	**+2**	**+1**	**+2**	**+2**	**+3**

Note: KN: Normal control (without treatment), P0: Negative control (without administration of aloe vera extract gel), P1: Administration of 5% dose of aloe vera extract gel, P2: Administration of 10% dose of aloe vera extract gel, P3: Administering 15% dose of aloe vera extract gel

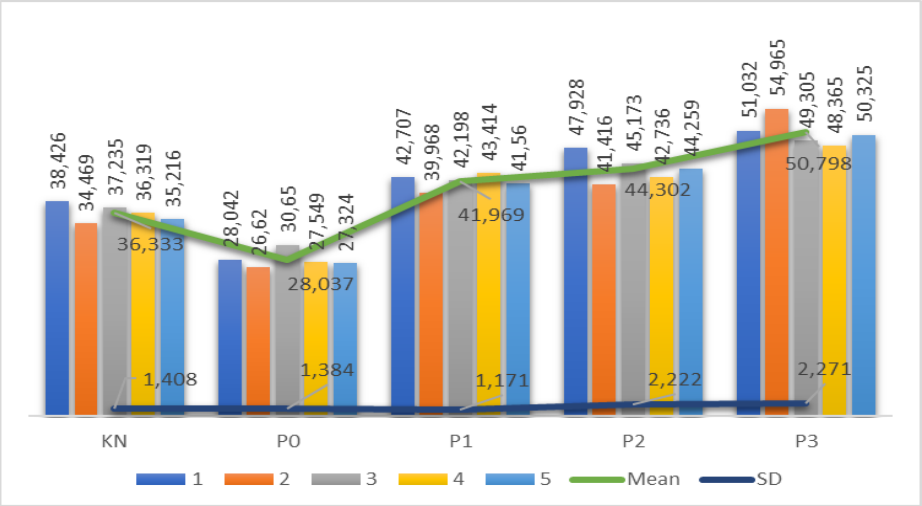


Fig 1. Graph of Collagen Density Test Results

Table 2 shows that the control group of mice (KN) had an average collagen density of 36.333 ± 1.408 without treatment. The mice exposed to UVB light without aloe vera extract gel (P0) had an average collagen density of 28.037 ± 1.384. Treatment group I (P1) animals, exposed to UVB exposure and administered 5% aloe vera extract gel, had an average collagen density of 41.969 ± 1.171. Group II (P2) mice exposed to UVB exposure and issued a 10% dose of Aloe vera extract gel had an average collagen density of 44.302 ± 2.222. In mice exposed to UVB exposure and given a 15% dose of aloe vera extract gel, the average collagen density was 50.798 ± 2.271 in treatment group III (P3). The statistics show that the P3 group, the treatment group given 15% aloe vera extract gel, had the highest average percentage of collagen density. Next, the group that was exposed to UVB light but not administered aloe vera extract gel (the "negative control group" or "P0") had the lowest average percentage of collagen density. The P0 group fared no better than the untreated, regular control group (KN).

Table 3. Results of the Shapiro-Wilk Normality Test

Group Extract	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Normal Control (KN)	.161	5	.200*	.980	5	.934
Negative Control (P0)	.299	5	.165	.849	5	.191
Treatment P1	.177	5	.200*	.961	5	.816
Treatment P2	.163	5	.200*	.979	5	.929
Treatment P3	.263	5	.200*	.895	5	.381

*. This is a lower bound of the true significance

a. Lilliefors Significance Correction

Table 3 displays the results of an SPSS normality test, which revealed statistically significant percentage increases in collagen density from day 1 to day 15 in both the negative control and treatment groups. Where the p-value from the Shapiro-Wilk test is more significant than 0.05, as in the case of Group KN (0.934), Group P0 (0.191), Group P1 (0.816), Group P2 (0.929), and Group 0.381. According to the results of the Shapiro-Wilk test, the supplied data follows a normal distribution.

