


The Effect of Administration of Rosella Flower (*Hibiscus Sabdariffa*) Extract Gel on The Expression Of TNF- α And Caspase-3 Expression in An In Vivo Study of Wistar Strain Rats Exposed to Ultraviolet B-Lights

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Article Info	ABSTRACT
<p>Article history:</p> <p>Received January 15, 2024 Revised January 24, 2024 Accepted February 05, 2024</p> <hr/> <p>Corresponding Author:</p> <p>Christina J.R. Esmaralda Lumbantobing Master Study Program in Biomedical Sciences, Faculty of Medicine, Dentistry and Health Sciences, Prima Indonesia University, Medan Email: christinajresmaraldalumbantobing@unprimdn.ac.id</p>	<p>UV rays are oxidative because they can produce Reactive Oxygen Species and free radical compounds. The dangers of sun exposure can cause skin disorders such as aging at an early age (photoaging). Rosella flower petals can be used in medicine, jams, and natural food coloring because they contain flavonoids as antioxidants or free radical scavengers. The research aimed to determine the effect of administering Rosella (<i>Hibiscus sabdariffa</i> L.) flower extract gel on TNF-α expression and Caspase-3 expression in an in vivo study of Wistar mice exposed to UVB. This research is an experimental study. The sample for each treatment group consisted of 6 individuals, so the total research sample was 24. Data analysis used descriptive, normality, and homogeneity tests with data analysis processing using SPSS 25.0 for Windows. The study found that administration of rosella flower extract gel with a concentration of 15% effectively reduced the expression of TNF-α and Caspase-3 in mice exposed to UVB light and produced filled and dense collagen. So, it can be concluded that rosella flower extract gel can act as an antioxidant that inhibits the growth of TNF-α and Caspase-3 expression.</p> <p>Keywords: Rosella Flower, TNF-α, Caspase-3, Ultraviolet B</p> <p>This article is licensed under a Creative Commons Attribution 4.0 International License.</p> 

1. INTRODUCTION

All living things on Earth rely on the sun's ultraviolet (UV) radiation for survival. The wavelength of ultraviolet-B radiation ranges from 290 to 315 nanometers [1]. Although some UV-B light is necessary for vitamin D production, skin can suffer damage from prolonged exposure to high doses [2]. Ultraviolet light is considered oxidative because it can generate free radical molecules known as reactive oxygen species (ROS). There is a belief that reactive oxygen species (ROS) cause cell damage, accelerated aging, and skin cancer [3]. When faced with potentially harmful environmental factors, the immune system launches an inflammatory response to keep homeostasis intact. When there is inflammation, neutrophil-type leukocytes release proteases and cytokines that promote inflammation, such as TNF- α , IL-1 β , and IL-6 [4].

The release of several pro-inflammatory chemicals, such as TNF- α , is a hallmark of inflammatory diseases, which might worsen when ROS generation is excessive. The NOXO1 (NADPH-Oxidase 1) pathway can cause DNA oxidative damage when TNF- α molecules are produced over an extended period. This, in turn, activates the p53 gene, which in turn promotes Caspase-3 activation and death of skin cells, including fibroblast cells [5]. According to multiple other studies, long-term exposure to UVB causes oxidative stress, which in turn activates a cascade of events that can degrade collagen and other components of the extracellular matrix (ECM), including the p38, JNK, ERK, and p53 pathways; finally, it can trigger the production of matrix metalloproteinases (MMPs)[6].

Because of its equatorial location, the island nation of Indonesia experiences scorching sunshine and very high humidity levels typical of tropical climates. Sunlight, which contains harmful ultraviolet radiation, can harm skin [7]. Nearly everyone is susceptible to photoaging, sunburns, and other skin problems [8]. Hyperpigmentation and wrinkles can be caused by other sun-related illnesses [9].

Some medications commonly used to treat skin damage caused by photoaging include topical retinoids, arbutin, glycolic acid, hydroquinone, and arbutin [10]. Several adverse effects, including skin irritation, skin cancer, and allergic contact dermatitis, have been associated with these medications.

