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Characterization of ultraviolet B light emitting diodes (UVB LED) irradiation device for Wistar rats as an experimental animal model

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ABSTRACT

Background: Light emitting diodes (LED) lamps are used widely in daily because of their many benefits, including their low cost, extended lifespan, low energy consumption, and low environmental impact. The use of LEDs, especially ultraviolet B LED (UVB LEDs) is quite extensive and has been developed for the treatment of skin diseases, irradiating pet reptiles, and conducting research on experimental animals. The use of light for therapy must be done carefully so that unwanted side effects do not occur because light also harms tissue. The purpose of this characterization is to determine the stability of temperature and irradiation of the device we make and to determine the duration of light exposure based on the characterization.

Methods: The irradiation device use UVB LEDs with a wavelength of 305-310 nm. The characterization carried out included temperature stability and irradiation stability, with observations every 30 minutes for 10 hours and no replication. Statistical analysis using the Kolmogorov-Smirnov (Asymptotic Approximation) testing technique. The test criteria state that if the probability value > level of significance (alpha (α) = 5%).

Results: The characterization results from 10 hours of observation with sampling data taken every 30 minutes showed stable LED temperature and room temperature while unstable cage temperature and irradiance.

Conclusion: This device can be considered to use in Wistar rat' experimental studies using UVB and perhaps developed further. The duration of exposure can be adjusted according to the distance of the object and the dose required.

Keywords: light emitting diodes (LED), ultraviolet B, irradiation device, irradiance stability, reduce waste generation.
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INTRODUCTION

Light emitting diodes (LED) are artificial light sources based on semiconductor (diode) junctions and will emit photons when an electric field is applied to the diode. The electric field will generate electrons and holes in the diode, which in turn will generate photons. The resulting wavelength depends on the energy range of the electron-hole combination.[1] The light produced by LEDs is non-collimated and non-coherent,[2] the further away from the center of the beam, the intensity will decrease.[3]

The light source that comes from the LED is very beneficial because the light exposure output can be adjusted and can be combined with other types of lights.[4] With the gallium nitride LED technique which can produce UV radiation, it is possible to produce LEDs that are efficient and have a wide range of commercial uses including for sterilization and therapy. The LEDs can be adjusted to produce the desired wavelength. LEDs have been developed to produce ultraviolet radiation.[5]

Natural light from the sun cannot provide the desired wavelength and stable intensity. This is because sunlight is highly dependent on latitude, the thickness of the ozone layer, air pollution, aerosols, and clouds,[6] so devices have begun to be developed that can replace sunlight, including using LED lights.[4] In the last few decades LED lighting has developed rapidly, this is because LEDs have many advantages when compared to other light sources.[4] LEDs are widely used in various aspects of life, one of which is a light source for phototherapy. Phototherapy is treatment using light, either natural light or artificial light.[7] In addition, LEDs have also been shown to be suitable for biomedical research.[8]

UV exposure gives effect under the skin. Changes occur in the number, phenotype, and function of circulating blood cells after UV exposure. Alterations in neutrophils, T cells, B cells and NK cells show increased immune balance by dampening the inflammatory process, and their immunoregulatory effects have been shown to be beneficial in the treatment of systemic diseases such as autoimmune diseases.[9]

Several studies on humans and experimental animals have proven that UVB LEDs can change the inflammatory response in skin diseases,[10,11] increase the level of vitamin D in the blood,[12–14] maintain bone health,[15] and prevent osteoporosis.[16] UV light also has been used in various medical application. Narrowband UVB is effective for treating vitiligo, psoriasis, mycosis fungoides, atopic dermatitis, photodermatoses, pruritus, generalized lichen planus, generalized granuloma annulare, pityriasis lichenoides et varioliformis acuta (PLEVA), and pityriasis lichenoides chronica (PLC). Narrowband UVB did not show carcinogenesis induction when compared to PUVA (psoralen ultraviolet A).[17]

