JURNAL MATEMATIKA, STATISTIKA DAN KOMPUTASI e–ISSN: 2614–8811
Published by Departement of Mathematics, Hasanuddin University, Indonesia
https://journal.unhas.ac.id/index.php/jmsk/index
Vol. 22, No. 3, May 2026, pp. 602-616
DOI: 10.20956/j.v22i3.49375

Analysis of the Effects of Light Emitting Diode (LED) Phototherapy on the Hematological and Biochemical Parameters of Rabbits Using Repeated Measures Longitudinal ANOVA

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Received: 11 January 2026, revised: 24 March 2026, accepted: 3 April 2026

Abstract

This study examined the longitudinal effects of Light Emitting Diode (LED) phototherapy on hematological (hemoglobin) and biochemical (creatinine) parameters in rabbits (*Oryctolagus cuniculus*). Although LED phototherapy is widely applied as a non-invasive treatment, its systemic effects in repeated-measure settings remain limited. Fourteen rabbits were randomly assigned to a control group (n = 7) and a treatment group (n = 7). The treatment group received two 12-hour LED phototherapy sessions on consecutive days, while the control group underwent identical conditions without LED activation. Hemoglobin and creatinine levels were measured at three time points and analyzed using Repeated Measures ANOVA, with assumption testing for normality, homogeneity, and sphericity; Greenhouse–Geisser correction was applied when necessary. The results showed that observation time significantly affected hemoglobin ($p = 0.004967$) and creatinine levels ($p = 0.03577$), indicating temporal physiological changes that occurred regardless of treatment exposure. However, no significant differences were observed between the control and treatment groups for hemoglobin ($p = 0.936$) or creatinine ($p = 0.357$), and no significant group–time interaction was detected, suggesting that the observed changes were independent of LED phototherapy. Post-hoc pairwise comparisons based on estimated marginal means (EMMs) with Tukey adjustment revealed a significant increase in hemoglobin from time 0 to time 1 and a significant difference in creatinine between time 1 and time 2, further supporting that these variations reflect natural physiological processes rather than treatment-induced effects. These findings indicate a lack of evidence of treatment effect under the studied conditions, although the relatively small sample size warrants cautious interpretation. Future studies with larger samples are recommended.

Keywords: *LED Phototherapy, Hematological, Biochemical, Repeated Measures ANOVA; Rabbit Model*

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Abstrak

Penelitian ini bertujuan untuk mengevaluasi efek jangka panjang fototerapi Light Emitting Diode (LED) terhadap parameter hematologi (hemoglobin) dan biokimia (kreatinin) pada kelinci (*Oryctolagus cuniculus*). Empat belas kelinci dibagi secara acak menjadi kelompok kontrol (n = 7) dan kelompok perlakuan (n = 7). Kelompok perlakuan menerima dua sesi fototerapi LED selama 12 jam berturut-turut, sedangkan kelompok kontrol menjalani kondisi yang sama tanpa aktivasi LED. Pengukuran hemoglobin dan kreatinin dilakukan pada tiga titik waktu dan dianalisis menggunakan *Repeated Measures* ANOVA dengan pengujian asumsi normalitas, homogenitas, dan sferisitas; koreksi Greenhouse–Geisser diterapkan bila diperlukan. Hasil menunjukkan bahwa waktu pengamatan berpengaruh signifikan terhadap hemoglobin ($p = 0{,}004967$) dan kreatinin ($p = 0{,}03577$), menandakan adanya perubahan fisiologis alami seiring waktu. Namun, tidak ditemukan perbedaan signifikan antara kelompok kontrol dan perlakuan untuk hemoglobin ($p = 0{,}936$) maupun kreatinin ($p = 0{,}357$), dan tidak terdapat interaksi kelompok–waktu yang signifikan, menunjukkan bahwa perubahan tersebut tidak terkait dengan fototerapi LED. Analisis *post-hoc* dengan perbandingan berpasangan *estimated marginal means* (EMMs) dengan penyesuaian *Tukey* menunjukkan peningkatan hemoglobin yang signifikan dari waktu 0 ke waktu 1 serta perbedaan kreatinin yang signifikan antara waktu 1 dan waktu 2, mendukung bahwa perubahan ini merupakan proses fisiologis alami, bukan akibat perlakuan. Temuan ini menunjukkan tidak adanya bukti efek fototerapi LED pada kondisi yang diteliti, meskipun ukuran sampel yang kecil perlu diperhatikan. Penelitian dengan sampel lebih besar disarankan untuk konfirmasi lebih lanjut.

Kata kunci: Fototerapi LED; Hematologis, Biokimia; Repeated Measures ANOVA; Kelinci

1. INTRODUCTION

Phototherapy, which involves the use of light to stimulate biological processes, has evolved into an increasingly important non-invasive therapeutic method in modern medicine, particularly with advances in near-infrared (NIR) technology (Pan, W. T., 2023). The basic principle is photobiomodulation (PBM), which involves the interaction of light with a wavelength of 600-1100 nm on cytochrome c oxidase in the mitochondria of cells, which increases ATP formation, reduces oxidative stress, and promotes cell proliferation and tissue healing (Hamblin, 2016). In this development, Light Emitting Diode (LED) technology has emerged as an important innovation that has transformed conventional phototherapy, with the advantages of high energy efficiency, ease of use, and lower costs, enabling wider application from skin wound treatment to transcranial neurological therapy (Glass, 2021). Globally, recent research demonstrates the ability of LED-based PBM in treating chronic conditions such as musculoskeletal pain and metabolic disorders (Moskvin & Kozlov, 2021).