Hibiscus sabdariffa L., more often known as Rosella, is a herb native to hot, subtropical regions of the world, including India, Malaysia, and other Asian countries [11]. Almost 300 species of this plant are known. Phytosterols, polyphenols, organic acids, and minerals abound in the vibrantly colored petals of rosella flowers, which are also a good source of fiber. The medical qualities of rose flower petals have led to their extensive usage in medicine. They are also a common ingredient in jam and are used as a natural food colorant [12]. Anthocyanin, a flavonoid, is abundant in rosella flower petals. Rosella flower petals, particularly red rosella, contain flavonoids and phenolic compounds with antioxidant and free radical scavenging potential [13]-[14].

Previous studies have shown that rosella flower petal methanol extract may help create a natural antioxidant [15]. Tested the antioxidant activity of rosella flower petals in a water-ethanol solvent with varying concentrations; the solution with the most significant antioxidant activity, with an IC50 of 67.3 ppm, was a 50:50V/v mixture of ethanol and water. Rosella leaf, stem, and flower methanol extracts were evaluated for their antioxidant activities [16]. According to the data, the antioxidant content of rosella flower petals was greater than that of the leaves and stems. [17], the antioxidant capabilities of rosella petals are partly due to the presence of phenolic chemicals, including delphinidin-3-glucoside, symbioses, and cyaniding 3-sambubioside.

Due to their potential antioxidant properties, Rosella flower petal extracts have been the subject of much research [18]. Nevertheless, the impact of rosella flowers on the expression of TNF- α and Caspase-3 in UVB-exposed skin has not been investigated. This study is to examine the effect of exposing Wistar mice to UVB and administering a gel made from Rosella (*Hibiscus sabdariffa L.*) flower extract on the production of TNF- α and Caspase-3, taking into consideration the backdrop of the problem.

2. METHOD

Using a control group that gets test results, this study represents a proper experimental design [19]. The research was conducted at the Anatomical Pathology Laboratory of the University of North Sumatra and the Laboratory of the Department of Pharmaceutical Pharmacology.

A straightforward way to look at it is that an independent variable impacts or brings about a dependent variable, so the two are directly related [20]. When an independent variable is introduced, the dependent variable transforms or directly results. This study used Rosella (*Hibiscus sabdariffa L.*) as the independent variable, while TNF- α expression and Caspase-3 expression were used as the dependent variables. As a prerequisite, the following factors have been considered: Sunlight's ultraviolet B rays cause the skin to age prematurely.

White male Wistar rats (*Rattus norvegicus*), around 2-3 months old, weighing 200-250 grams, and evaluated by a veterinarian as healthy and fit for research, made up the study's sample [21]. Rats were exposed to 302 nm UVB radiation at a distance of 20 cm for five days or until their collagen density decreased. The mean effective dose (MED) was 160 mJ/cm²/day 3.121.

The study of the data made use of descriptive tests, normality, and homogeneity. Then, we checked for homogeneity of variances and normality of distribution using the Levene test. A One-Way ANOVA test was utilized for group comparisons due to the non-normal distribution of the data [22]. We utilized SPSS 25.0 for Windows for data processing.

3. RESULTS AND DISCUSSION

Results from the study that examined the effects of UVB light exposure on the production of TNF- α and Caspase-3 in rats exposed to rosella flower extract gel in Wistar white rats (*Rattus norvegicus*) showed many highlights, such as:

Table 1. Characteristics Test Animals

Component	Group			
	Control -	Control +	P1	P2
Types of Rats	<i>Rattus norvegicus</i> white Wistar strain			
General circumstances	The coat color is white, healthy, and active.			
Average Initial Weight	211gr	217gr	219gr	213gr
Average Final Weight Loss	212gr	215gr	216gr	211gr

Both before and after the 14-day therapy, the mice in this study appeared to be in good health, according to the features of the test animals. There was no dropout among the 24 test animals in the trial. Fourteen experimental animals were weighed.

