Administration Of Eucalyptus To Inhibit The Skin Aging Process Of Rats (Rattus Norvegicus) Wistar Strains Exposed To Ultraviolet-B Light

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Abstract.

Ageing weakens the skin's protective layer, causing pigmentation, inflammation, and cancer. Environmental hazards like pollution and UV radiation harm the skin. Antioxidants, like Eucalyptus globulus, protect against free radicals, making them valuable in medicinal use. The study aimed to investigate the efficacy of Eucalyptus administration in inhibiting skin ageing in rats exposed to ultraviolet-B light. A true experiment was conducted to examine the effectiveness of Eucalyptus in reducing skin ageing in Wistar rats exposed to ultraviolet-B radiation. The study used the "3R Principle" and SPSS to analyse the relationship between collagen quantity, Eucalyptus, and UVB radiation. The study found normal distribution for the control and treatment groups, with a significance of 0.200 and 0.091, respectively. The Levene test showed homogeneity and a T-test result 0.000, indicating a significant difference. The control group had a moderate collagen density, while the treatment group with Eucalyptus extract had a tighter collagen density. The study concludes that the Eucalyptus tree extracts, including tannins, saponins, and flavonoids, have been found to have anti-ageing and collagen-increasing effects in mice exposed to UVB light. The treatment group showed significantly increased collagen density, indicating potential anti-ageing properties in the treatment group.

Keywords: Eucalyptus, Ultraviolet-B, Aging and Collagen.

I. INTRODUCTION

All living things go through the progressive aging process, which affects every organ. Aging is a natural part of life; as we age, our cells become less efficient in producing new skin cells and collagen, weakening the skin's internal support system and protective outer layer [1], [2]. Air pollution, ultraviolet radiation, chemicals, and cigarette smoke are some environmental hazards that can harm the skin, the body's outermost layer [3]. Skin aging can be caused by various external sources, including exposure to ultraviolet light from the sun [4]. Sunlight harms the skin through its ultraviolet (UV) radiation. Its rays can induce skin problems, including pigmentation changes, inflammation, and even cancer [5]. Additionally, sun exposure can hasten the skin's aging process, a phenomenon known as photaging. Over time, chronic reactivity to UV rays can cause skin structural issues, such as premature skin aging and skin cancer [6]. Sunlight and ultraviolet radiation (UV-B) are the leading causes of photoaging and skin aging [7]. Reactive oxygen species (ROS) generation accounts for approximately half of the skin damage caused by ultraviolet B radiation, accelerating aging [8]. Radical oxidants cause skin damage by directly modifying mitochondrial DNA (mtDNA), lipid bilayers, DNA strands, dermal tissue protein matrix, and collagen layers [9]. UV rays damage skin by creating sunburn cells, triggering an inflammatory response, creating thymine dimers, and producing matrix metalloproteinases (MMPs). These enzymes hydrolyze extracellular matrix proteins, and exposure to UV radiation can decrease TNF-α and TGF-β production.

According to [6], MMP-1 is the sort of matrix metalloproteinase most impacted by sun-induced UV light and is accountable for the breakdown of collagen in the skin that has been photoaged. Photoaged skin, triggered by UV light exposure, undergoes collagen degradation, affecting skin strength and suppleness. MMP-1, responsible for collagen degradation, produces enzymes like collagenase and gelatinase, leading to decreased dermal collagen content [10]. Oxidation stress theory explains that UV-B exposure causes skin structure changes, including connective tissue and extracellular matrix, through lipid peroxidation, enzyme

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activation, and increased free radical production [11]. Wrinkles appear when enzymes like collagenase and elastase, which break down proteins in the subcutaneous extracellular matrix, are activated [9]. A collagen deficit causes signs of premature aging, including decreased skin elasticity, fine lines, and wrinkles [12]. Collagen is a protein abundant in the human body and responsible for forming connective tissues, particularly in the skin, joints, and bones [13]. Aging skin and collagen go hand in hand. The human body uses a variety of collagen types in its tissues. The dermal layer of human skin is home to the most abundant kind of collagen, type 1. According to [12], type 1 collagen accounts for around 80% to 85% of the dermal collagen. Wrinkles and fine lines manifest as a decrease in dermal collagen levels. The most apparent way that sun-damaged skin ages is through the formation of wrinkles. [10] found that antioxidants can prevent ROS production.