In recent preclinical studies, LED phototherapy has been used in animals to identify cellular mechanisms prior to clinical trials, particularly in assessing long-term safety (Hamblin, 2016). Rabbits (Oryctolagus cuniculus) are used as experimental models in translational research due to similarities in physiology with humans, including vascular and lipid metabolic processes (Fan et al., 2018). In animals such as rabbits, it is possible to observe the effects of light on blood vessels and metabolic processes, such as increased blood flow and endothelial cell activation after LED exposure. Furthermore, low-level laser therapy has demonstrated anti-inflammatory effects and promoted tissue healing in rabbit models, supporting its potential for medical applications (Park et al., 2023).

Although preclinical results in rabbits are promising, many studies are still limited to short-term observations. This often overlooks differences in impact caused by varying light intensities or exposure durations. For example, red LED light (around 660 nm) has been reported to activate endogenous protective mechanisms, including the reduction of oxidative stress and inflammatory responses. However, without continuous monitoring, its effects on blood components and systemic metabolism may vary due to dose and time dependent responses (Maghfour., 2024).Therefore, long-term research is needed to understand the overall changes. This rabbit model is suitable for establishing a safety basis before application in humans, especially for vulnerable groups such as newborns (neonates).

Light Emitting Diode (LED) phototherapy is a standard treatment for neonatal hyperbilirubinemia that has been proven to be safe and effective (Bhutani *et al.*, 2024) . However, exposure to high-intensity LED light for a certain duration has the potential to affect other systemic physiological parameters, including hemoglobin levels as an indicator of hematological function and creatinine as a biomarker of renal function. Phototherapy exposure can generate reactive oxygen species and induce apoptosis, thereby potentially affecting blood cells. Therefore, evaluating changes in hematological parameters is important to minimize complications and improve outcomes (Kassie *et al.*, 2025). Although several studies have evaluated the biocompatibility of LED phototherapy, most have used a single or short-term measurement approach, for example, only comparing before and after phototherapy in neonates, so the longitudinal pattern of blood parameter changes has not been well characterized.

Research with a longitudinal design using repeated measures ANOVA is important to understand the dynamics of changes in hematological and biochemical biomarkers over time. Repeated measures ANOVA is an appropriate method for analyzing repeated measurement data in medical research. Muhammad (2023) explains that this method is able to overcome the problem of correlation between measurements from the same subject, noting that approximately 50% of biomedical research uses a repeated measurement design. The effectiveness of this method was demonstrated by Hassan (2021) through a study of 50 patients with depression who underwent psychotherapy for four months, which showed that repeated measures ANOVA has better statistical power and is able to control for inter-subject variation.

Additionally, preclinical studies in rabbits, which have metabolic characteristics similar to humans, can provide reliable physiological indicators of systemic responses to repeated LED exposure (Pessini *et al.*, 2020). This longitudinal data is not only important for identifying potential long-term side effects, but also for optimizing safer and more effective treatment protocols. With repeated analysis at multiple time points, research can capture more detailed patterns of change, helping to determine the ideal duration and intensity of phototherapy to achieve maximum benefits with minimal risk. In comparison, several studies on rabbits evaluating hematological and biochemical responses still rely on a single-time-point design using conventional one-way analysis of variance (ANOVA). For example, Adewale *et al.* (2021) investigated the effects of Rauvolfia vomitoria root extract on the blood profiles of growing rabbits, but blood samples were collected only once at the end of the experiment, limiting the ability to observe physiological changes over time. This cross-sectional approach cannot capture dynamic trends, unlike repeated measures ANOVA, which allows for the assessment of temporal patterns across measurement points.

Research on the effects of LED phototherapy on hemoglobin and creatinine levels in rabbits using repeated measures ANOVA contributes to SDG 3 (Good Health and Well-being) and SDG 9 (Industry, Innovation and Infrastructure). This non-invasive health technology has the potential to improve the quality of medical care and expand access to services, in line with target 3.8 on universal health coverage. The environmentally friendly and cost-effective innovation of LED phototherapy supports the development of efficient and sustainable medical technology, as well as the achievement of SDG 9 (Hernández-Bule, *et al*., 2024). Additionally, the use of repeated measures ANOVA is reinforced by Muhammad (2023) findings, which show that this method can overcome correlations between measurements on the same subject. Thus, this research contributes to health, technological innovation, and the strengthening of sustainable scientific capacity.