Table 2. Average TNF- α Expression (pg/ml)

No	Group	Repetition	TNF- α expression before treatment (pg/ml)	TNF- α expression after treatment (pg/ml)
1	K-	1	40.2	40.1
2		2	41.3	41.1
3		3	41.1	40.9
4		4	43.5	43.2
5		5	40.1	39.8
6		6	43.5	43.1
		Average	41.6	41.4
7	K+	1	61.2	57.7
8		2	62.3	59.5
9		3	62.6	58.3
10		4	64.1	56.7
11		5	61.5	58.5
12		6	63.7	59.1
		Average	62.6	58.3
13	P1	1	62.1	53.3
14		2	63.2	54.2
15		3	62.9	54.8
16		4	64.1	52.1
17		5	63.3	53.7
18		6	61.3	52.6
		Average	62.8	53.5
19	P2	1	62.3	43.2
20		2	64.1	44.3
21		3	61.3	42.1
22		4	60.9	40.1
23		5	65.3	41.3
24		6	61.1	41.8
		Average	62.5	42.1

Table 3. Average Caspase-3 expression (ng/ml)

No	Group	Repetition	Caspase-3 levels before treatment (ng/ml)	Caspase-3 Levels After Treatment (ng/ml)
1	K-	1	3.3	3.2
2		2	3.5	3.1
3		3	4.3	4.1
4		4	3.9	3.6
5		5	3.2	2.9
6		6	2.9	2.5
		Average	3.5	3.2
7	K+	1	12.2	9.2
8		2	13.1	8.5
9		3	12.9	7.8
10		4	11.9	8.1
11		5	13.5	7.5
12		6	11.5	8.7
		Average	12.5	8.3
13	P1	1	11.3	6.2
14		2	12.7	6.9
15		3	13.6	7.1

No	Group	Repetition	Caspase-3 levels before treatment (ng/ml)	Caspase-3 Levels After Treatment (ng/ml)
16		4	13.1	5.5
17		5	12.8	5.9
18		6	12.2	6.1
Average			12.6	6.3
19	P2	1	11.3	4.1
20		2	12.7	4.5
21		3	13.5	3.9
22		4	13.2	3.3
23		5	12.5	2.9
24		6	12.1	2.4
Rata-rata			12.6	3.5

A decrease in caspase-3 expression was seen in both the negative control group and the second group of mice exposed to UVB light and covered with 15% rosella flower extract gel. This decrease was particularly pronounced in the second group, which was also coated. Mice exposed to UVB radiation but not treated in any other way formed the positive control group, whereas the treatment group showed the most significant decrease in caspase-3 expression.

Rosella flower extract (*Hibiscus sabdariffa L.*) was used in phytochemical screening tests to lower the levels of TNF- α and caspase-3, which are high in Wistar strain white rats exposed to UVB light. Here are the results of the screening:

Table 4. Phytochemical Test

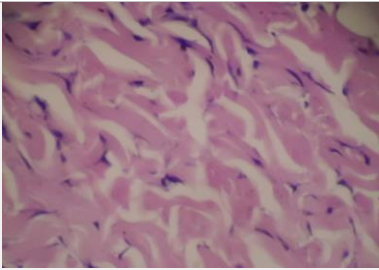
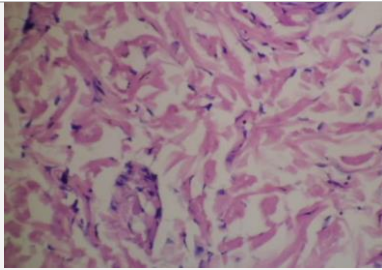
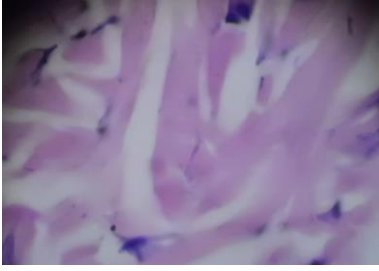
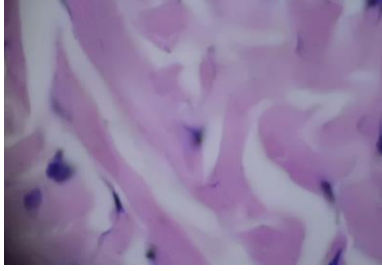
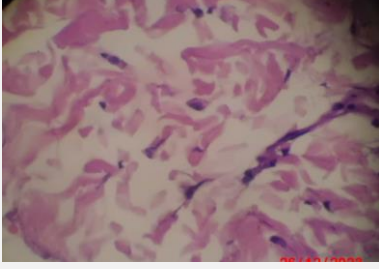
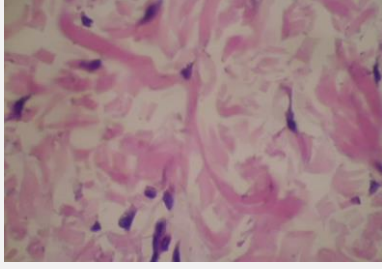
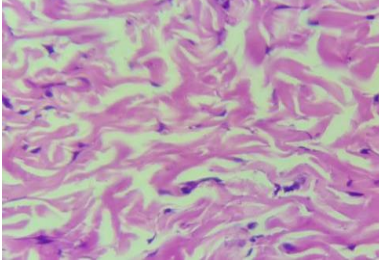
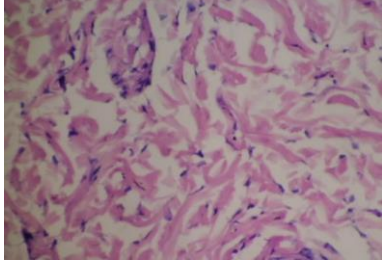
Secondary Metabolites	Testing	Color	Results
Flavonoid	Mg dan HCl	Yellow	+
Saponin	Forth	Foam forms	+
Tannin	FeCl ₃	Blackish green	+
Alkaloid	Mayer	White precipitate	-

