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Effect of oyster mushroom powder on total leukocyte during inflammatory and proliferative phases of the wound healing process of third-degree burns model in Wistar rat *(Rattus norvegicus)*

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ABSTRACT

Burn wounds are challenging to treat because they often heal slowly and are susceptible to complications. The inflammatory phase plays an essential role in the repair process. However, prolonged inflammation can delay tissue healing. The total leukocyte count is an important parameter to evaluate the process, as it reflects the balance between inflammation and the initiation of tissue regeneration. Oyster mushrooms (*Pleurotus ostreatus*) contain β-glucans and phenolic compounds with anti-inflammatory and antioxidant properties, which may help regulate leukocyte activity and promote wound healing. This study aimed to evaluate the effect of oyster mushroom powder on total leukocyte counts during burn wound healing in *Rattus norvegicus*. Twenty-seven healthy male rats aged 2-3 mo were randomly divided into 3 groups, with 9 rats in each group. The negative control group received aquadest, the positive control group received bovine serum albumin, and the treatment group received 10% oyster mushroom powder. All groups received identical topical wound management with 1% silver sulfadiazine ointment to prevent infection and maintain a moist wound environment during healing. Blood samples were collected on days 0, 4, 8, and 12 after burn induction, and leukocyte counts were measured. The results showed that the treatment group consistently had lower leukocyte counts compared with control groups. On day 0, the treatment group had significantly lower leukocyte levels compared with the negative control ($p = 0.019$). On day 8, leukocyte counts in the treatment group were also significantly lower than the positive control ($p = 0.030$). By day 12, all groups showed a decrease, but the treatment group demonstrated the most gradual and stable reduction over time. In conclusion, oyster mushroom powder reduces leukocyte levels and supported a more controlled inflammatory phase, allowing faster progression to proliferation and improved burn wound healing.

Keywords:
burn wound;
inflammation;
leukocyte;
oyster mushroom;
wound healing

ABSTRAK

Luka bakar merupakan jenis luka yang sulit ditangani karena proses penyembuhannya cenderung berlangsung lambat dan rentan terhadap komplikasi. Fase inflamasi berperan penting dalam proses perbaikan jaringan. Namun, apabila berlangsung terlalu lama, proses penyembuhan dapat terhambat. Jumlah leukosit total merupakan parameter penting untuk dievaluasi karena mencerminkan keseimbangan antara proses inflamasi dan awal regenerasi jaringan. Jamur tiram putih (*Pleurotus ostreatus*) mengandung senyawa β-glukan dan senyawa fenolik yang memiliki efek antiinflamasi serta antioksidan, sehingga berpotensi membantu mengatur aktivitas leukosit dan mempercepat penyembuhan luka. Penelitian ini bertujuan untuk mengkaji pemberian serbuk jamur tiram putih terhadap jumlah leukosit total pada fase inflamasi dan proliferasi penyembuhan luka bakar pada tikus putih (*Rattus norvegicus*). Sebanyak 27 ekor tikus jantan sehat berusia 2-3 bulan dibagi secara acak menjadi 3 kelompok, masing-masing 9 ekor. Kelompok kontrol negatif menerima akuades, kelompok kontrol positif menerima albumin serum sapi, dan kelompok perlakuan menerima serbuk jamur tiram putih 10%. Seluruh kelompok mendapatkan perawatan luka topical dengan

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salep silver sulfadiazine 1% untuk mencegah infeksi dan menjaga kelembapan luka selama proses penyembuhan. Sampel darah diambil pada hari ke-0, 4, 8, dan 12 setelah induksi luka bakar, kemudian diperiksa jumlah leukosit. Hasil penelitian menunjukkan bahwa kelompok perlakuan secara konsisten memiliki jumlah leukosit lebih rendah dibandingkan kelompok kontrol. Pada hari ke-0, kelompok perlakuan memiliki jumlah leukosit yang secara signifikan lebih rendah dibandingkan kontrol negatif (p = 0,019). Pada hari ke-8, jumlah leukosit kelompok perlakuan juga lebih rendah secara signifikan dibandingkan kontrol positif (p = 0,030). Pada hari ke-12, seluruh kelompok menunjukkan penurunan jumlah leukosit, namun kelompok perlakuan memperlihatkan penurunan yang paling stabil dan bertahap dari waktu ke waktu. Dapat disimpulkan, pemberian serbuk jamur tiram putih 10% dapat menurunkan jumlah leukosit total dan membantu menciptakan fase inflamasi yang lebih terkontrol, sehingga mempercepat transisi menuju fase proliferasi serta mendukung penyembuhan luka bakar yang lebih optimal.