2. MATERIAL AND METHODS

2.1 Repeated Measures ANOVA

Repeated Measures ANOVA is a statistical method specifically designed to analyze data involving the same subject under several different conditions or times. Repeated Measures ANOVA statistical model considers the fixed effects of treatment or time, the random effects of individuals, and errors. The general model of Repeated Measures ANOVA for cases with no treatment or ANOVA random blocks is written as follows (Blanca *et al*., 2023).

$$Y_{ij} = \mu + \pi_i + \alpha_j + \varepsilon_{ij} \quad (1)$$

Where, Y_{ij} is the subject measurement value to-i at the time-j ; μ is the general average ; α_j is the effect of time or time treatment j ; π_i is a random effect of the subject i ; and ε_{ij} is the effect of the error associated with the subject i at the time j.

Meanwhile, the model that is commonly used when there is a treatment of the data is called ANOVA split-plots which are written as follows:

$$Y_{hij} = \mu + \gamma_h + \alpha_j + (\gamma\alpha)_{hj} + \pi_{i(h)} + \varepsilon_{hij} \quad (2)$$

Where, μ is the overall average ; γ_h is the group h effect with $\sum_{h=1}^{H} \gamma_h = 0$; α_j is the effect of time with j $\sum_{j=1}^{J} \alpha_j = 0$; $(\gamma\alpha)_{hj}$ is the effect of the interaction between time and the group with $\sum_{h=1}^{H}\sum_{j=1}^{J}(\gamma\alpha)_{hj} = 0$; $\pi_{i(h)}$ is an individual influence i who are in the group h at the time j ; and ε_{hij} is an error for individuals i who are in the group h at the time j.

This study employed Repeated Measures ANOVA due to the balanced experimental design, with complete observations for all subjects across three time points. This method is suitable for examining within-subject (time), between-subject (group), and interaction effects in a relatively simple longitudinal structure. To ensure the validity of the analysis, key assumptions such as normality, homogeneity, and sphericity were evaluated, and the Greenhouse–Geisser correction was applied when violations were detected.

While more flexible approaches for longitudinal data analysis exist, Repeated Measures ANOVA was considered adequate for addressing the objectives of this study. Effect sizes were calculated using partial eta squared (η^2p) to quantify the magnitude of the effects of time, group, and their interaction. Estimated marginal means (EMMs) along with 95% confidence intervals were computed to summarize the mean responses across time and groups. Post-hoc pairwise

comparisons were performed based on estimated marginal means (EMMs) with Tukey adjustment when significant effects were detected.

2.2 Assumption Test Repeated Measures ANOVA

2.2.1 Normality Test

The normality test is performed to check whether the residues in the model have a normal distribution, as the normality assumption in ANOVA theoretically applies to residuals, not to raw data (Midway & White, 2025). One commonly used approach to assess residual normality is the Kolmogorov–Smimov test**.** The hypotheses used in the residual normality test with Kolmogorov–Smirnov are as follows:

H_0 : Residual data is normally distributed

H_1 : Residual data is not normally distributed

Test statistics for Kolmogorov-Smirnov are given on the equation (3) as follows:

$$D = \sup_Y |F_n(Y) - F(Y)| \tag{3}$$

With $F_n(Y)$ as the residual cumulative distribution value and $F(Y)$ is the cumulative distribution value below H_0 for distribution $P(Z < Z_i)$. With its critical areas, reject H_0 if $D > D_\alpha$, where D_α is the Kolmogorov–Smirnov critical value at the 0.05 significance level. Or the data can be said to be normally distributed if the value $Asymp.\,Sig > 0.05$.

2.2.2 Homogeneity Test

In ANOVA analyses of most clinical trials with continuous response, variance homogeneity is often an important assumption in testing whether the mean of several groups is the same (Zhou *et al*., 2023). If the variance between groups is different, then the test results are invalid. One of the homogeneity tests is the Levene Test. The hypotheses used in this test are:

H_0 : $\sigma_1^2 = \sigma_2^2 = \cdots = \sigma_k^2$

H_1 : $\sigma_i \neq \sigma_j$ for at least one pair (i, j)

Test statistics for the Levene Test are given on the equation (4) as follows:

$$W = \frac{(N-k)\sum_{i=1}^{k} n_i (\bar{Z}_i - \bar{Z}..)^2}{(k-1)\sum_{i=1}^{k}\sum_{j=1}^{n_i}\left(Z_{ij} - \bar{Z}_{i.}\right)^2} \tag{4}$$

Where, N is the total number of observations ; k is the number of groups ; n_i is the sample size of group i ; $Z_{ij} = \left|Y_{ij} - \bar{Y}_{i.}\right|$; $\bar{Y}_{i.}$ is the average of the group to-i ; $\bar{Z}_{i.}$ is the average group of Z_i ; $\bar{Z}..$ is the overall average of Z_{ij}. With critical areas reject H_0 if $W > F_{(\alpha;k-1,N-k)}$.

2.2.3 Mauchly Test of Sphericity

In repeated measures ANOVA, the main assumption for within-subject factors is sphericity, tested using Mauchly's test, while Levene's test is only used to check homogeneity between groups at each time point. Therefore, *the Mauchly Test of Sphericity* needs to be used to test whether these assumptions are met, both in the testing

of the main effect and the interaction(Langenberg *et al.*, 2023). The hypothesis of the *Mauchly Test of Sphericity* is as follows.