Rosella flower extract (*Hibiscus sabdariffa L.*) includes secondary metabolites such as tannins, alkaloids, flavonoids, and saponins, according to the findings of the phytochemical studies. These findings were confirmed, which aligns with earlier research [23]. Rosella flower ethanol extract contains phytonutrient-rich substances such as alkaloids, tannins, saponins, and flavonoids.

Collagen densities ranging from 75% to 100% were present in the negative control group. Since the negative control group did not get UVB radiation, their skin histology was normal. This group compared to the treatment group that received UVB light and rosella flower extract gel (*Hibiscus sabdariffa L.*) and characterized the other groups.

The positive control group was treated with base gel (0%) and exposed to UVB rays. Collagen growth in this group is fragile, measuring less than 25%—the lack of visible collagen results from the skin tissue's ongoing inflammatory state. The collagen growth rate was modest, at 50% in the first treatment group. Among the groups tested, the histopathological image from treatment two rosella (*Hibiscus sabdariffa L.*) flower extract gel, 15% concentration) exhibited the densest collagen structure, while the others ranged from 75% to 100%. Separating the parts of rosella flower extract (*Hibiscus sabdariffa L.*) from collagen production in white Wistar rats exposed to UVB is impossible.

Table 5. Histopathological Image of Skin Tissue

No	Group	Histopathological Image of Skin Tissue	
1	Negative Control		
2	Positive Control (Gel base)		
3	Treatment 1 (10%)		
4	Treatment 2 (15%)		

Observation Results of TNF- α Levels

Table 6. TNF- α Normality Test

	Groups	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Results	K-	.239	6	.200*	.866	6	.211
	K+	.167	6	.200*	.970	6	.892
	P1	.135	6	.200*	.983	6	.966
	P2	.176	6	.200*	.987	6	.979

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Testing for a normal distribution is the goal of the normalcy test. Using the Kolmogorov-Smirnov test, we checked for normalcy in this research. To ensure that the data is representative of the population as a whole, it must pass the normalcy test. This data is said to be normally distributed if the p-value is more significant than 0.05 and not normally distributed if the p-value is less than 0.05 [22]. There is a table with the results of the data normality test that was put into this study:

The Kolmogorov-Smirnov normality test gave all groups a significance score of 0.200. A normal distribution is assumed if the p-value is more significant than 0.05 [22]. This takes a normal distribution. After ensuring the data follows a normal distribution, perform the Levene test to check for homogeneity in all study population subgroups.

Table 7. TNF- α Homogeneity Test

		Levene Statistic	df1	df2	Sig.
Hasil	Based on Mean	.645	3	20	.595
	Based on Median	.369	3	20	.776
	Based on the Median and with adjusted df	.369	3	16.623	.776
	Based on trimmed mean	.624	3	20	.608

The table above shows the outcomes of the homogeneity test conducted using the Levene test. In the significance column, the probability value is 0.595. Given that the calculated significance probability value is more significant than 0.05, it may be inferred that all groups originate from a homogenous or similarly distributed population.