INTRODUCTION

Wound healing is a complex biological process crucial for restoring tissue integrity following injury. Among the different types of wounds, burns pose significant challenges, often resulting in prolonged healing times and increased healthcare costs.[1] The inflammatory and proliferative phases of wound healing play vital roles in recovery; however, if inflammation persists, it can lead to delayed healing and additional complications.[2] Consequently, there is a pressing need for effective strategies and therapeutic interventions that can enhance the healing process.

Recent studies have explored the use of natural products, particularly from fungi, as potential wound-healing agents. One such candidate is the oyster mushroom (*Pleurotus ostreatus*), which is known for its rich nutritional profile, including high levels of protein, essential amino acids, and bioactive compounds such as β-glucans. These components have demonstrated significant biological activities, such as anti-inflammatory and antioxidant effects, which are essential for promoting wound healing.[3] Previous research has shown that oyster mushroom powder can positively influence various health parameters, including immune response and wound-healing dynamics, making it a noteworthy focus for further investigation.[4]

Leukocytes represent one of the most critical parameters in evaluating wound healing, particularly during the inflammatory and proliferative phases. Total leukocyte count (TLC) is a well-established marker of the systemic inflammatory response after thermal injury and has been used to monitor early immune activation in burn models.[5] Total peripheral leukocyte counts increase markedly in the early inflammatory stage (days 0–3), dominated by neutrophils and monocytes. These cells mediate phagocytosis of necrotic debris, release antimicrobial peptides, and secrete pro-inflammatory cytokines such as IL-6, IL-8, and MCP-1, which correlate with burn severity.[6] As the healing process progresses, macrophages take over the dominant role by producing growth factors (VEGF, TGF-β, PDGF, EGF) that stimulate angiogenesis, fibroblast proliferation, and extracellular matrix deposition, thereby driving the transition into the proliferative phase.[7] Thus, monitoring leukocyte dynamics provides a meaningful indicator of wound progression, particularly in burns where prolonged inflammation can hinder epithelialization and tissue remodeling.[8]

The present study aimed to analyze the effect of 10% oyster mushroom (*P. ostreatus*) powder on total leukocyte counts during the inflammatory and proliferative phases of burn wound

healing in Wistar rats. The 10% concentration was selected based on the experimental findings of previous study, who demonstrated that a diet containing 10% *Pleurotus eous* produced the greatest increase in serum albumin, globulin, and total protein levels in Wistar rats compared with 2.5% and 5% formulations.[9] Because albumin synthesis and immune-cell activity share overlapping metabolic pathways regulated by hepatic protein turnover and cytokine signaling, this dose was considered physiologically effective and safe for evaluating the immunomodulatory effects of oyster mushroom powder.[10] Building upon this evidence, the current research further investigates whether the same concentration can modulate leukocyte dynamics and inflammatory resolution in burn wounds, thereby providing insight into its potential as an adjunctive therapy to improve wound-healing outcomes.

MATERIAL AND METHODS

Study design and animals

This study employed a true experimental design with a multiple point post-test only control group approach. A total of 27 healthy male *Rattus norvegicus* (aged 2–3 mo, weighing 200–250 g) were housed under standard laboratory conditions (temperature 22–24 °C, 12 h light/dark cycle) with ad libitum access to food and water.[11] Prior to the experimental procedures, all animals underwent a one-week acclimatization period to minimize environmental stress.

The sample size was determined using Federer's rule of thumb for experiments with multiple treatment groups. Therefore, 9 rats per group (total n = 27) were used to satisfy this criterion and to provide adequate degrees of freedom for statistical analysis. Moreover, utilizing Wistar as a model organism allows for controlled experimentation, providing a robust framework to evaluate the effectiveness of oyster mushroom supplementation in a biologically relevant context.[12] The focus on leukocyte dynamics is critical, as these immune cells orchestrate the wound-healing response, influencing both inflammation and tissue formation.[6] As such, this investigation seeks not only to contribute to the understanding of natural remedies in wound management but also to highlight the significant potential of oyster mushrooms in enhancing the healing process, thereby offering a cost-effective and accessible treatment option for burn wounds.

The experimental protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine, University of Jember (No. 2795/UN/25.1.10.2/KE/2025).

