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RESEARCH ARTICLE

Auricularia (Wood Ear Mushroom) Extract on Leukocyte and Lymphocyte Levels in Male Wistar Rats

Pemberian Ekstrak Auricularia (Jamur Kuping) terhadap Kadar Leukosit dan Limfosit pada Tikus Jantan Galur Wistar

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Abstract

The immune system protects the body through the activation of immune cells, which can be supported by polysaccharides from wood ear mushrooms as potential immunomodulators. This study aimed to determine the effect of wood ear mushroom extract on leukocyte count and lymphocyte percentage in male Wistar rats. A true experimental post-test-only control group design was used with 25 male Wistar rats divided into five groups: extract doses of 300, 400, and 500 mg/kg body weight, a positive control group received an immune booster supplement, and a negative control group. The treatments were administered orally for 10 days. Leukocyte counts and lymphocyte percentages were measured using a hematology analyzer. Data were analyzed using the Shapiro–Wilk test, one-way ANOVA, and DMRT as a post hoc test. Leukocyte counts showed no significant differences among groups ($p > 0.05$), whereas lymphocyte percentages differed significantly ($p = 0.0005$). Bonferroni post hoc analysis showed significant differences ($p < 0.05$). Based on the DMRT results, 400 mg/kg body weight was considered the optimal dose because it belonged to the same subset as the positive control. Wood ear mushroom extract has potential as a natural immunomodulator that may enhance adaptive immune responses. Further studies are recommended to assess immune parameters before and after treatment and to identify bioactive compounds involved in its immunomodulatory effects.

Keywords: leukocytes, lymphocytes, wood ear mushroom

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Abstrak

Sistem imun melindungi tubuh melalui aktivasi sel-sel imun, yang dapat didukung oleh polisakarida dari jamur kuping sebagai imunomodulator potensial. Penelitian ini bertujuan untuk mengetahui pengaruh ekstrak jamur kuping terhadap jumlah leukosit dan persentase limfosit pada tikus jantan galur Wistar. Rancangan penelitian yang digunakan adalah true experimental post-test-only control group design dengan 25 ekor tikus jantan galur Wistar yang dibagi menjadi lima kelompok: kelompok dosis ekstrak 300, 400, dan 500 mg/kg berat badan, kelompok kontrol positif yang menerima suplemen immune booster, serta kelompok kontrol negatif. Perlakuan diberikan secara oral selama 10 hari. Jumlah leukosit dan persentase limfosit diukur menggunakan hematology analyzer. Data dianalisis menggunakan uji Shapiro–Wilk, ANOVA satu arah, dan uji lanjut DMRT. Hasil penelitian menunjukkan bahwa jumlah leukosit tidak mengalami perbedaan signifikan antarkelompok ($p > 0{,}05$), sedangkan persentase limfosit menunjukkan perbedaan yang signifikan ($p = 0{,}0005$). Analisis uji lanjut Bonferroni menunjukkan adanya perbedaan signifikan ($p < 0{,}05$). Berdasarkan hasil uji DMRT, dosis 400 mg/kg berat badan dinilai sebagai dosis optimal karena berada pada subset yang sama dengan kontrol positif. Ekstrak jamur kuping memiliki potensi sebagai imunomodulator alami yang dapat meningkatkan respons imun adaptif. Penelitian lebih lanjut direkomendasikan untuk mengevaluasi parameter imun sebelum dan sesudah perlakuan, serta mengidentifikasi senyawa bioaktif yang berperan dalam efek imunomodulator tersebut.

Kata Kunci: *leukosit, limfosit, jamur kuping*

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Highlight:

- Administration of wood ear mushroom (*Auricularia*) extract had no significant effect on increasing leukocyte counts.
- Administration of wood ear mushroom extract significantly increased lymphocyte counts, with an optimal dosage of 400 mg/kg BW.
- Wood ear mushroom extract exhibits immunomodulatory activity without elevating total leukocyte counts.

INTRODUCTION

An immunocompromised state refers to a condition in which the immune system suffers from functional impairment, rendering it incapable of optimally combating infectious pathogens. Immune system dysfunctions can stem from a reduction in immune cell counts, such as neutropenia or lymphopenia, thereby weakening the immune response to pathogens even when cells are present within the body. Consequently, immunocompromised individuals face a high risk of opportunistic infections, rapid disease progression, and atypical inflammatory responses. Immediate therapeutic intervention is required to manage immunocompromised conditions and restore optimal immune function (Azoulay et al., 2019).

Immunity represents the body's defense system against foreign agents that threaten tissue and organ integrity. The immune system protects the host by activating constitutive immune cells to neutralize invading pathogens, thereby preventing tissue damage and preserving physiological function. Tissues constituting the immune system are categorized as lymphoid tissues, divided into central and peripheral lymphoid organs. Central lymphoid organs comprise the bone marrow, thymus, and bursa of Fabricius. The thymus provides an appropriate microenvironment for the maturation of small lymphocytes into T lymphocytes, whereas the bursa of Fabricius facilitates cell maturation required for B-cell antibody production (Simatupang et al., 2022).