H_0 : The variance of the difference between group pairs is the same

H_1 : The variance of the difference between group pairs is not the same

The test statistics of the *Mauchly Test of Sphericity* are written in the following equation.

$$W = \frac{|\boldsymbol{T}|}{\frac{tr(\boldsymbol{T})}{p}\times d} \quad (5)$$

Where, $\boldsymbol{T}$ is a matrix with a shape $\boldsymbol{T} = \boldsymbol{M}'\widehat{\boldsymbol{\Sigma}}\,\boldsymbol{M}$; $\widehat{\boldsymbol{\Sigma}}$ is the sample covariance matrix (estimate of the population); $\boldsymbol{M}$ is an orthogonal contrast matrix that measures $d \times p$ with $d = p - 1$; $\boldsymbol{\Sigma}$ is the covariance matrix of the measured data $p \times p$; σ^2 is the population variance of each variable; ρ is the correlation between variables; and p is the number of the groups.

After the calculation of the value W , Statistical calculation of the Chi-Square test was carried out to test the significance of the W with the following equation:

$$C = -(n - r)\log(W)\,.D \quad (6)$$

Where, n is the number of rows of the experimental design matrix ; r is the rank of the experimental design matrix ; and D is a correction factor with $D = \frac{1-2d^2+d+2}{6d(n-r)}$. H_0 rejected if test statistics $C > C_{(\alpha,df)}$ with $df = \frac{p(p-1)}{2} - 1$.

2.2.4 Greenhouse Geisser

If the sphericity assumption tested using the Mauchly's Test is not met, the results of the analysis may be invalid due to a bias in the calculation of the degree of freedom. To overcome this, correction methods are applied, one of which is Greenhouse Geisser correction. The Greenhouse Geisser is made by calculating the epsilon value (ε) written in the equation as follows:

$$\varepsilon = \frac{(k-1)*\Sigma(s_j-s)^2}{(\sum_{i=1}^{k}\sum_{j=1}^{k}(s_{ij}-s)^2 - 2\sum_{j=1}^{k}(s_j-s)^2 + ks^2)} \quad (7)$$

Where, k is number of repeated measurements ; s_j is the average of the element on the line to j of the covariance matrix; and s is the average of all elements of the covariance matrix. In general, the value of the sphericity index is in the range of 0 to 1. If $\varepsilon = 1$, this indicates that the sphericity assumption is fully met. Conversely, when the value ε is between $0 \leq \varepsilon < 1$, then the sphericity assumption is not fully met.

2.3 Pairwise Comparisons Based on Estimated Marginal Means

Pairwise comparisons between time points were conducted based on estimated marginal means (EMMs) derived from the fitted repeated measures ANOVA model. Adjustments for multiple comparisons were performed using the Tukey method. This approach accounts for the correlation among repeated observations within the same subjects, providing more reliable inference compared to classical post-hoc procedures based on independent observations. The hypothesis for pairwise comparisons are defined as follows:

$H_0 : \mu_i EMM = \mu_j EMM$

$H_1 : \mu_i EMM \neq \mu_j EMM$

The null hypothesis states that there is no difference between the estimated marginal means (EMMs) at two time points, while the alternative hypothesis states that a difference exists.

2.4 Statistical Software

All statistical analyses were performed using R software (version 4.5.1). Repeated measures ANOVA was conducted using the afex package. Prior to model interpretation, assumption tests including normality, homogeneity, and sphericity were evaluated using the rstatix and car packages. Post-hoc pairwise comparisons between time points were conducted based on estimated marginal means (EMMs) derived from the fitted model using the emmeans package, with Tukey adjustment applied to control the family-wise error rate. This method accounts for within-subject correlations and provides more reliable results compared to classical post-hoc procedures based on independent observations.

3. RESULT AND DISCUSSION

3.1 Data Exploration

This study utilized secondary data obtained from the Mendeley Data repository (Version 2, DOI: 10.17632/s56gr964h6.2, available at https://data.mendeley.com/datasets/s56gr964h6/2), published on February 8, 2024. The dataset was derived from a phototherapy experiment using Light Emitting Diode (LED) on rabbits (*Oryctolagus cuniculus*). The dataset involved 14 rabbits, divided into two groups: a control group (n = 7) and a treatment group (n = 7). The LED device used was a blanket-type device placed in direct contact with the rabbit skin after ventrolateral trichotomy, with a mean irradiance of 19.3 (13.0–22.0) µW/cm²/nm. The treatment group was exposed to LED phototherapy in two sessions (each lasting 12 hours over two consecutive days), while the control group was kept under the same conditions without device activation. Rectal and skin temperatures were monitored throughout the experiment. Each rabbit was observed at three time points: before treatment (T0), after the first day of treatment (T1), and after the second day of treatment (T2). Blood samples were collected at these three time points for hematological and biochemical analysis. Therefore, the dataset is arranged in a repeated measures format, with each individual having three observations recording hemoglobin and creatinine levels at each time point. The authors were responsible for data acquisition, preprocessing, and statistical analysis, and no additional experimental procedures were conducted in this study.

a. Hematological

In this section, we present an exploration of longitudinal data related to hematological parameters, namely hemoglobin levels in each rabbit.