Preparation of mushroom powder

Oyster mushroom (*P. ostreatus*) powder was obtained from PT Mitra Jamur, Jember, Indonesia. Fresh oyster mushrooms were cleaned and dried using a food dehydrator at 90 °C until a constant weight was achieved, ensuring complete removal of moisture. The dried mushrooms were then finely ground into powder form. For the treatment protocol, the powder was administered orally as a suspension, not as feed pellets. The suspension was prepared by dissolving 10% of the total daily feed equivalent (approximately 1.5 g per rat per day) in 8 mL of distilled water, which was then given via oral gavage in two divided doses every 8 hr. No additional ingredients or binders were mixed with the mushroom powder to avoid interference with its bioactive components.

Burn induction

A third-degree burn, also known as a full-thickness burn, is characterized by the destruction of the epidermis and dermis, often extending into the subcutaneous tissue, resulting in a dry, leathery appearance and loss of skin sensation. The degree of burn injury was assessed macroscopically based on these visual and textural characteristics, including the presence of a pale or brownish eschar, absence of bleeding, and loss of pain response, to confirm full-thickness involvement.[13]

Standardized third-degree burn wounds were then induced under ketamine–xylazine anesthesia. A 3 × 3 cm metal rod was heated in boiling water (100 °C) for 15 min to stabilize the temperature, wiped dry, and applied to the shaved dorsal skin of each rat for 10 sec.[14] This procedure consistently produced full-thickness burn injuries.

This procedure consistently produced full-thickness burn injuries, as visually confirmed by the formation of a dry, leathery eschar and absence of dermal elasticity. Representative macroscopic appearances of the standardized third-degree burn wounds are shown in FIGURE 1.

Treatment protocol

Following burn induction, all animals were randomly assigned into three groups, each consisting of nine rats. The negative control group received 2 mL of aquadest orally once daily. The positive control group received bovine serum albumin (BSA) at a dose of 0.32 g/kg BW/day, administered orally once daily after dissolving the BSA powder in 2 mL of distilled water. The bovine serum albumin used as a reference protein supplement and the dosage was calculated from the human equivalent dose using the FDA conversion formula. The treatment group received 10% oyster mushroom (*P. ostreatus*) powder equivalent to approximately 1.5 g per rat per day. The mushroom powder was suspended in 8 mL of distilled water and administered orally via gastric gavage in two divided doses given eight hours apart. This division was necessary because the suspension, when prepared in less than 8 mL, became too viscous for accurate administration through the oral gavage tube, while the rat's gastric capacity is approximately 4 mL. Therefore, the dose was split to allow sufficient gastric emptying and ensure complete delivery of the intended amount without causing discomfort or regurgitation

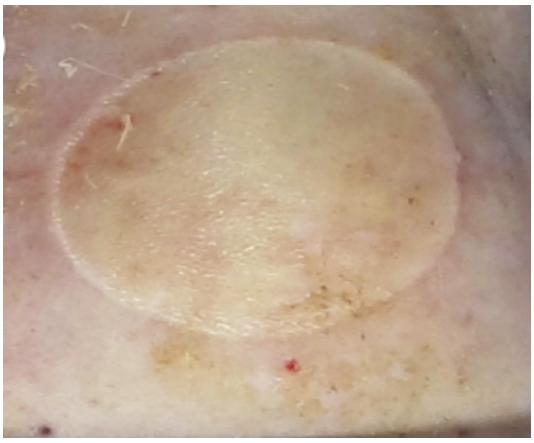

FIGURE 1. Representative image of a standardized third-degree burn wound immediately after induction.[11]

In addition to oral treatments, all groups received identical local wound management consisting of mechanical debridement and topical application of 1% silver sulfadiazine ointment to prevent infection and maintain a moist wound environment conducive to optimal healing.[15] The wounds were covered with sterile tulle, gauze, and adhesive dressing, and care was taken to prevent animals from scratching, licking, or removing the ointment. Topical treatment and dressing changes were performed every four days throughout the study period. This approach ensured that the only experimental variable among groups was the type of oral supplementation, while topical care was standardized to eliminate confounding effects from differences in wound management.

Blood sampling procedure

Peripheral blood samples were collected from each rat at four time points: day 0, 4, 8 and 12 following burn induction. On day 0, blood sampling was performed prior to burn induction and before the first administration of any treatment to establish baseline leukocyte counts. Subsequent samples were collected at the same time each day between 08:00 and 10:00 AM. to minimize circadian variations in leukocyte counts.