Table 4. Results of the ANOVA Test of Homogeneity of Variances

	Levene Statistical	df1	df2	Sig.
Base on Mean	.568	4	20	.689
Base on Median	.485	4	20	.747
Based on the Median & with the adjusted df	.485	4	15.967	.747
Based on trimmed mean	.544	4	20	.705

The One-Way ANOVA Test was used to examine the consistency of the collagen-growth or collagen-density-increase processes across the KN, P0, P1, P2, and P3 groups after 15 days of treatment. According to Table 4, there is no statistically significant difference between the variances of the research results for the standard control group (KN), the negative control group (P0), group P1, group P2, and group

P3; all five groups have the same variance of 0.689 (p>0.05).Table 5 shows the percentage of collagen density among the five study groups; this led us to conduct additional tests to see whether or not there were any significant differences between the groups, according to the "Sig" column data in the table. The p-value (p-value) that was calculated is 0.000. Therefore, Ho is rejected at the proper level = 0.05, and a significant difference in the n mean percentage of collagen density across the five groups is found.

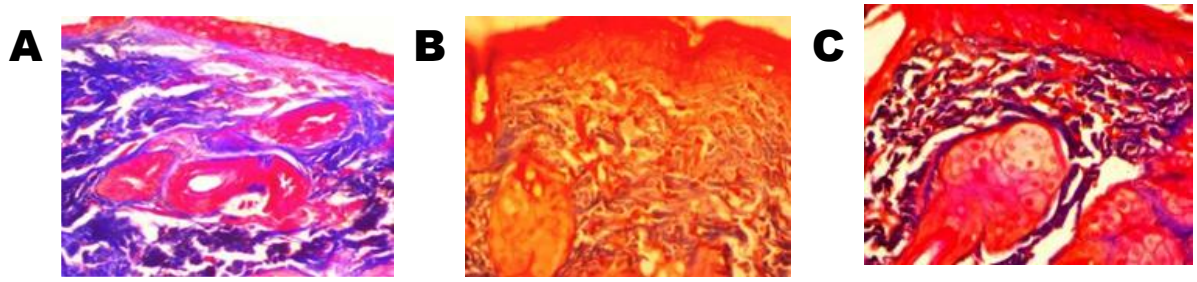
Table 5. Results of the ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1475.700	4	368.925	96.029	.000
Within Groups	76.836	20	3.842		
Total	1552.536	24			

Table 6. Post Hoc Bonferroni Test Results

Test	Experimental Group (I)	Experimental Group (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Bonferroni	Normal Control (KN)	Negative Control (P0)	8.29600*	1.23964	.000	4.3891	12.2051
		Treatment P1	-5.63640*	1.23964	.002	-9.5455	-1.7273
		Treatment P2	-7.96940*	1.23964	.000	-11.8785	-4.0603
		Treatment P3	-14.46540*	1.23964	.000	-18.3745	-10.5563
	Negative Control (P0)	Normal Control (KN)	-8.29600*	1.23964	.000	-12.2051	-4.3869
		Treatment P1	-13.93240*	1.23964	.000	-17.8415	-10.0233
		Treatment P2	-16.26540*	1.23964	.000	-20.1745	-12.3563
		Treatment P3	-22.76140*	1.23964	.000	-26.6705	-12.3563
	Treatment P1	Normal Control (KN)	5.63640*	1.23964	.002	1.72731	9.5455
		Negative Control (P0)	13.93240*	1.23964	.000	10.0233	17.8415
		Treatment P2	-2.33300	1.23964	.745	-6.2421	1.5761
		Treatment P3	-8.82900*	1.23964	.000	-12.7381	-4.9199
	Treatment P2	Normal Control (KN)	7.96940*	1.23964	.000	4.0603	11.8785
		Negative Control (P0)	16.26440*	1.23964	.000	12.3563	20.1745
		Treatment P1	2.33300	1.23964	.745	-1.5761	6.2421
		Treatment P3	-6.49600*	1.23964	.000	-10.4051	-2.5869
	Treatment P3	Normal Control (KN)	14.46540*	1.23964	.000	10.5563	18.3745
		Negative Control (P0)	22.76140*	1.23964	.000	18.8523	26.6705
		Treatment P1	8.82900*	1.23964	.000	4.9199	12.7381
		Treatment P2	6.49600*	1.23964	.000	2.5869	10.4051

Bonferroni Post Hoc Test (Table 6) comparison results between groups I and J show that there is a difference in the average percentage of moisture in the skin of mice between the negative control group (K) and the P3 treatment group and the P4 treatment group, and vice versa, marked with an asterisk "*."According to Figure 1, the negative control group (P0) had the lowest percentage of collagen density compared to the other groups when observing collagen density preparations using Image J software with the area fraction method utilizing five fields of view at 400x magnification. Collagen density (shown by the blue hue) is nearly undetectable in the P0 group.