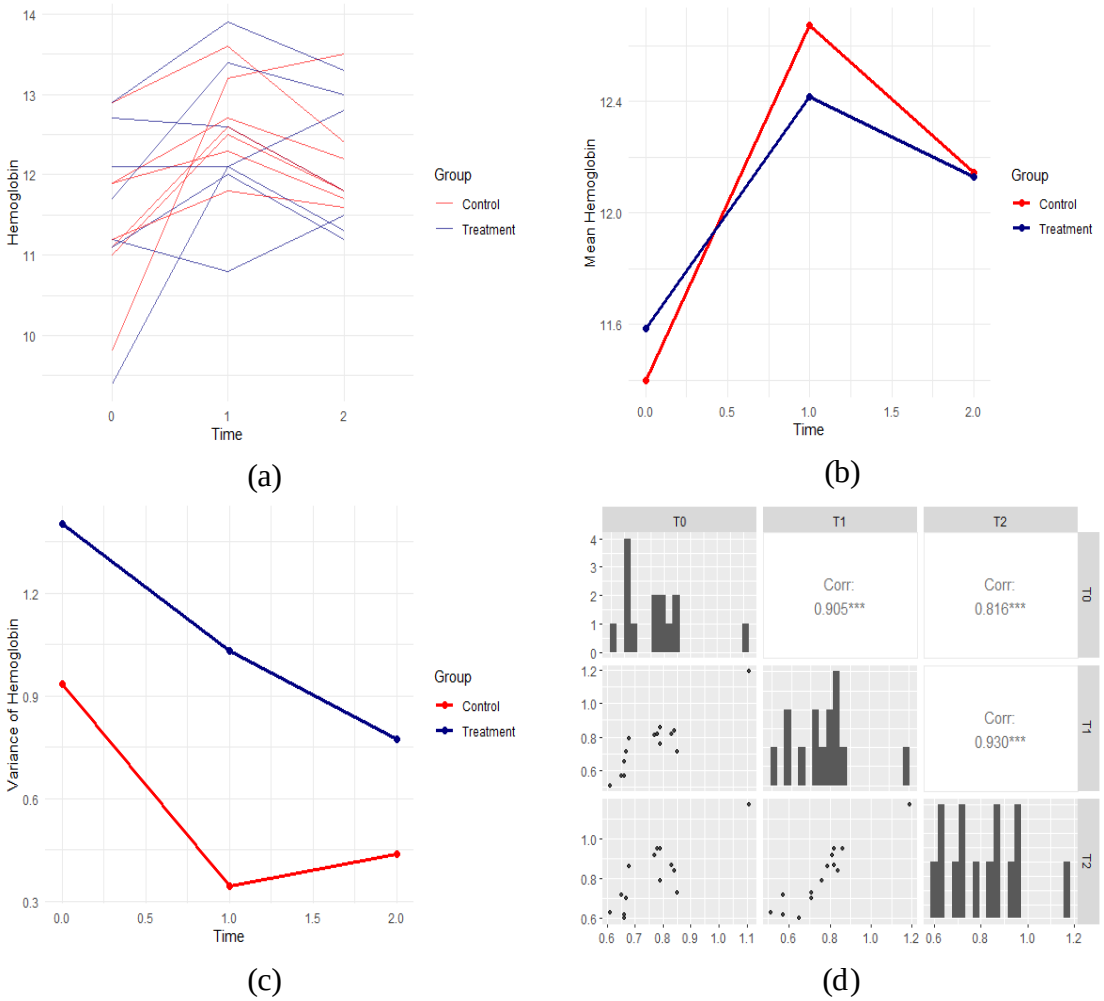


Figure 1. (a) Profile Individu Hematological (b) Mean Structure by Group (c) Variance Structure by Group (d) Scatterplot and Correlation within Time

The longitudinal exploration indicates that hemoglobin levels in both groups follow a similar pattern, increasing at the first measurement and decreasing at the second. The Control group consistently shows slightly higher mean levels, while variances in both groups decline over time, suggesting reduced inter-individual heterogeneity. Correlations are strongest between the first and second measurements, whereas correlations between the Control and Treatment groups at each time point are weak and nonsignificant. These findings suggest that changes in hemoglobin occur independently across groups.

b. Biochemical

In this section, we present an exploration of longitudinal data related to biochemical parameters, namely creatinine levels in each rabbit.