Another great way to stop the skin from aging is to use antioxidants. According to [14], antioxidants are commonly utilized to lessen the effects of UV radiation and shield the skin from its potential hazards. According to [15], fewer adverse effects are associated with antioxidants derived from plants and fruits than synthetic ones. Antioxidants protect the skin from free radicals, including glutathione peroxidase, superoxide dismutase, catalase, vitamin E, coenzyme Q10, ascorbate, and carotenoids [16]. But when we're in the sun for too long, our antioxidant stores get drained, and oxidative stress sets in. So, to enhance the skin's antioxidant reserves, antioxidants administered topically are also necessary. The Eucalyptus plant, rich in antioxidants, has a long history of medicinal use. *Eucalyptus* globulus, also known as *Eucalyptus* in Indonesia, is extensively used due to its rapid-fire recovery and thriving nature. According to [17], *Eucalyptus* contains chemical components such as tannins, flavonoids, and terpenoids. *Eucalyptus* leaves contain antioxidant-active phenolic compounds used in pharmaceuticals for essential oils like 1,8-cineole, α-terpineol acetate, and alloaromadendrene, which treat diseases. Both as an antioxidant and an antibacterial, the essential oil of *Eucalyptus* globulus leaves is highly effective [18]. Researchers are encouraged to conduct laboratory experiments on *Eucalyptus* leaf extract administration to reduce the aging process of rat skin (*Rattus Norvegicus*) Wistar strain exposed to ultraviolet-B light, based on the backdrop provided above. The study utilized the rise in skin collagen following UV-B exposure to measure what slows the skin's aging process.

II. METHODS

This experiment was an actual experiment that aimed to analyze the efficacy of administering Eucalyptus in reducing skin aging in rats subjected to ultraviolet-B radiation [19]. The specimens utilized were adult male rats of the Wistar strain, namely of white coloration, and were between the ages of 2 and 3 months. The selection of these rats was based on their resemblance to humans in terms of traits and their ability to adapt in a laboratory setting[20]. The study consisted of 20 animals separated into two groups, each containing ten animals. The "reduction" point was employed to minimize the number of animals utilized while ensuring the validity of the data. The study used the “3R Principle” (Replacement, Reduction, and Refinement) for in vivo research [21], [22]. The study's variables were a combination of independent and dependent variables. The researchers used the administration of Eucalyptus and UVB rays as their independent variables. This study's dependent variable is the skin's aging process, specifically the rise in collagen quantity. Researchers look at the relationship between the dependent and independent variables[23]. The research involved acclimatizing Wistar male white rats to a new climate, environment, food, and drink. *Eucalyptus* leaf extract was prepared by washing and draining *Eucalyptus* leaves, drying them in an oven, and mashing them. The powder was then filtered and concentrated using a rotary evaporator. Phytochemical testing on *Eucalyptus* extract revealed antioxidants and anti-aging compounds beneficial for Wistar rats exposed to UV-B light [24].

The extract also contains alkaloids, sediments, and foams that persist when HCl 2 N is added. The study involved 20 Wistar male white rats divided into a Control Group (K) and a Treatment Group (P). The rats were exposed to UV-B light and smeared with *Eucalyptus* leaf extract for 14 days. The *Eucalyptus* oil was administered twice, 20 minutes before and 4 hours after UVB exposure. The UV-B exposure was repeated twice for 15 days, resulting in a total UV-B exposure of 800 mJ/cm². The exposure dose was...
measured using a UVmeter tool. Skin biopsies were taken 24 hours after the last irradiation, and histopathological preparations were made using Sirius Red micro staining. The study aimed to determine the effects of UV-B exposure on skin and hair. This study examined skin aging by analyzing collagen levels in test animals' skin. Digital analysis was performed using LC Evolution cameras and Olympus Bx51 microscopes. Collagen tissue was selected, and histogram results were recorded. The percentage of collagen area pixels compared to the entire tissue was calculated by comparing collagen area pixels to other tissue area pixels.

\[
\text{Collagen Count} = \frac{\text{collagen area pixel}}{\text{pixel area of the entire network}} \times 100\%
\]

Description:
0= No collagen fibres found
+1= Low collagen fiber density (less than 10%)
+2= Medium collagen fiber density (10-50%)
+3= Dense collagen fibres (50-90%)
+4= Very tight collagen fiber density (90-100%)

The study's (collagen count) data were analyzed using the SPSS program [25]. The data normality test was analyzed using the Kolmogorov-Smirnov test approach (p > 0.05). The significant difference or effect between the test groups was analyzed by t-test or independent sample T-Test approach at a 95% confidence level (p < 0.05).