The wavelength, temperature stability, and irradiation exposure must be considered to obtain the expected therapeutic results with no side effects or minimal side effects. Ultraviolet (UV) light is electromagnetic waves with a wavelength of less than 400 nm and cannot be seen by the human eye. UV light has a shorter wavelength than visible light, which is 400-200 nm. UV light is divided into ultraviolet A (UVA) (400-320 nm), ultraviolet B (UVB) (320-280 nm) and ultraviolet C (UVC) (280-200 nm).[4,18,19] When the light hits the skin, the light will be reflected, forwarded, scattered, or absorbed. Wavelength is very important in light therapy because biological components in the skin have several absorption ranges for certain wavelengths. These tissue components include water, blood, protein, and fat, which have a spectrum between 200 and 1200 nm.[20]

The use of light as therapy requires special and careful attention because excessive exposure to light will cause dangerous side effects, including sunburn and DNA damage.[21] To conduct research using UV light, a device that has temperature and irradiation stability is needed. Measurement of UV LED irradiance is very important because with increasing time the UV LED irradiance will change gradually.[22]

From the background above, we characterized the UVB LED irradiation device used for the Wistar rat by observing temperature and irradiance stability. The purpose of this characterization is to determine the stability of temperature and irradiation of the device we make and to determine the duration of light exposure based on the characterization.

METHODS

Irradiation source and observation

The irradiation source used is a UVB LED lamp with the following specifications: supply constant voltage 6V-7V, supply constant current 80 mA – 385 mA, luminous corner 115°, wavelength 305 – 310 nm. The UVB LED lamp is mounted on the top of the black acrylic box with dimensions of 22 cm x 22 cm x 20 cm, equipped with a fan at the top of the UVB LED lamp, to avoid an increase in temperature during irradiation. A black acrylic box was used as a UVB LED simulation device. We use black acrylic because acrylic is not expensive, easy to get and easy to construct to the desired shape, and black color does not reflect light, so that the beam of light received by objects will not increase.

LED temperature, cage temperature, room temperature, and irradiance were observed at 5 cm from the UVB LED. Observations were made for 10 hours with recording every 30 minutes and without replication. The duration of observation is based on the estimated total duration of irradiation in experimental animal. Wavelength was measured using a High-Resolution Spectrometer - Aurora 4000. Irradiance was measured using a UV light meter. Measurements were made at each point with 5 mm with a grid guide on the bottom of the acrylic box as shown in Figure 1. The value of each point is added up and the average is calculated.

Statistical analysis

Statistical analysis using the Kolmogorov-Smirnov (Asymptotic Approximation) testing technique. The test criteria state that if the probability value > level of significance (alpha (α) = 5%).

RESULTS

Wavelength spectrum

The observation of the wavelength spectrum can be seen in Figure 2.

Irradiance and irradiance distribution

Observations at an exposure distance of 5 cm obtained the following results; peak irradiation of 200 ± 2 μW/cm², average irradiation 53.16 μW/cm2, radian power 0.05316 mJ. The coverage area of LED

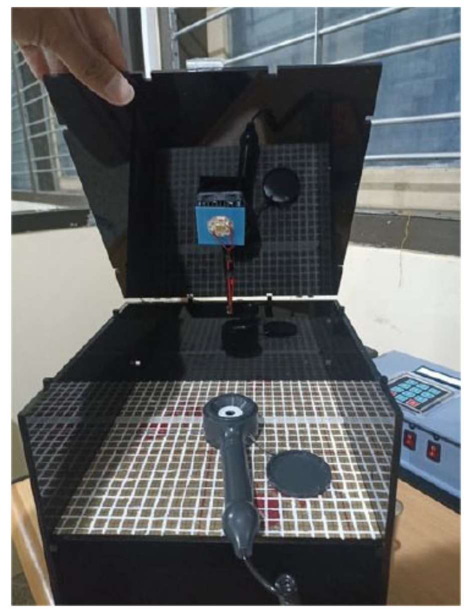

Figure 1. Simulation device of UVB LED irradiation. Measurement of irradiance distribution using a UV light meter. The test distance is at 5 cm from the LED. The dimension of the test surface is 22 cm x 22 cm, with 5 mm test grid line.