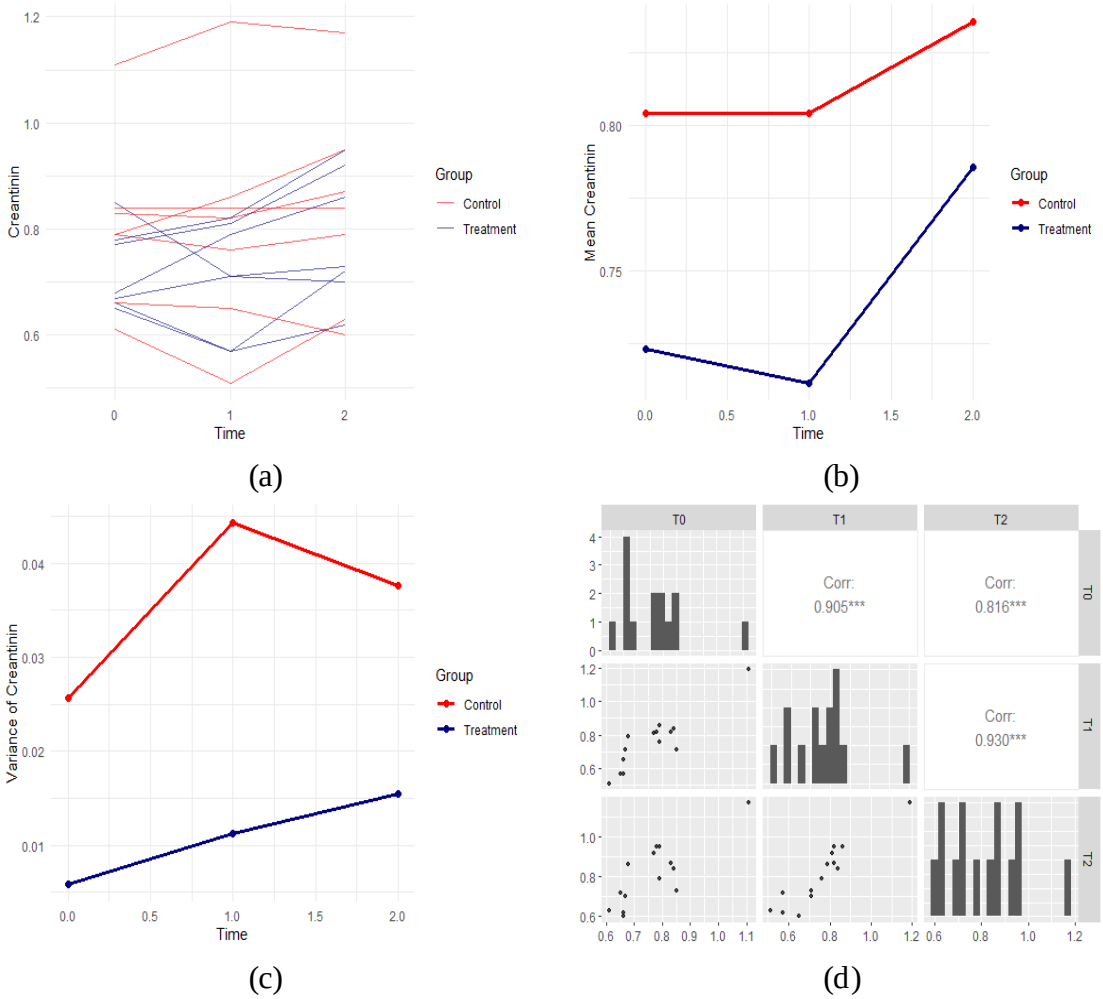


Figure 2. (a) Profile Individu Biochemical (b) Mean Structure by Group (c) Variance Structure by Group (d) Scatterplot and Correlation within Time

The exploratory analysis shows that creatinine levels in both groups change over time with similar directional patterns, characterized by a decrease at the first measurement followed by an increase at the second. Mean creatinine levels in the Control group remain relatively stable, whereas the Treatment group exhibits a decline at time 1 and an increase at time 2, with the Control group consistently presenting higher mean values. Variance patterns differ across groups: the Control group shows an initial increase followed by a decrease, while the Treatment group maintains lower and more stable variance. Correlations between repeated measurements are strongest between time 1 and time 2, with time 0 showing moderately lower yet still positive correlations. Inter-group correlations at each time point are weak and mostly nonsignificant, indicating that creatinine changes in the two groups evolve independently.

3.2 Classical Assumption

a. Mauchly's Test of Sphericity

The results of Mauchly's Test performed on the hematological and biochemical parameters are summarized below. For the hematological parameter (hemoglobin), Mauchly's Test yielded a p-value of 0.0018099, which is lower than the significance threshold of $\alpha = 0.05$. This result indicates that the null hypothesis of equal variances is rejected, meaning that the assumption of sphericity is violated. Due to this violation, the Greenhouse–Geisser correction must be applied to adjust the degrees of freedom and avoid inflated Type I error rates. Therefore, the repeated measures ANOVA results for hemoglobin are reported using Greenhouse–Geisser corrected degrees of freedom. The obtained Greenhouse–Geisser epsilon value of 0.59426 reflects a moderate deviation from perfect sphericity, and the corrected results ensure that the repeated measures ANOVA for hemoglobin remains valid and statistically sound.