Blood was drawn via the retro-orbital plexus using a sterile capillary tube under light anesthesia with ketamine–xylazine to minimize pain and stress. Prior to sampling, the periocular area was sterilized with 70% ethanol. Approximately 0.5–1.0 mL of blood was collected from each rat and immediately transferred into EDTA-coated microtubes to prevent coagulation. After collection, gentle pressure was applied to the eyelid using sterile gauze to control any potential bleeding and reduce discomfort. This retro-orbital method was selected because it allows for repeated sampling from small laboratory animals while providing an adequate blood volume for hematological analysis.[16]

The selection of sampling days (day 0, 4, 8 and 12) was based on the temporal characteristics of the wound-healing process, particularly the leukocyte response during each phase. Day 0 represents the acute inflammatory phase (0–3 days post-injury), when neutrophil and monocyte infiltration peaks following tissue damage. Day 4 marks the transition from inflammation to proliferation, characterized by a gradual decline in neutrophil activity and an increase in macrophage-mediated cytokine signaling. Days 8 and 12 correspond to the proliferative phase, during which fibroblast proliferation, angiogenesis, and extracellular matrix deposition dominate the healing response. Sampling at these four points therefore allows the evaluation of leukocyte dynamics across the major phases of burn wound healing and provides insight into how oyster mushroom supplementation may modulate the inflammatory to proliferative transition.

Leukocyte Count Measurement

The total leukocyte count was measured manually using a Neubauer hemocytometer under a light microscope. Before counting, Turk's solution was prepared as a diluent in a 1:20 ratio (e.g., 20 µL blood + 380 µL Turk's solution) to lyse erythrocytes and stain leukocyte nuclei for easier visualization. The diluted sample was mixed gently, then introduced into the counting chamber of the hemocytometer.

Counting was performed manually in the four large corner squares of the Neubauer hemocytometer grid under a light microscope at 400× magnification. The following standard formula was used to calculate the total leukocyte count:

$$\text{Total leukocyte count (cells/µL)} = \frac{\text{Number of cells counted} \times \text{dilution factor} \times 10}{\text{number of squares counted}}$$

Where the dilution factor was set at 20, based on a blood-to-Turk's solution ratio of 1:20. The counting chamber had a depth of 0.1 mm, and leukocytes were counted within four large corner squares of the Neubauer grid, corresponding to a total area of 4 mm.² To ensure accuracy and minimize inter-observer variability, each sample was counted twice by two independent observers, and the average value was used for subsequent statistical analysis.

Statistical Analysis

All analyses were performed using IBM SPSS Statistics version 29, with a two-tailed significance threshold set at $\alpha = 0.05$. Data were analyzed using repeated measures analysis of variance (Anova) to evaluate the effects of treatment and time on total leukocyte counts. This approach was selected because it accounts for the correlation among repeated measurements taken from the same subjects across different time points, improving accuracy for within-subject comparisons.

Before analysis, data were examined to ensure that the statistical assumptions of repeated measures Anova were satisfied. The Shapiro–Wilk test was used to confirm the normality of residuals, and Levene's test assessed the homogeneity of variances among groups. The assumption of sphericity, which requires the variances of the differences between all pairs of repeated measurements to be equal, was tested using Mauchly's test. When this assumption was violated, the Greenhouse–Geisser correction was applied to adjust the degrees of freedom and maintain valid F-statistics.

The model included two factors: time (within-subject factor: day 0, 4, 8, and 12) and group (between-subject factor: negative control, positive control, and treatment). Interaction effects between time and group were also tested to determine whether changes in leukocyte counts across days differed between groups.

When significant main or interaction effects were detected ($p < 0.05$), post hoc pairwise comparisons were performed using the Bonferroni adjustment. This correction method divides the significance level (α) by the number of comparisons, thereby controlling the family-wise Type I error rate and preventing false-positive results from multiple testing. Bonferroni-adjusted p-values were then used to identify significant differences both between groups at each time point and within groups across different time points.

RESULTS

The effect of 10% oyster mushroom powder on total leukocyte count during the wound healing process was evaluated at four different time points: day 0, 4, 8, and 12. The descriptive analysis (TABLE 1) showed that the negative control group consistently had the highest leukocyte levels across all time points, while the treatment group had the lowest. At baseline day 0, the mean leukocyte count was highest in the negative control group (17,212.50 ± 4,244.47 cells/μL) and lowest in the treatment group (11,975.00 ± 1,815.61 cells/μL). By day 12, the leukocyte count decreased in all groups, with the treatment group showing the greatest reduction (10,737.50 ± 1,416.17 cells/μL).

Repeated measures Anova indicated a significant difference in leukocyte levels between groups over time ($p < 0.05$). Post hoc Bonferroni analysis (TABLE 2) revealed that on day 0, there was a significant difference between the treatment group and the negative control group ($p = 0.019$). On day 8, a significant difference was observed between the treatment group (Treatment) and the positive control group/Control (+) ($p = 0.030$). However, there were no significant differences between groups

at days 4 and 12 ($p > 0.05$). These results suggest that the administration of 10% oyster mushroom powder was associated with a reduction in leukocyte count, particularly during the inflammatory to proliferative transition phase (day 8). Significant intergroup differences were confirmed by Bonferroni post hoc analysis, showing lower leukocyte levels in the treatment group compared with negative control on day 0 ($p = 0.019$) and with positive control on day 8 ($p = 0.030$).