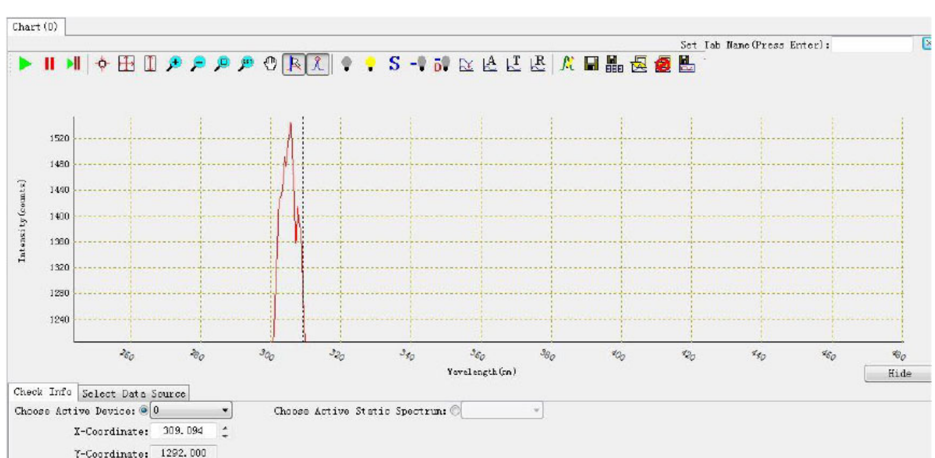

Figure 2. The wavelength spectrum with a peak of 309.094 nm. Wavelength was measured using a High-Resolution Spectrometer - Aurora 4000.

radiation exposure based on specifications is 193.5 cm², whereas based on tests is 153.86 cm² (Figure 3).

Simulation of dose calculation and irradiation time

The results for simulation of dose calculation and irradiation time depicted in Table 1.

Temperature and irradiance observation

Descriptive analysis was carried out to determine the characteristics of the variables studied, namely, to determine the minimum, maximum, average, and standard deviation values. The results of the descriptive analysis inform that the lowest LED temperature data is 25.30°C and the highest is 26.10°C. The average LED temperature data is 25.58°C with a standard deviation of 0.16°C. Furthermore, the lowest data cage temperature is 23.30 °C and the highest is 24.20°C. The average data cage temperature is 23.42°C with a standard deviation of 0.17°C. Next, the lowest room temperature data is 23.30°C and the highest is 23.80°C. The average room temperature data is 23.48°C with a standard deviation of 0.14°C. For irradiance data, the lowest is 201 μW/cm2 and the highest is 202 μW/cm2. The average irradiance data is 201.62 μW/cm2 with a standard deviation of 0.50 μW/cm2. The results can be seen at Figure 4 and Table 2.

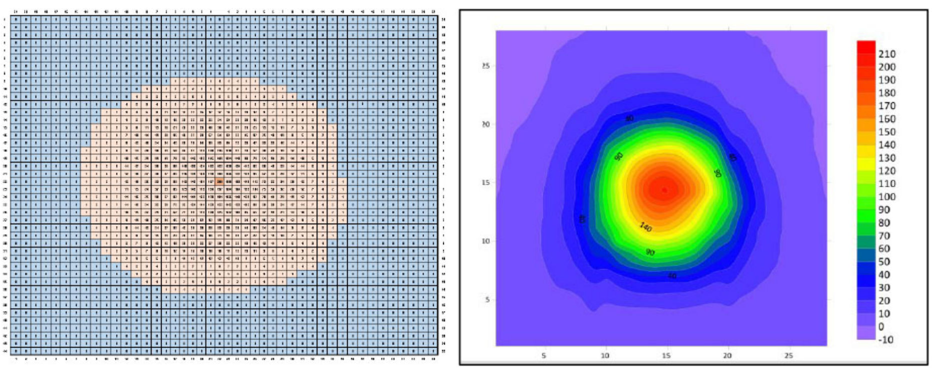

Figure 3. Results of area test and contour map of irradiation distribution (a) Results of exposure area test at 5 cm. (b) Simulation of a 2D irradiation distribution contour map at a height of 5cm. The orange color is the exposed area. The total area of the exposure test area is 22cm x 22cm. The exposed area (orange color) has a radius of ±7cm, L = 3.14 * r² = 153.86 cm².