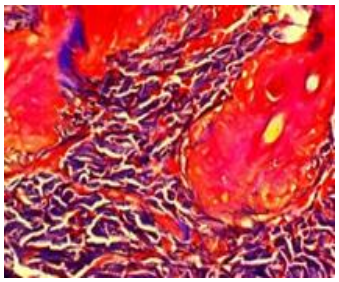


A: Normal group collagen density is marked in blue

B: Collagen density of the negative control group (P0) is marked in blue. (slight

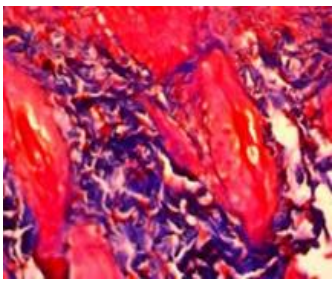
D: Collagen density in the 5% dose treatment group (P1) is marked in blue.

D



D: Collagen density in the 10% dose treatment group (P2) is marked in blue.

E



E: Collagen density in the 15% dose treatment group (P3) is marked in blue.

Fig 1. Histopathological Observation of Collagen Density

The blue hue of the collagen density photo in the table indicates that the area % of collagen density in the standard control group is more stable than in the hostile control groups P0, P1, P2, and P3. This was because collagen production in the usual control treatment was unaffected by UV light exposure, in contrast to the other groups.After UVB light exposure, the collagen density of the treatment groups improved in the P3 group, which received aloe vera extract gel at a dose of 15%. This occurs because a higher concentration of aloe vera extract is required to produce the same volume of gel. According to Sari's (2021) research, which found that anti-aging creams containing aloe vera and green algae extract were more effective, this is the case.

Discussion

Extrinsic skin aging also referred to as photoaging, encompasses modifiable alterations in both the structure and function of the skin, primarily resulting from prolonged and unprotected exposure to UV radiation. UVB radiation largely contributes to direct DNA damage and inflammation in photoaging. UVB radiation can permeate the epidermis and higher dermis layers of the skin. Consequently, apart from its role in inducing typical aging processes, UVB-induced harm also plays a significant role in the destruction of collagen and the generation of elastotic substances within the skin [32]–[34].This study measured collagen growth using Image J's collagen growth area percentage. Aloe vera was extracted for this investigation. Fresh, dark-green aloe vera leaves are used. Maceration produces aloe vera leaf extract. Maceration involves blending 1000 grams of fresh aloe vera leaves, placing them in a vessel, adding 7500 liters of 70% ethanol, and stirring for five days. Scrub/stain, squeeze, and wash the dregs with 2500 ml 70% ethanol. Next, concentrate at 500C with a rotary evaporator until the water stops dripping. The liquid extract was evaporated in a 500C water bath. I am manually controlling the temperature with a thermometer. Ethanol meets extracting requirements. Ethanol dissolves most polar and non-polar compounds. The aloe vera extract is then made into a gel. The more solvents utilized, the higher the yield.Flavonoids, saponins, phenolics, and tannins are found in phytochemically positive aloe vera or extract. Flavonoids are exhibited by adding 1 mL of thick aloe vera extract to a test tube, 2 mg magnesium powder, and three drops of concentrated HCl, which turns the solution orange and yellow. Shaking 10 mL of thick aloe vera extract vertically in a test tube for 10 seconds and leaving it for 10 seconds produced foam as high as 3.8 cm, indicating saponin component presence.

A dark blue or greenish-black tint was detected when 1 mL of thick aloe vera extract was added to a test tube with a few drops of 10% iron (III) chloride solution to test for tannins and phenolics.Male white rats were utilized in this study because they are easy to handle and have human-like physiology and anatomy. Twenty-five mice—5 in each group—were used. To adapt, mice were acclimatized for one week before treatment. Each rat was grouped and marked: regular control group (KN); negative control group (P0), which received no treatment; treatment group I (P1), which received 5% topically aloe vera extract gel; group II (P2), which received 10% topically, and group III (P3), which received 15% topically.Skin/tissue samples were taken on the 16th day before mice were sacrificed via chloroform inhalation. The back area was cleaned of hair, and the skin was cut with a thickness of ± 3 mm up to the subcutaneous and a length of 2.5 cm—