Normal lymphocyte levels range from 20% to 40%. Lymphocytes play a pivotal role in host defense against microorganisms, tumors, and foreign cellular entities by mediating immune responses to foreign molecules and establishing immunological memory to counteract subsequent challenges (Prakoeswa, 2020). Lymphocytes belong to the leukocyte lineage. Leukocytes are immune defense cells that rapidly respond to foreign entities causing inflammation or infection, maintaining a normal human reference range of approximately 4,000 to 11,000 cells/mm^3 (Ainu'rohmah et al., 2020).

Leukocyte counts and lymphocyte percentages serve as key parameters for evaluating immune responses, with leukocytes representing general systemic immunity and lymphocytes constituting a crucial component of adaptive immunity. A previous study by Ibe et al. (2020) on *A. auricula-judae* demonstrated that the administration of extracts and β-D-glucan-rich polysaccharide fractions significantly increased lymphocyte counts, as well as cellular and humoral immune responses in animal models.

Wood ear mushroom (*Auricularia auricula*) is a widely consumed natural ingredient whose health benefits remain underappreciated. It is a nutritious food source rich in bioactive compounds, particularly polysaccharides, which possess strong potential for immunomodulation. Although this potential has been reported, experimental evidence regarding the effects of wood ear mushroom extract on the cellular components of the immune system requires further validation, highlighting the necessity to investigate its specific impacts on leukocyte counts and lymphocyte percentages (Chen et al., 2020).

Wood ear mushroom is rich in carbohydrates, particularly various polysaccharide fractions, including chitin, hemicellulose, mannan, α-glucan, and β-glucan. Notably, 1,3-1,6-β-D-glucan is the most prominent component, accounting for approximately 16.76 g/100 dry weight or roughly 47.9\% of the total polysaccharides in wood ear mushroom (Mirończuk-Chodakowska et al., 2017). Absorbable β-glucan can stimulate antitumor activity and enhance host immunity (Elfirta et al., 2023). Furthermore, polysaccharides derived from wood ear mushroom act as immunomodulators that fortify the immune system, thereby conferring enhanced protection against infections and disease (Adha et al., 2025).

Ethanol 70% was utilized to obtain the crude extract of wood ear mushroom, which contains various bioactive compounds, as reported by Sangphech et al. (2021) demonstrating that the ethanolic extract of *Auricularia polytricha* suppressed nitric oxide production in RAW264.7 macrophages. Therefore, the biological activity of the *Auricularia* ethanolic extract should not be attributed solely to polysaccharides, but rather to the synergistic contribution of diverse bioactive constituents extracted by the hydroalcoholic solvent. However, because this study did not perform identification and quantification of polysaccharides or β-glucan in the 70% ethanolic extract, the observed immunomodulatory effects cannot be directly attributed to β-glucan. Polysaccharides β-glucans are discussed herein as recognized bioactive components of *Auricularia* with reported immunomodulatory activities, rather than as components proven to exist at specific concentrations within the extract investigated in this study.

Previous research has demonstrated that the administration of edible mushroom extracts can alter hematological parameters, such as total leukocyte and lymphocyte levels. However, specific data regarding the effect of wood ear mushroom extract on hematological profiles, particularly leukocytes and lymphocytes in male Wistar rats, remain scarce in the scientific literature. This investigation is crucial, as leukocyte and lymphocyte counts represent primary hematological parameters that reflect white blood cell responses to the administration of wood ear mushroom extract. The aim of this study is to determine whether the administration of wood ear mushroom (*Auricularia auricula*) extract influences total leukocyte and lymphocyte counts in male Wistar rats. By providing more specific data, this study aims to contribute to the understanding of the biological functions of wood ear mushroom regarding hematological components and advance knowledge regarding its potential applications in medical science.

METHODS

This study employed a laboratory experimental approach utilizing a true experimental post-test-only control group design to evaluate the efficacy of wood ear mushroom (*Auricularia auricula*) extract administration on total leukocyte and lymphocyte counts in male Wistar rats. The study was conducted from September to December 2025 at the Biology Laboratory, Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto for extract preparation, treatment intervention, and blood sampling. Hematological analyses were performed at the Regional Health Laboratory of Banyumas (Labkesda).

The independent variables consisted of various treatment doses of wood ear mushroom extract—specifically 300 mg/kg BW, 400 mg/kg BW, and 500 mg/kg BW—alongside positive and negative control groups. The positive control group received Imboost Original (containing *Echinacea purpurea* extract and zinc) as a reference standard, with the dosage determined based on human-to-rat dose conversion utilizing body surface area ratios. The dependent variables measured were total leukocyte and lymphocyte counts in male Wistar rats.