Table 1. Simulation of calculating the time needed to get a certain dose with an irradiance of 53.16 mW/cm2 at 5 cm from the UVB LED

Average irradiance (μW/cm²)	Average dose (mJ/cm²)	Time (minutes)
53.16	100.0	31.35
53.16	50.0	15.67
53.16	25.0	7.84
53.16	12.5	3.92

μW, microwatt; mJ, millijoule; cm, centimeter

Suitability test of LED temperature, cage temperature, room temperature, and irradiance

The suitability test for LED temperature, cage temperature, room temperature, and irradiance were carried out using the Kolmogorov-Smirnov (Asymptotic Approximation) testing technique. The test criteria state that if the probability value > level of significance (alpha (α) = 5%) then there is no difference in LED temperature, cage temperature, room temperature, and irradiance in each observation

Table 3 informs that the suitability test at LED temperature produces a Kolmogorov-Smirnov test statistic of 0.185 with a p-value of 0.058. Thus, it can be stated that there is no difference in the LED temperature data at each observation. Furthermore, testing the suitability of the cage temperature produces a Kolmogorov-Smirnov test statistic of 0.397 with a p-value of 0.000. Thus, it can be stated that there is a difference in the data cage temperature in each observation. The suitability test at room temperature produces a Kolmogorov-Smirnov test statistic of 0.182 with a p-value of 0.068. Thus, it can be stated that there is no difference in room temperature data for each observation. Furthermore, the suitability test on irradiance produces a Kolmogorov-Smirnov test statistic of 0.397 with a p-value of 0.000. Thus, it can be stated that there are differences in the irradiance data for each observation.

DISCUSSION

The LED emission pattern is in the form of a Lambertian emission pattern, that is, the distribution of light is not the same at each point on the surface that receives the light. At an angle of 60° from the centre of the beam, the intensity decreases to half of the maximum value,[3] so we averaged

Table 2. Descriptive analysis of LED temperature, cage temperature, room temperature and irradiance, which includes minimum value, maximum value, average and standard deviation

Variable	Minimum	Maximum	Average	Standard Deviation
LED Temperature (⁰C)	25.30	26.10	25.58	0.16
Cage Temperature (⁰C)	23.30	24.20	23.42	0.17
Room Temperature (⁰C)	23.30	23.80	23.48	0.14
Irradiance (μW/cm²)	201.00	202.00	201.62	0.50

μW, microwatt; cm, centimeter; C, Celsius

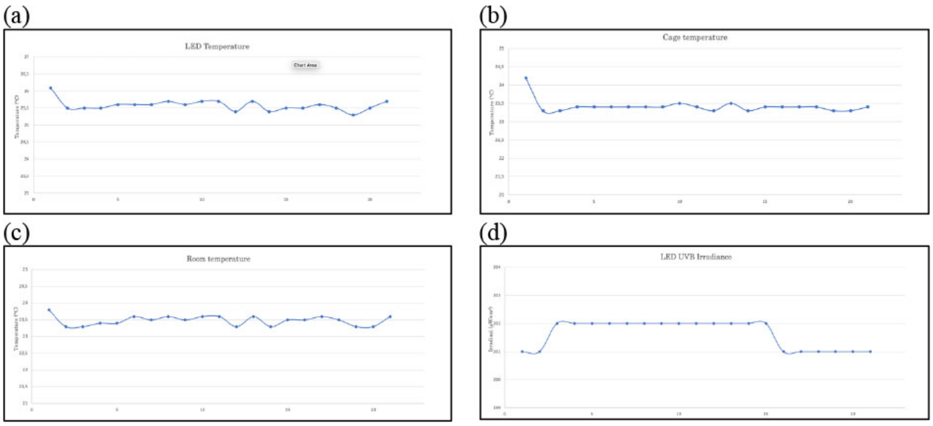

Figure 4. Graph of temperature and irradiation test results (a) LED temperature (b) Cage temperature (c) Room temperature (d) Irradiance. Observation of device endurance test for 10 hours, data sampling was taken every 30 minutes.