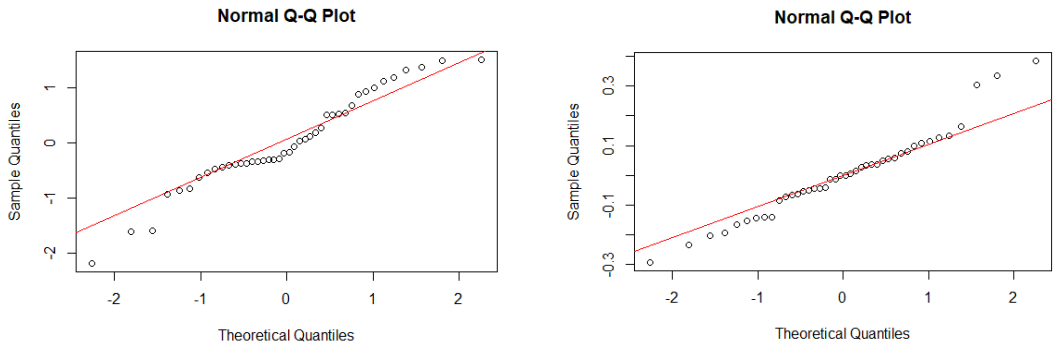
In contrast, for the biochemical parameter (creatinine), Mauchly's Test produced a p-value of 0.21249, which is greater than 0.05. This indicates that the assumption of sphericity is satisfied, allowing the repeated measures ANOVA to be conducted without applying any corrections such as the Greenhouse–Geisser adjustment. Thus, the creatinine analysis can proceed using the standard repeated measures ANOVA framework.

b. Normality Test

In repeated measures ANOVA, the normality assumption applies to the residuals of the fitted model rather than to the raw data. In this study, the normality of residuals was evaluated using the Shapiro–Wilk test, which is more appropriate for small sample sizes. The residuals were obtained from the full repeated measures ANOVA model including the effects of Group, Time, and the Group × Time interaction.

Table 1. Shapiro–Wilk Test Kolmogorov–Smirnov

Normality Test Shapiro–Wilk	Sig.
Hemoglobin	0.1692
Creatinine	0.2203



(a) (b)

Figure 3. Normal Q–Q plots of residuals from the repeated measures ANOVA model: (a) Hematological (hemoglobin), (b) Biochemical (creatinine)

Table 1 shows that the Shapiro–Wilk test produced a p-value of 0.1692 for hemoglobin residuals and 0.2203 for creatinine residuals. Since both p-values are greater than 0.05, there is no statistical evidence to reject the null hypothesis of normality. This indicates that the residuals from the repeated measures ANOVA model are approximately normally distributed.

The Q–Q plots for both hemoglobin and creatinine residuals also show that most of the points lie close to the reference line, with only minor deviations at the tails. This graphical result supports the conclusion that the residuals follow a normal distribution. Therefore, the normality assumption required for repeated measures ANOVA is considered satisfied for both hemoglobin and creatinine variables.

c. Homogeneity Test

Levene's test was used to evaluate the homogeneity of variances between groups at each observation time. This test does not assess covariance structure across repeated measurements, but only checks whether the variance of the response variable is equal between control and treatment groups at each time point.

Table 2. Levene's Test

Time	Sig.	
	Hemoglobin	Creatinine
0	0.657	0.408
1	0.362	0.365
2	0.341	0.499

As shown in Table 2, all p-values exceed 0.05. Therefore, the null hypothesis cannot be rejected, indicating that the residual variances across all time points are homogeneous. With the homogeneity assumption fulfilled, the repeated measures ANOVA can be appropriately conducted without further adjustment.

3.3 Repeated Measures ANOVA

Repeated measures analysis was conducted to assess significant differences across repeated measurements of the same variable within the same subjects. Once all underlying assumptions were met, a comprehensive Repeated Measures ANOVA was performed to test for significance.

a. Hematological

To evaluate changes in hemoglobin levels across time and treatment groups, a repeated measures ANOVA was conducted. Because Mauchly's test indicated that the sphericity assumption was violated ($p < 0.05$), the Greenhouse–Geisser correction was applied to adjust the degrees of freedom. The corrected results are presented in Table 3.

Table 3. Results of Repeated Measures ANOVA on Rabbit Hemoglobin Levels

Observartion	Sum Sq	df	Error SS	den df	F-Test Statistic	P-Value	η^2p
Group	0.0	1	15.428	12	0.0067	0.936	0.00055

Time (GG)	7.8	1.19	14.115	14.26	6.671	0.005	0.36
Group*Time(GG)	0.3	2	14.115	24	0.293	0.749	0.024

Based on the results in Table 3 using Greenhouse–Geisser corrected degrees of freedom, a Repeated Measures ANOVA was conducted at a significance level of 0.05. The time effect was significant ($F = 6.671$, $p = 0.005$), indicating differences in hemoglobin levels across observation times. This suggests that variations in hemoglobin among time points are unlikely due to chance, warranting post-hoc analysis, such as Tukey's HSD, to identify specific time points with significant differences. The group effect was not significant ($F = 0.0067$, $p = 0.936$), indicating no difference in hemoglobin levels between treatment groups. Likewise, the interaction effect between group and time was not significant ($F = 0.293$, $p = 0.749$), suggesting that the combined effect of group and observation time does not influence hemoglobin levels.

In terms of effect size, the time factor showed a moderate to large effect ($\eta^2p = 0.36$), indicating that time contributed substantially to the variation in hemoglobin levels. In contrast, the group ($\eta^2p = 0.00055$) and interaction effects between group and time ($\eta^2p = 0.024$) were small, suggesting that their contributions to the variability in hemoglobin levels were minimal.

b. Biochemical

Similarly, to assess changes in creatinine levels across time and treatment groups, a Repeated Measures ANOVA was performed. The results are summarized in Table 7.