The within-group analysis was performed using repeated measures Anova with Bonferroni adjustment to evaluate changes in leukocyte counts over time in each group (TABLE 3). In the negative control group/Control (–), leukocyte levels decreased significantly from day 0 to day 4 ($p = 0.019$) and from day 0 to day 8 ($p = 0.032$). However, there was no significant difference between day 8 and day 12 ($p > 0.05$), indicating that leukocyte levels stabilized in the later phase of wound healing.

In the positive control group/Control (+), a significant decrease was observed between day 8 and day 12 ($p = 0.030$), suggesting that the reduction in leukocyte counts occurred later compared to the treatment group.

In the treatment group (Treatment), there were no statistically significant changes in leukocyte counts between time points ($p > 0.05$), although there was a consistent downward trend from baseline to day 12. This gradual and stable decrease may reflect an accelerated resolution of the inflammatory phase, resulting in a smoother transition to the proliferative phase.

TABLE 1. Mean ± SD of total leukocyte counts (cells/µL) in each group at different time points.

Time point	Group	Mean ± SD (cells/µL)
Day 0	Control (-)	17,212.50 ± 4,244.47
	Control (+)	14,612.50 ± 3,827.88
	Treatment	11,975.00 ± 1,815.61
Day 4	Control (-)	13,625.00 ± 3,875.10
	Control (+)	13,175.00 ± 2,983.65
	Treatment	11,687.50 ± 3,004.02
Day 8	Control (-)	13,787.50 ± 2,920.59
	Control (+)	14,400.00 ± 2,801.53
	Treatment	10,650.00 ± 2,173.21
Day 12	Control (-)	14,212.50 ± 3,855.40
	Control (+)	11,450.00 ± 2,142.10
	Treatment	10,737.50 ± 1,416.17

TABLE 2. Pairwise comparison of leukocyte levels between groups at each time point.

Time point	Group comparison	Mean difference	p
Day 0	Control (-) vs Treatment	5,237.50	0.019
	Control (-) vs Control (+)	2,600.00	0.444
	Control (+) vs Treatment	2,637.50	0.428
Day 4	Control (-) vs Treatment	1,937.50	0.766
	Control (-) vs Control (+)	450.00	1.000
	Control (+) vs Treatment	1,487.50	0.766
Day 8	Control (-) vs Treatment	3,137.50	0.083
	Control (-) vs Control (+)	-612.50	1.000
	Control (+) vs Treatment	3,750.00	0.030
Day 12	Control (-) vs Treatment	2,762.50	0.154
	Control (-) vs Control (+)	-3,475.00	0.050
	Control (+) vs Treatment	712.50	1.000

TABLE 3. Pairwise comparison of leukocyte counts over time by group.

Group	Time point comparison (I-J)	Mean difference	Std. error	p
Control (-)	D0 – D4	3587.50	1078.29	0.019
	D0 – D8	3425.00	1104.59	0.032
	D0 – D12	3000.00	1112.21	0.081
	D4 – D8	-162.50	769.25	1.000
	D4 – D12	-587.50	969.92	1.000
	D8 – D12	-425.00	939.11	1.000
Control (+)	D0 – D4	1437.50	1078.29	1.000
	D0 – D8	212.50	1104.59	1.000
	D0 – D12	3162.50	1112.21	0.058
	D4 – D8	-1225.00	769.25	0.757
	D4 – D12	1725.00	969.92	0.539
	D8 – D12	2950.00	939.11	0.030
Treatment	D0 – D4	287.50	1078.29	1.000
	D0 – D8	1325.00	1104.59	1.000
	D0 – D12	1237.50	1112.21	1.000
	D4 – D8	1037.50	769.25	1.000
	D4 – D12	950.00	969.92	1.000
	D8 – D12	-87.50	939.11	1.000