Table 3. Testing the LED temperature, cage temperature, room temperature, and irradiance using the Kolmogorov-Smirnov (Asymptotic Approximation) testing technique

Variable	Kolmogorov-Smirnov Statistic	Probability Value
LED Temperature	0.185	0.058
Cage Temperature	0.397	0.000
Room Temperature	0.182	0.068
Irradiance	0.397	0.000

the scatter on the emitted surface of the LED. The Lambertian pattern on this LED causes an unequal distribution of light on the surface of the rat's body. We have calculated the average irradiation distribution but there is a possibility that the exposure received by each rat is not the same, because during irradiation the rats were not anesthetized, so they could keep moving even though they were not free. Anesthesia in experimental animals must also be considered because there are still side effects from anesthesia. The average irradiance during the observation was 53.16 μW/cm², so it can be determined how long it will take to get the desired dose. For example, for a dose of 100 mJ/cm2, it takes 31.35 minutes.

The UVB LED wavelength spectrum is expected to be within specifications, namely in the range of 305-310 nm. From the observation results obtained a spectrum with a peak of 309,094 nm. Possible causative this condition could be tolerance of UVB LED. The value in the datasheet is the value in the test under ideal conditions. So that when applied in other conditions, it will allow the value to change. But the change in value will not come out far from the original value. So, there is a tolerance value, it doesn't exactly match the datasheet value. Another possibility are the differences in the LED driver used compared to the driver used during the test for the datasheet, accuracy of the spectrometer, the accuracy of the measurement method, temperature, humidity,[23] air pollutant and the present of corrosive gases,[24] and several other environmental factors that are not the same as the ideal test conditions in the datasheet. Air pollutant can reduce the light output power and high humidity can contributed to LED's failure.[23]

From the test results, it is proven that the LED temperature and room temperature are compatible, which means that the LED temperature and room temperature are stable. As for the cage temperature and irradiance, it was stated that there was no match, which could mean that the cage temperature and irradiance were unstable.

The advantages of LED when compared to conventional lamps are that they possess lower energy consumption, longer lifetime, and environmental safety.[8] 95% of the energy from an LED lamp is used to produce light and 5% to generate heat. This is inversely proportional to other types of lamps such as fluorescent incandescent lamps which produce 95% heat and only 5% is converted to light. From research by Rammohan et al it is known that the halogen temperature is almost 300% higher compared to LED bulb.[25]

Although LEDs are efficient power-to-light converters, they still generate heat. LEDs differ from other light sources in that they are more efficient at lower temperatures and can be effectively cooled through the structure into which they are installed.[8] High temperature usually contributed to LED's failure.[23] The emission intensity of LEDs decreases with increasing temperature.[3] We put a fan above the LED to maintain the stability of the LED temperature. We put a fan above the LED to maintain the stability of the LED temperature. The fan placed above the LED will not affect the condition of the experimental animals. This is different from research conducted on cell culture where air movement will increase the risk of contamination of the sample.[8]

This study still has limitation, namely in the number of replications, observation time and ideal observation place. We suggest for further improvement or modification to the UVB LED irradiation device with more detail in material and methods.

CONCLUSION

We have designed, built, and then tested a device for animal model (Wistar rats) with a UVB LED irradiation source. Observations were made on LED temperature, cage temperature, room temperature, and irradiance. Observations were made for 10 hours with recording every 30 minutes. Irradiance measurements were carried out at 5 cm from the LED lamp. The average irradiance is 53.16 μW/cm², so we can determine how long it will take to get the desired dose. This special device with a UVB LED source can be used for research on Wistar rats, and maybe another UVB LED irradiation source design will be developed for other types of experimental animals and specific purposes.

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ETHICAL STATEMENT

This study has been approved by Animal Care and Use Committee of Brawijaya University with Ethical Clearance No: 142-KEP-UB-2022.

CONFLICT OF INTEREST

The author reports no conflicts of interest in this work.

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None.

AUTHORS' CONTRIBUTION

DD responsible for concept of the study, definition of intellectual content, literature search, clinical studies, experimental studies, data analysis, statistical analysis, manuscript preparation, manuscript editing, and guarantor. CP responsible for concept of the study, definition of intellectual content, literature search, manuscript preparation, manuscript editing, and guarantor. RPR responsible for concept of the study, literature search, data analysis, statistical analysis, manuscript preparation, manuscript editing, manuscript review, and guarantor. VRW responsible for concept and design of the study, clinical studies, experimental studies, data acquisition, manuscript preparation, and guarantor.

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