Table 4. Results of Repeated Measures ANOVA on Rabbit Creatinine Levels

Observation	Sum Sq	df	Error SS	den df	F-Test Statistic	P-Value	η^2p
Group	0.0587	1	0.76846	12	0.9165	0.3573	0.07
Time	0.0236	2	0.07366	24	3.8386	0.04759	0.24
Group*Time	0.0034	2	0.07366	24	0.5617	0.54236	0.04

Based on Table 4, a Repeated Measures ANOVA was performed at a significance level of 0.05. The time effect was significant ($F = 3.8386$, $p = 0.04759$), indicating differences in creatinine levels across observation times. This suggests that variations in creatinine among time points are unlikely due to chance, and post-hoc analysis, such as Tukey's HSD, may be conducted to identify specific time points with significant differences. The group effect was not significant ($F = 0.9165$, $p = 0.357$), indicating no difference in creatinine levels between treatment groups. Similarly, the interaction effect between group and time was not significant ($F = 0.5617$, $p = 0.54236$), suggesting that the combined effect of group and observation time does not influence creatinine levels.

In terms of effect size, the time factor showed a moderate effect ($\eta^2p = 0.24$), indicating that time contributed meaningfully to the variation in creatinine levels. In contrast, the group ($\eta^2p = 0.07$) and the interaction effect between group and time ($\eta^2p = 0.04$) were small, suggesting that their contributions to the variability in creatinine levels were limited.

3.4 Pairwise Comparisons Based on Estimated Marginal Means

a. Hematological

Following the significant time effect detected by Repeated Measures ANOVA, pairwise comparisons between time points were conducted based on estimated marginal means (EMMs) derived from the fitted model. Adjustments for multiple comparisons were performed using the Tukey method.

Table 5. Pairwise Comparison Based on Estimated Marginal Means for Time Effect on Rabbit Hemoglobin Levels

Time (I)	Time (J)	Estimate	SE	T-Ratio	P-Value
T0	T1	-1.050	0.286	-3.670	0.0083
	T2	-0.643	0.381	-1.687	0.2498
T1	T2	0.407	0.158	2.574	0.0589

Pairwise comparisons based on EMMs indicated a significant difference in hemoglobin levels between T0 (pre-treatment) and T1 (one day post-treatment) ($t = -3.670$, $p = 0.0083$), suggesting a meaningful increase in hemoglobin after one day of treatment. Comparisons between T0 and T2 ($t = -1.687$, $p = 0.2498$) and between T1 and T2 ($t = 2.574$, $p = 0.0589$) were not statistically significant, indicating that hemoglobin levels tended to stabilize after the initial change.

b. Biochemical

Following the significant time effect detected by the Repeated Measures ANOVA, pairwise comparisons between time points were conducted based on estimated marginal means (EMMs), with Tukey adjustment applied for multiple testing.

Tabel 6. Pairwise Comparisons Based on Estimated Marginal Means for Time Effect on Rabbit Creatinine Levels

Weeks (I)	*Weeks* (J)	Estimate	SE	T-Ratio	P-Value
T0	T1	0.00571	0.0208	0.275	0.9593
	T2	-0.04714	0.0251	-1.878	0.1872
T1	T2	-0.05286	0.0159	-3.317	0.0157

The results showed a significant difference in creatinine levels between week 1 and week 2 ($t = -3.317$, $p = 0.0157$), indicating a meaningful change during this period. In contrast, comparisons between week 0 and week 1 ($t = 0.275$, $p = 0.9593$) and between week 0 and week 2 ($t = -1.878$, $p = 0.1872$) were not statistically significant, suggesting that creatinine levels remained relatively stable in the early phase of treatment.

4. CONCLUSION

Based on the analysis and discussion in this study, it can be concluded that time had a significant effect on hemoglobin and creatinine levels in rabbits, as indicated by ANOVA p-values of 0.004967 and 0.03577, respectively. However, LED phototherapy did not produce significant differences compared to the control group, with p-values of 0.936269 for hemoglobin and 0.35730 for creatinine. Additionally, no statistically significant interaction was found between time and treatment for both biomarkers. These findings should be interpreted with caution given the study's

limitations, particularly the small sample size (n = 7 per group) and short observation period (two days). Therefore, future studies are recommended to use larger sample sizes and longer observation periods to obtain more comprehensive longitudinal patterns. Within these limitations, LED phototherapy did not show significant adverse effects on the measured parameters; however, definitive conclusions regarding its safety require further investigation. Continuous biomarker monitoring remains important for early detection of physiological changes. Furthermore, collaboration among academics, practitioners, and the health industry is encouraged to support the development of more effective and efficient LED devices in line with SDG 3 (Good Health and Well-being) and SDG 9 (Industry, Innovation and Infrastructure).

ACKNOWLEDGEMENT

The author would like to express sincere gratitude to Universitas Airlangga for the guidance and support provided throughout the completion of this research.

CONFLICT OF INTEREST

All of authors declare that there is no conflict of interest

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