III. RESULTS AND DISCUSSION

Table 1. Characteristics Of Test Animals

<table>
<thead>
<tr>
<th>Component</th>
<th>Group P1</th>
<th>Group P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Types of Rats</td>
<td>White Rattus Norvegicus Wistar strain</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>General Condition</td>
<td>White fur, healthy and active</td>
<td></td>
</tr>
<tr>
<td>Average Initial Body Weight</td>
<td>201gr</td>
<td>200gr</td>
</tr>
<tr>
<td>Average Final Body Weight</td>
<td>197.2gr</td>
<td>196.7gr</td>
</tr>
</tbody>
</table>

The study involved 20 healthy white rats divided into control and treatment groups. The control group received UVB exposure and distilled water, while the treatment group received UVB exposure and Eucalyptus leaf extract.

Table 2. Phytochemical Test

<table>
<thead>
<tr>
<th>Secondary Metabolites</th>
<th>Testing</th>
<th>Colour</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>Wilstater</td>
<td>Red</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>Forth</td>
<td>Blue and effervescent</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>FeCl3</td>
<td>Blackish green</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>Wagner</td>
<td>Red</td>
<td>-</td>
</tr>
</tbody>
</table>

Based on the results of phytochemical tests conducted, it can be concluded that Eucalyptus extract contains secondary metabolites in the form of flavonoids, saponins, and tannins.

Table 3. Collagen Density Test Result (%)

<table>
<thead>
<tr>
<th>Repetition</th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33.13</td>
<td>59.33</td>
</tr>
<tr>
<td>2</td>
<td>44.12</td>
<td>55.14</td>
</tr>
<tr>
<td>3</td>
<td>39.45</td>
<td>52.55</td>
</tr>
<tr>
<td>4</td>
<td>38.22</td>
<td>49.31</td>
</tr>
<tr>
<td>5</td>
<td>46.13</td>
<td>56.72</td>
</tr>
<tr>
<td>6</td>
<td>31.82</td>
<td>50.78</td>
</tr>
<tr>
<td>7</td>
<td>29.12</td>
<td>58.12</td>
</tr>
<tr>
<td>8</td>
<td>30.98</td>
<td>52.81</td>
</tr>
<tr>
<td>9</td>
<td>32.78</td>
<td>53.14</td>
</tr>
</tbody>
</table>
Description: Control: Exposed to UVB light and treated with distilled water.
Treatment: Exposed to UVB light and treated with *Eucalyptus* extract.

Based on the data obtained, it can be observed that the average percentage of collagen density in the control group of rats, which were exposed to UVB light and only smeared with distilled water, was 32.57 ± 0.92. At the same time, the average percentage of collagen density in the treatment group of rats smeared by *Eucalyptus* extract was 53.70 ± 7.21. A comparison of the percentage of collagen density between each experimental rat according to each group can also be observed through the following diagram.

**Fig 1.** Collagen Density Diagram

The observations in both groups show differences in collagen density in white rats (*Rattus Norvegicus*) and Wistar strains exposed to UVB light. Researchers concluded that the control group received a score of 2, which means it has a moderate collagen density (10-50%), while the treatment group smeared with *Eucalyptus* extract received a score of 3 or has a tight collagen density (50-90%).

**Fig 2.** Histopathologic features of skin tissue

The study found that *Eucalyptus* extract can affect collagen density in white rats exposed to UVB rays. The control group showed moderate collagen growth, while the treatment group showed dense and
thick collagen growth, scoring 3 (50-90%). The collagen density in the histopathology of white rat skin exposed to UVB light is linked to the content of phytochemical compounds in *Eucalyptus* extract.

**Table 4.** Normality Test Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Kolmogorov-Smirnov*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Statistic df Sig.</td>
</tr>
<tr>
<td>Control</td>
<td>.208 10 .200*</td>
</tr>
<tr>
<td>Treatment</td>
<td>.163 10 .200*</td>
</tr>
</tbody>
</table>

* This is a lower bound of the true significance.

The One-Sample Kolmogorov-Smirnov Test yielded a significance of 0.200 for each group, indicating normal distribution. The data was then subjected to a homogeneity test using the Levene test to determine if each variant of the study population group was homogeneous.

**Table 5.** Homogeneity Test Results

<table>
<thead>
<tr>
<th>Levene static</th>
<th>df1</th>
<th>df2</th>
<th>Sig</th>
<th>Desc</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.189</td>
<td>1</td>
<td>18</td>
<td>.091</td>
<td>Homogeneous</td>
</tr>
</tbody>
</table>

The Levene test results show a homogeneity test with a significance value of 0.091, indicating that the control and treatment groups are from populations with the same variance.