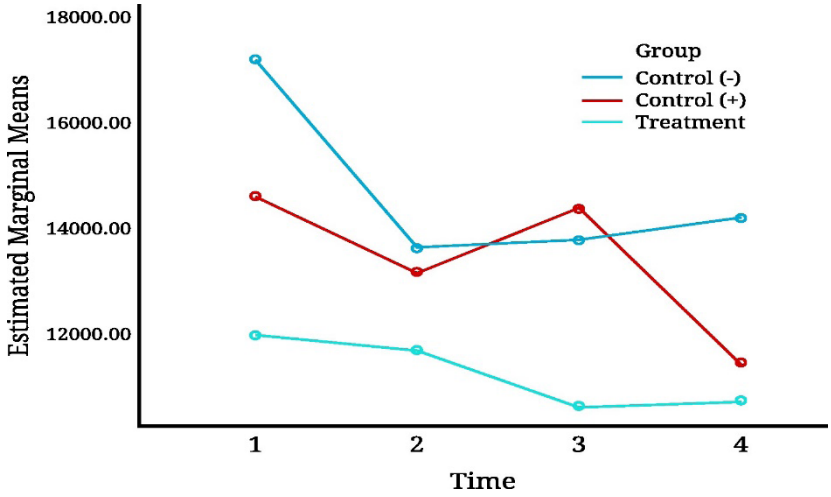

FIGURE 2. Estimated marginal means of blood leukocyte counts across experimental groups over time.

Estimated marginal means of blood leukocyte counts measured at four different time points during the wound healing process (FIGURE 2): day 0 (time 1), day 4 (time 2), day 8 (time 3), and day 12 (time 4). The three experimental groups include the negative control/ Control (–), positive control/Control (+), and treatment group (Treatment).

At time 1 (day 0), the negative control group/Control (–) exhibited the highest leukocyte count compared to the other groups, indicating an initial inflammatory response immediately following thermal injury. The positive control group/Control (+) showed a moderately high leukocyte count, while the treatment group (Treatment) demonstrated the lowest baseline level.

From time 1 to time 2 (day 4), a notable decrease was observed in the Control (-) group, followed by a slight stabilization from day 8 to day 12. In the Control (+) group, leukocyte counts initially decreased from day 0 to day 4, peaked slightly on day 8, and then sharply declined by day 12. Conversely, the treatment group (Ttreatment) exhibited a gradual and consistent decrease in leukocyte counts throughout the observation period, reflecting a potentially controlled inflammatory response.

DISCUSSION

This study investigated the effect of 10% oyster mushroom (*P. ostreatus*) powder on the total leukocyte count during the wound healing process in rats, focusing on the inflammatory and proliferative phases. The results showed that the treatment group (Treatment) consistently had lower leukocyte levels compared to both the positive control/ Control (+) and negative control/ Control (–) groups. The most significant reduction occurred on day 8, where leukocyte counts in the treatment group were significantly lower than in the positive control group ($p = 0.030$). By day 12, the differences between groups were no longer significant, suggesting that the wound healing process reached a more stable phase across all groups.

The elevated leukocyte counts observed at baseline (day 0), especially in the negative control group, likely reflect the acute inflammatory response immediately following burn induction. According to previous study, the initial phase of wound healing is characterized

by a rapid influx of neutrophils and monocytes to the injury site, resulting in a transient systemic leukocytosis.[17] This pattern was consistent in our study, where the negative control/Control (–) group had the highest leukocyte levels at day 0 (17,212.50 ± 4,244.47 cells/μL). In contrast, the treatment group exhibited a slightly lower baseline leukocyte count, which likely reflects individual physiological variation rather than any treatment effect, as blood sampling on day 0 was conducted prior to both burn induction and the initial administration of oyster mushroom powder.

Oyster mushrooms are rich in β-glucans, phenolic compounds, and polysaccharides, which have been reported to possess immunomodulatory and antioxidant properties.[18–20] These compounds can downregulate pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), while upregulating anti-inflammatory cytokines like interleukin-10 (IL-10). This mechanism explains the significant decrease in leukocyte counts observed in the treatment group on day 8. The timing of this reduction is consistent with the transition from the inflammatory phase to the proliferative phase of wound healing, where excessive leukocyte activity subsides, and fibroblast proliferation along with collagen deposition becomes predominant.[2]

Interestingly, the leukocyte count in the positive control group/Control (+) increased slightly on day 8, indicating a prolonged inflammatory response. This suggests that without the modulating effect of oyster mushroom powder, the inflammatory phase was sustained longer, potentially delaying the onset of tissue repair.[1] Prolonged inflammation can lead to excessive release of reactive oxygen species (ROS) and proteolytic enzymes, which may damage surrounding healthy tissue and impair healing.[21] In contrast, the treatment group showed a smoother transition to the proliferative phase, likely due to the antioxidative properties of phenolic compounds in oyster mushrooms that scavenge ROS and protect regenerating tissues.[3]