hematoxylin-stained tissue for 5 minutes and washed for 10 minutes with running water. After staining with eosin for 2 minutes, the sample was placed in a graded alcohol solution, cleared with still, and covered with an adhesive-coated cover glass.After observing collagen density preparations using Image J software with the area fraction method in 5 fields of view at 40x magnification, the normality and homogeneity tests showed that the Shapiro-Wilk test significance value for each group was > 0.05, so the trial was valid. Further statistical analysis was conducted using the parametric ANOVA test with a p-value < 0.05. Parametric ANOVA yielded 0.000 significance. The significance value is ($p<0.05$), indicating substantial differences among groups. Statistical analysis proceeded with Bonferroni.The negative control group (P0) had the lowest collagen density in the Bonferroni post hoc difference test. Significant differences ($p<0.05$) were seen between negative control group P0 and groups P1, P2, and P3, compared to standard control. The negative control group P0 had a collagen density score of +1 and an average density of 28.037%, lower than groups P1, P2, P3, and standard control KN.

The negative control group had the most deficient collagen synthesis since it was exposed to UVB rays directly without gel treatment.Collagen density significantly differed between the standard control group and hostile control groups P0, P1, P2, and P3 ($p<0.05$). The average group had a collagen density score of +2 and an average of 36.333%, more significant than the negative control group. The usual control treatment had stable collagen development since it was not exposed to UVB rays.The average collagen density in the aloe vera extract gel treatment groups P1, P2, and P3 was 41.969%, 44.302%, and 50.798% higher than the standard control and negative control. The collagen density scores in groups P1 and P2 were +2, indicating that the three formula groups had moderate collagen fiber density (10-50% per field of view). In the P3 group, the collagen density score was +3, indicating dense collagen.The considerable difference in results between groups P1, P2, and P3 and the negative control group P0 reveals that UVB light destroys mouse skin tissue and increases collagen in mouse skin. The gel preparation's aloe vera extract dose increased in groups P1, P2, and P3, with 5%, 10%, and 15% doses, respectively, as aloe vera extract dose increases in gel formation, collagen fiber density between P1, P2, and P3 increases. This is because aloe vera extract gel includes antioxidant and anti-inflammatory flavonoid and polyphenol components. According to other research, flavonoids, polyphenols, carotenoids, vitamins C and E, and natural extracts can prevent or accelerate collagen degradation or production due to their anti-inflammatory and anti-oxidative effects [13], [14], [35].As an astringent, aloe vera gel's tannin and phenolic content reduce mucosal permeability and strengthen mucosal bonds, preventing irritants. Tannins also shrink and kill bacteria by affecting bacterial walls. Aloe vera's phenolic component prevents free radical-induced cell damage and inflammation [25], [27], [29].

IV. CONCLUSION

Aloe vera gel's effect on collagen growth in Wistar strain rats (Rattus norvegicus) that were untreated and exposed to ultraviolet-B light for 15 days can be inferred from the phytochemical test of the extract, which found flavonoids, saponins, tannins, and phenolics. Because of its antioxidant and anti-inflammatory compounds, aloe vera extract can be utilized as a medicine.The average percentage of collagen development in the negative control group (P0) following UVB radiation exposure differed significantly from P1, P2, and P3. The negative control group (P0) was not administered gel with active ingredients to stimulate skin tissue collagen formation.

This study found that the standard control group had no collagen growth and was stable. This was because the typical control treatment was not exposed to UVB rays; hence, collagen formation was stable. The group administered 15% aloe vera extract gel had a more substantial effect on rat skin tissue collagen development after UVB light exposure than the 5% and 10% groups. This is because a higher dose of aloe vera extract includes more chemical components that promote mouse skin collagen formation. Additional research is needed employing a positive control group (for instance, those given bioplacentons) and a higher concentration or dose of aloe vera extract or aloe vera to be utilized as a therapy preparation.

V. ACKNOWLEDGMENTS

The author would like to thank the Faculty of Medicine, Dentistry and Health Sciences, Prima Indonesia University, for supporting this project, the head of the Animal House Center, the Faculty of Mathematics and Natural Sciences, Medan State University, and the supervisor. We also thank the research partners for their valuable advice.

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