**Table 6.** T-Test Results

<table>
<thead>
<tr>
<th>Result</th>
<th>F</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equal variances assumed</td>
<td>3.189</td>
<td>18</td>
<td>.000</td>
<td>-22.17 to -13.20</td>
</tr>
<tr>
<td>Equal variances are not assumed</td>
<td>15.07</td>
<td>18</td>
<td>.000</td>
<td>-22.23 to -13.13</td>
</tr>
</tbody>
</table>

The table shows that the probability value (sig.2-tailed) with the t-test is 0.000. The significance value obtained is more significant than 0.05, so the result is accepted, or there is a substantial difference between the control and treatment groups.

**Discussion**

The initial phenomenon showed how UV light accelerates photoaging. Skin structure issues, including premature aging and skin cancer, can result from chronic UV ray reactions, especially UVB [6]. Nearly 50% of aging skin damage is caused by reactive oxygen species (ROS) from UVB exposure. ROS directly chemically damages mtDNA, fat layer, DNA strand, dermis tissue protein matrix, and collagen layer [9]. Dermis collagen decrease causes fine lines and wrinkles. The most noticeable signs of sun-induced skin aging are wrinkles. Antioxidants prevent ROS production [10]. They are also effective in preventing skin aging. Many people use antioxidants to protect their skin from UV radiation [14]. Natural antioxidants from plants and fruits have fewer adverse effects than synthetic ones [15]. One antioxidant-rich plant is *Eucalyptus*. The researcher hypothesizes that *Eucalyptus* extract enhances collagen in male Wistar white rats (*Rattus Norvegicus*) exposed to UVB. Researchers tested white rats (*Rattus Norvegicus*) male Wistar strains to prove these claims. This study used male Wistar strain white rats (*Rattus Norvegicus*) weighing 160-200g and 2-3 months old. In vivo, researchers must follow the "3R Principle" (Replacement, Reduction, and Refinement) to reduce the number of animals used in the study without compromising results [21], [22].

This study had 20 rats separated into two groups: the control group, which received only distilled water, and the treatment group, which received *Eucalyptus* extract. Treatment observation data was collected for the study. We started with the phytochemical test. Phytochemical investigations show that *Eucalyptus* extract contains flavonoids, saponins, and tannins. [26] found these results. The study found secondary metabolites in flavonoids, saponins, and tannins in *Eucalyptus* ethanol extract. After studying *Eucalyptus* extract, researchers began an investigation. This 14-day research approach produced data that needed to be processed and assessed; therefore, normality, homogeneity, and significance tests were required. The Kolmogorov-Smirnov test in SPSS determined normality. All urea and creatinine test groups had typically distributed data with a significance value of 0.200. Thus, the data represents the population or is regularly

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distributed. After that, a homogeneity test assessed subject variance. The control and treatment groups had the same or homogenous variance, with a significance value of 0.91.

Finally, a t-test determined significance. The significance value is 0.000 or less than 0.05. Thus, the control group, smeared with distilled water, varies significantly from the treatment group, smeared with *Eucalyptus* extract. The average collagen density of histopathological pictures of skin tissue exposed to UVB light and treated with distilled water and *Eucalyptus* extract differs between the control and treatment groups. The data shows that the control group of rats exposed to UVB light and smeared with distilled water had an average collagen density of 32.575 ± 0.92. The treatment group of rats smeared with *Eucalyptus* extract had an average collagen density of 53.70 ± 7.21. A score of 2 indicates a moderate collagen density (10-50%) for the control group, whereas a score of 3 indicates a tight collagen density (50-90%). UVB radiation changes collagen density in rats’ skin, linked to *Eucalyptus* extract's secondary metabolites, flavonoids, saponins, and tannins, which become free radical transporters. Flavonoids increase collagen density in UVB-exposed rats. Flavonoids fight free radicals that produce wrinkles and other indications of aging. Research suggests that flavonoids have anti-aging properties. Flavonoid chemicals remove aged cells in vitro, improve physical function, and extend mouse lifetime in vivo [27].

IV. CONCLUSION

Extracts from *Eucalyptus* trees, including tannins, saponins, and flavonoids, have anti-aging and collagen-increasing effects in mice exposed to ultraviolet B light. The phytochemical analysis, which revealed the concentration of these secondary metabolites, lends credence to this. The significance test results, which used an independent T-test, likewise showed that the treatment group had significantly increased collagen density compared to the control group. The results were further supported by histopathological examinations, which revealed that the control group did not have the same level of collagen growth density as the treatment group. Since *Eucalyptus* extracts increased collagen density in UVB-exposed rats, it may be inferred that they may have anti-aging properties.

V. ACKNOWLEDGMENTS

The authors sincerely thank the Laboratory of the Department of Pharmacology and Therapeutics, affiliated with the University of North Sumatra Faculty of Medicine.

REFERENCES


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