By day 12, no statistically significant differences were observed between groups. This indicates that the natural healing process eventually progresses toward resolution, even without treatment, as the inflammatory phase subsides and tissue remodeling begins.[22] However, the earlier reduction in leukocyte levels in the treatment group suggests that oyster mushroom powder may accelerate the overall healing timeline by shortening the inflammatory phase. These findings are supported by previous study, who demonstrated that oyster mushroom extracts enhanced wound closure rates and modulated inflammatory responses in rat models.[4]

The decrease in leukocyte levels observed in the treatment group corresponds with the expected resolution of inflammation during the proliferative phase of wound healing. Although leukocyte dynamics provide indirect evidence of phase transition, leukocyte count alone is not sufficient to clinically confirm proliferation. In this study, the inference was made based on the physiological timeline of burn wound healing, where the reduction of inflammatory cells typically precedes fibroblast activation, granulation tissue development, and reepithelialization. Direct histological or macroscopic evaluation of granulation tissue was not performed; thus, the observed leukocyte reduction should be interpreted as a systemic indicator consistent with the proliferative phase rather than as definitive proof of it. Previous studies have reported that decreased systemic leukocyte activity is associated with enhanced fibroblast function and collagen deposition during this stage,

supporting the interpretation that oyster mushroom supplementation may help establish a more regulated inflammatory environment conducive to proper tissue regeneration.[23]

One limitation of this study is the initial baseline variation in leukocyte counts across groups. The high baseline leukocyte level in the negative control group may have influenced the statistical outcomes, especially at the early time point (day 0). This variation could be attributed to individual biological differences among subjects or a heightened stress response due to the retro-orbital blood collection method, which is known to transiently increase circulating leukocyte levels. Future studies should include larger sample sizes and standardized handling procedures to minimize such variability.

For future research, it is recommended to explore different dosages and administration routes of oyster mushroom powder to determine the most effective therapeutic window. In addition, further studies should evaluate other key biomarkers of wound healing, such as fibroblast activity, collagen deposition, and angiogenesis, to better elucidate the underlying mechanisms. Expanding the model to include chronic wounds or different types of burn injuries, as well as conducting clinical trials in humans, would also provide valuable insights into the translational potential of oyster mushroom–based therapies in wound management.

CONCLUSION

In conclusion, oyster mushroom powder has a significant effect on modulating leukocyte dynamics during the wound healing process. By reducing leukocyte levels during the critical inflammatory phase, oyster mushrooms may accelerate the transition to the proliferative phase, thereby promoting faster and more efficient healing. These findings suggest that oyster mushroom powder has potential as a natural therapeutic agent in wound care management.

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REFERENCES

1. Mulder PPG, Vlig M, Fasse E, Stoop MM, Pijpe A, van Zuijlen PPM, *et al.* Burn-injured skin is marked by a prolonged local acute inflammatory response of innate immune cells and pro-inflammatory cytokines. Front Immunol 2022; 13:1034420. https://doi.org/10.3389/fimmu.2022.1034420
2. Landén NX, Li D, Ståhle M. Transition from inflammation to proliferation: a critical step during wound healing. Cell Mol Life Sci 2016; 73(20):3861-85. https://doi.org/10.1007/s00018-016-2268-0
3. Selvamani S, El Enshasy H, Dailin DJ, Malek RA, Hanapi SZ, Ambehabati KK, *et al.* Antioxidant compounds of the edible mushroom pleurotus ostreatus. Int J Biotech Wellness Ind 2018; 7:1-14. https://doi.org/10.6000/1927-3037.2018.07.01
4. Abdulhameed OA, Kadhim HM. Exploring the role of pleurotus ostreatus as an ointment formulation in inducing wound healing in mice skin. J Pharm Bioallied Sci 2024; 16: S243-6. https://doi.org/10.4103/jpbs.jpbs_480_23
5. Rizzo JA, Thomas JM, Aden JK, Schauer SG, Van Gent JM, Peterson WC, *et al.* Decrease in total leukocyte count is associated with acute kidney injury after severe burn. J Burn Care Res 2025; 46(4):850-3. https://doi.org/10.1093/jbcr/iraf039
6. Mulder PPG, Vlig M, Boekema BKHL, Stoop MM, Pijpe A, van Zuijlen PPM, *et al.* Persistent systemic inflammation