Table 8. One-Way ANOVA TNF- α Test

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1269.425	3	423.142	268.306	.000
Within Groups	31.542	20	1.577		
Total	1300.966	23			

The significant value is 0.000, less than 0.05, according to the one-way ANOVA test findings in the table above [22]. These results suggest that the treatment group differs significantly from the control group. The average differences between groups were analyzed using the post hoc LSD further test. The following table displays the outcomes of the post-hoc LSD additional test:

Table 9. Post Hoc LSD TNF- α Test

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
K-	K+	-16.93333*	.72505	.000	-18.4458	-15.4209
	P1	-12.08333*	.72505	.000	-13.5958	-10.5709
	P2	-.76667	.72505	.303	-2.2791	.7458
K+	K-	16.93333*	.72505	.000	15.4209	18.4458
	P1	4.85000*	.72505	.000	3.3376	6.3624
	P2	16.16667*	.72505	.000	14.6542	17.6791
P1	K-	12.08333*	.72505	.000	10.5709	13.5958
	K+	-4.85000*	.72505	.000	-6.3624	-3.3376
	P2	11.31667*	.72505	.000	9.8042	12.8291
P2	K-	.76667	.72505	.303	-.7458	2.2791
	K+	-16.16667*	.72505	.000	-17.6791	-14.6542
	P1	-11.31667*	.72505	.000	-12.8291	-9.8042

*. The mean difference is significant at the 0.05 level.

If two groups are significantly different from one another, the LSD post hoc test will reveal it. Analysis revealed a statistically significant difference between treatment group 1 and positive and negative control groups ($p = 0.000$). At the same time, there was no statistically significant difference between the negative control group and treatment group 2 ($p = 0.303$).

Observation Results of Caspase-3 Levels

Table 10. Caspase-3 Normality Test

	Groups	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Result	K-	.191	6	.200*	.980	6	.954
	K+	.126	6	.200*	.983	6	.966
	P1	.221	6	.200*	.940	6	.661
	P2	.186	6	.200*	.968	6	.878

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

All groups achieved a significance level of 0.200 according to the normalcy test, which was conducted using the Kolmogorov-Smirnov test. If the p-value exceeds 0.05, we say the data follows a normal distribution [24]. The data is thus assumed to follow a normal distribution. After confirming that the data follows a normal distribution, the next step in checking for homogeneity is to use the Levene test to see if all of the subsets of the study population are similar.

Table 11. Caspase-3 Homogeneity Test

		Levene Statistic	df1	df2	Sig.
Result	Based on Mean	.595	3	20	.626
	Based on Median	.571	3	20	.640
	Based on the Median and with adjusted df	.571	3	19.392	.641
	Based on trimmed mean	.601	3	20	.622

The table above shows the outcomes of the homogeneity test conducted using the Levene test. In the significance column, the probability value is 0.626. Given that the calculated significance probability value is more significant than 0.05, it may be inferred that all groups originate from a homogenous or similarly distributed population.

Table 12. One-Way ANOVA Caspase-3 Assay

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	104.483	3	34.828	82.238	.000
Within Groups	8.470	20	.423		
Total	112.953	23			

The significant value is 0.000, less than 0.05, according to the one-way ANOVA test findings in the table above. These results suggest that the treatment group differs significantly from the control group. The average differences between groups were analyzed using the post hoc LSD further test. The following table displays the outcomes of the post-hoc LSD in Table 13.

If two groups are significantly different from one another, the LSD post hoc test will reveal it. Analysis revealed a statistically significant difference between treatment group 1 and positive and negative control groups ($p = 0.000$). Additionally, no statistically significant difference ($p = 0.460$) between the negative control and treatment groups 2.

Table 13. Post Hoc LSD Caspase-3 Assay

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
K-	K+	-5.06667*	.37572	.000	-5.8504	-4.2829
	P1	-3.05000*	.37572	.000	-3.8337	-2.2663

	P2	-.28333	.37572	.460	-1.0671	.5004
K+	K-	5.06667*	.37572	.000	4.2829	5.8504
	P1	2.01667*	.37572	.000	1.2329	2.8004
	P2	4.78333*	.37572	.000	3.9996	5.5671
P1	K-	3.05000*	.37572	.000	2.2663	3.8337
	K+	-2.01667*	.37572	.000	-2.8004	-1.2329
	P2	2.76667*	.37572	.000	1.9829	3.5504
P2	K-	.28333	.37572	.460	-.5004	1.0671
	K+	-4.78333*	.37572	.000	-5.5671	-3.9996
	P1	-2.76667*	.37572	.000	-3.5504	-1.9829

*. The mean difference is significant at the 0.05 level.

Discussion

This study aimed to determine the impact of ultraviolet B radiation on tumor necrosis factor- α and caspase-3 levels in Wistar white rats (*Rattus norvegicus*) after administering rosella flower extract gel. The research utilized samples of male Wistar white rats (*Rattus norvegicus*) ranging in weight from 200 to 250 g. For this study's sample computation, twenty-four mice were utilized. Each group was given six mice according to the Ferderer formula. Four sets of test animals were used. To start, the group of mice served as a control and did not get any UVB radiation treatment. Two groups were tested: one that did nothing and the other that used base gel and UVB radiation to treat the mice. Before exposure to UVB rays, all three treatment groups were given a gel that included 10% rosella flower extract. A gel containing 15% rosella flower extract was subsequently applied to the second group exposed to UVB radiation.

The process of producing reactive oxygen species (ROS) by exposure to ultraviolet B radiation is known to have a direct impact on nuclear factor kappa B (NF- κ B) activation [25], which in turn promotes the synthesis of pro-inflammatory proteins such as tumor necrosis factor- α (TNF- α). Continuous TNF- α production from UVB radiation exposure triggers caspase eight and caspase three activation [26]. Consequently, this may lead to the death of skin cells. Caspase 3 and the TNF- α gene upregulation demonstrate DNA oxidative damage [27].

Rosella is an excellent source of bioactive compounds, including polyphenols, organic acids, phytosterols, and minerals rich in antioxidants [28]. The plant produces glucoside hibiscitrin, gossipetin, and hibiscus (anthocyanin) from the rosella flower petals; flavanol is another name for it [18]. The theory goes like this: once mice's skin is exposed to UVB, a gel consisting of rosella flower extract may be applied to it. The antioxidant capabilities of the flower extract will supposedly limit the production of TNF- α and caspase-3, which will help fix any damage to the skin and maintain its health. According to scientists, given rosella flower extract, exposing Wistar white rats (*Rattus norvegicus*) to UVB light reduces the synthesis of TNF- α and caspase-3. Using Wistar white rats (*Rattus norvegicus*) allowed the researchers to verify this hypothesis.

The first step is to put the test animal to sleep by injecting it with xylazine (20 mg/kg BW) and ketamine (60 mg/kg BW) via the injection site. We then smoothed the rat's back using a razor and veet cream. For five days, mice were exposed to UVB at a distance of 20 cm, with a Minimum Erythema Dose (MED) of 160 mJ/cm², for about 15 minutes daily. The distinctive reddening of the skin on the backs of the infected mice was brought on by the cumulative effect of Ultraviolet B (UVB) radiation absorbed by the skin. The mice in each group were given the prescribed amounts of rosella flower extract gel. The results of this study on TNF- α and caspase-3 expression can only be confirmed by doing several data analyses, including tests for normality, homogeneity, and significance.

The normality test data was gathered using SPSS's Kolmogorov-Smirnov test. In all test groups, the significance threshold was 0.200, and the findings showed that the TNF- α and caspase-3 data followed a normal distribution. This suggests that the data may follow a normal distribution or be statistically representative of the community. If data with a normal distribution is homogenous, meaning it comes from a population with the same variance, then the Levene test will return a yes. The results of the homogeneity test for TNF- α and Caspase-3, which were obtained, were 0.595 and 0.626, respectively. All categories are valid representations of the same population, as the computed significance probability value is more significant than 0.05.