in patients with severe burn injury is accompanied by influx of immature neutrophils and shifts in t cell subsets and cytokine profiles. Front Immunol 2021; 11:621222. https://doi.org/10.3389/fimmu.2020.621222
7. Chandra P, Faizan M, Porwal M, Sharma H, Sachan N. An overview and review of growth factors in wound healing: emerging trends and innovations. Curr Diabetes Rev 2025; 27. https://doi.org/10.2174/0115733998332692241202072249
8. Mulder PPG, Koenen HJPM, Vlig M, Joosten I, de Vries RBM, Boekema BKHL. Burn-induced local and systemic immune response: systematic review and meta-analysis of animal studies. J Invest Dermatol 2022; 142(11):3093-109.e15. https://doi.org/10.1016/j.jid.2022.05.004
9. Saravanan KG, Murugan SS, Eswaran A. Effect of pleurotus eous supplemented diets on the albumin, globulin and total proteins in experimental animal (male albino Wistar rats). Int J Pure App Biosci SPI 2018; 6(2):239-44.
10. Duran-Güell M, Flores-Costa R, Casulleras M, López-Vicario C, Titos E, Díaz A, Alcaraz-Quiles J, *et al.* Albumin protects the liver from tumor necrosis factor α-induced immunopathology. FASEB J 2021; 35(2):e21365. https://doi.org/10.1096/fj.202001615RRR
11. Venter NG, Monte-Alto-Costa A, Marques RG. A new model for the standardization of experimental burn wounds. Burns 2015; 41(3):542-7. https://doi.org/10.1016/j.burns.2014.08.002
12. Elhusseiny SM, El-Mahdy TS, Elleboudy NS, Farag MMS, Aboshanab KM, Yassien MA. Immunomodulatory activity of extracts from five edible basidiomycetes mushrooms in Wistar albino rats. Sci Rep 2022; 12(1):12423. https://doi.org/10.1038/s41598-022-16349-2
13. Abazari M, Ghaffari A, Rashidzadeh H, Badeleh SM, Maleki Y. A systematic review on classification, identification, and healing process of burn wound healing. Int J Low Extrem Wounds 2022; 21(1):18-30. https://doi.org/10.1177/1534734620924857
14. Zheng B, Shen C, Sun J, Guo W, Jin Y, Niu Y. Developing a Simple burn model in rats of different ages. J Burn Care Res 2019; 40(5):639-47 https://doi.org/10.1093/jbcr/irz072
15. Devi MV, Poornima V, Sivagnanam UT. Wound healing in second-degree burns in rats treated with silver sulfadiazine: a systematic review and meta-analysis. J Wound Care 2022; 31(Sup4):S31-45. https://doi.org/10.12968/jowc.2022.31.Sup4.S31
16. Hoggatt J, Hoggatt AF, Tate TA, Fortman J, Pelus LM. Bleeding the laboratory mouse: Not all methods are equal. Exp Hematol 2016; 44(2):132-7.e1. https://doi.org/10.1016/j.exphem.2015.10.008
17. Beserra FP, Gushiken LFS, Hussni MF, Pellizzon CH. Regulatory mechanisms and chemical signaling of mediators involved in the inflammatory phase of cutaneous wound healing. Wound healing - current perspectives. Intech Open; 2019. https://doi.org/10.5772/intechopen.81731
18. Devi PV, Islam J, Narzary P, Sharma D, Sultana F. Bioactive compounds, nutraceutical values and its application in food product development of oyster mushroom. J Fut Foods 2024; 4(4):335-42. https://doi.org/10.1016/j.jfutfo.2023.11.005
19. Lam YS, Okello EJ. Determination of lovastatin, β-glucan, total polyphenols, and antioxidant activity in raw and processed oyster culinary-

medicinal mushroom, *Pleurotus ostreatus* (Higher Basidiomycetes). Int J Med Mushrooms 2015; 17(2):117-28. https://doi.org/10.1615/IntJMedMushrooms.v17.i2.30

20. Nworu CS, Ihim SA, Okoye FB, Esimone CO, Adikwu MU, Akah PA. Immunomodulatory and immunorestorative activities of β-D-glucan-rich extract and polysaccharide fraction of mushroom, Pleurutus tuberregium. Pharm Biol. 2015; 53(11):1555-66. https://doi.org/10.3109/13880209.2014.991838
21. Strudwick XL, Cowin AJ. The Role of the inflammatory response in burn injury [Internet]. Hot Topics in Burn Injuries. InTech; 2018. https://doi.org/10.5772/intechopen.71330
22. Cañedo-Dorantes L, Cañedo-Ayala M. Skin acute wound healing: a comprehensive review. Int J Inflam 2019; 2019: 3706315. https://doi.org/10.1155/2019/3706315
23. Pinar AA, Samuel CS. Immune mechanisms and related targets for the treatment of fibrosis in various organs. Curr Mol Med 2022; 22(3):240-9. https://doi.org/10.2174/1566524022666220114122839