Using this homogeneous and normally distributed data, the one-way ANOVA test was run to see if it was influential and significant. Using the one-way ANOVA test results on the TNF- α and caspase-3 data, we determined that the significance value was 0.000, which is more than 0.05. Although a post hoc LSD test is necessary to validate these results, these results do indicate a substantial difference between the treatment and control groups.

A post hoc LSD test was conducted to investigate differences in the average total cholesterol levels across the groups. The results of the study's post hoc LSD test showed that there was a statistically significant difference between all of the groups (p 0.000, less than 0.05). Treatment group 1 (p = 0.000), the positive control group (p = 0.000), and

the negative control group ($p = 0.000$) were all found to vary significantly based on the results of the TNF- α expression data analysis. Meanwhile, treatment group 2 did not differ significantly from the negative control group ($p = 0.303$). Differences between treatment group 1 and the negative and positive control groups were found to be statistically significant ($p = 0.000$) when analyzing data on caspase-3 expression. The second treatment group did not vary significantly from the negative control group ($p = 0.460$). In this test, the researchers discovered that treatment group 2 had significantly higher levels of TNF- α and caspase-3 compared to the negative control group, which had normal levels.

One of the initial findings was the need to document the presence of TNF- α . The researchers found that the group that received treatment 2 (rosella flower extract gel at a concentration of 7%), based on the average value of TNF- α expression, had the most significant reduction in TNF- α expression. The group receiving only base gel, the positive control group, had the slightest reduction in TNF- α expression.

Second, caspase-3 expression was averaged. Based on the average value of caspase-3 expression, the researchers observed that the 2-treatment group, which got a 7% concentration of rosella flower extract gel, had the most notable decrease in caspase-3 expression. The amount of caspase-3 expression that was reduced was the least in the positive control group, which had just base gel.

Thirdly, skin tissue may be examined under a microscope using histological photographs. These findings show that rosella flower extract gel (*Hibiscus sabdariffa L.*) may modify the collagen exposure to UVB rays in male Wistar white rats. Different collagen densities in the treatment and positive and negative control groups provided evidence of this. A table shows the microscopic appearance of treated histopathological skin tissue. The negative control group's 75-100% collagen density indicated thick and dense tissue. Normal skin histology was seen in the negative control group due to the absence of ultraviolet B exposure. Aside from characterizing the other groups, this compared to the treatment group that got UVB light and rosella flower extract gel (*Hibiscus sabdariffa L.*).

A 0% base gel was applied to the group that acted as the positive control after exposure to UVB radiation. This group's collagen growth is thin, coming in at less than 25%. This is because the skin is still inflammatory, so less collagen is visible. In the first group to receive therapy, collagen development was minimal, occurring at 50%. Histopathological images taken from treatment group 2 (rose (*Hibiscus sabdariffa L.*) flower extract gel) at a concentration of 15% showed the densest structure when compared to the other groups (75-100% collagen structure). A thick collagen layer cannot be separated from the compound content of rosella flower extract in the histopathology of white Wistar rats (*Rattus norvegicus*) subjected to UVB radiation.

Phytochemical analysis revealed the presence of secondary metabolites, including alkaloids, tannins, saponins, and flavonoids in rosella flower extract (*Hibiscus sabdariffa L.*) [23]. According to this study, rosella flower ethanol extract has antioxidant-rich phytochemical components such as flavonoids, saponins, tannins, and alkaloids. Flavonoids inhibit the generation of reactive oxygen species (ROS), leading to their anti-inflammatory abilities. In addition to that, flavonoids can boost the synthesis of SOD and other anti-oxidative stress enzymes, which in turn can hinder the expansion of TNF- α and caspase-3 expression [29]. [30], who also discovered that a gel made from rosella leaf extract had anti-inflammatory, antioxidant, and antibacterial characteristics that sped up the healing process of wounds.

4. CONCLUSION

The expression of TNF- α and caspase-3 was reduced in mice exposed to UVB radiation after they were given rosella flower extract gel at a dosage of 15%. The histological examination revealed that collagen development was fragile in the positive control group compared to thick and filled in the treatment group that received 15% rosella flower extract gel. The phytochemical analysis of rosella flower extract reveals the presence of alkaloids, tannins, saponins, and flavonoids, among other secondary metabolites. These substances suppress the increase of TNF- α and Caspase-3 expression by acting as antioxidants.

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