A sample size of 25 male Wistar rats was selected using non-probability sampling combined with a simple random assignment approach. Inclusion criteria required healthy rats aged 2–3 months with a body weight ranging from 140 to 200 grams. The treatment grouping is detailed in the table below:

Table 1. Experimental animal treatment groups

Treatment	Number of Animals	Extraxt Concentration
Dose 1	5	300 mg/kg BW
Dose 2	5	400 mg/kg BW
Dose 3	5	500 mg/kg BW
Positive Control	5	Immune booster supplement
Negative Control	5	Untreated

The equipment utilized in this study included a drying cabinet, analytical balance, blender, glass jars, filter cloth, glass stirring rods, evaporating dishes, water bath, beakers, porcelain mortar and pestle, oral gavage needles, 3 mL syringes, cloth gloves, microhematocrit tubes, EDTA vacutainer tubes, cooler box, and an automated hematology analyzer. The materials used comprised wood ear mushroom (*Auricularia auricula*), 70% ethanol, and standard rat feed.

The selection of doses—300, 400, and 500 mg/kg BW—was based on prior experimental studies regarding the application of *Auricularia* in animal models. The 300–400 mg/kg BW dosage range is supported by previous research demonstrating the biological and immunomodulatory activities of *Auricularia*, whereas the 500 mg/kg BW dose was included as a higher dosage tier to evaluate dose-dependent responses. Based on this range, the present study employed 300, 400, and 500 mg/kg BW as graded doses to assess potential dose-response dynamics on leukocyte and lymphocyte parameters (Ibe et al., 2020).

This study evaluated the effects of wood ear mushroom extract on hematological parameters in test animals. The main raw material, wood ear mushroom, was dried sequentially in a drying cabinet and ground into a fine powder. The powdered mushroom was weighed and extracted with 70% ethanol via maceration at a crude drug-to-solvent ratio of 1:10 for three days, with solvent replacement every 24 hours (remaceration). The remacerated liquid was evaporated over a water bath to yield a dry extract. Subsequently, the extract was weighed according to the required dosages and reconstituted in water for oral administration to the animals.

The test animals underwent a 7-day acclimatization period prior to treatment, during which initial body weights were measured to ensure baseline homogeneity across groups. The extract was administered orally via gavage every 24 hours for 10 days at specified group dosages. Following the treatment period, blood samples were collected from the retro-orbital sinus using microhematocrit tubes and transferred into EDTA-containing vacutainer tubes as an anticoagulant. Total leukocyte and lymphocyte counts were subsequently analyzed using an automated hematology analyzer.

Data were analyzed using univariate and bivariate statistical methods. Univariate analysis was performed to describe data characteristics for each variable—specifically leukocyte and lymphocyte counts in male Wistar rats—presented as mean ± standard deviation. Prior to bivariate analysis, normality testing was conducted on leukocyte and lymphocyte datasets; normally distributed data ($p > 0.05$) proceeded directly to bivariate analysis, whereas non-normal data ($p < 0.05$) underwent data transformation followed by re-testing for normality. Data fulfilling normality assumptions met the criteria for parametric testing.

Homogeneity of variance was subsequently evaluated. Upon meeting homogeneity assumptions, bivariate analysis was performed using one-way Analysis of Variance (ANOVA) to assess mean differences in leukocyte and lymphocyte counts across treatment groups. Statistical significance was set at $p < 0.05$. This study received ethical approval from the Health Research Ethics Committee of Universitas Muhammadiyah Purwokerto on August 3, 2025 (Registration No. KEPK/UMP/11/VIII/2025).

RESULTS AND DISCUSSION

The quantitative evaluation of total leukocyte and lymphocyte counts in male Wistar rats treated with wood ear mushroom (*Auricularia auricula-judae*) extract for 10 days is summarized in Table 2 and Table 4.

Table 1. Analysis of total leukocyte counts in experimental groups

Variable	n	Mean ± SD (cells/mm^3)	95% CI	p-value
D1	5	4.1381 ± 0.10922	4.0025 – 4.2737	
D2	5	4.0882 ± 0.16701	3.8809 – 4.2956	0.349
D3	5	4.0375 ± 0.13794	3.8662 – 4.2088	
K(+)	5	4.1627 ± 0.19520	3.9204 – 4.4051	

Variable	n	Mean ± SD (cells/mm^3)	95% CI	p-value
K(−)	5	3.9858 ± 0.11781	3.8396 – 4.1321	

Note: One-way ANOVA was used to assess differences among experimental group; D1: Group administered wood ear mushroom extract at dose 1 (300 mg/kg bw); D2: Group administered wood ear mushroom extract at dose 2 (400 mg/kg bw); D3: Group administered wood ear mushroom extract at dose 3 (500 mg/kg bw); K(+): Positive control group administered an immune booster supplement; K(-): Negative control group (untreated). n: number of animals per group; SD: standard deviation; CI: confidence interval.

As presented in Table 2, the one-way ANOVA for total leukocyte counts yielded a p-value > 0.05 (p = 0.349), indicating no statistically significant difference across the experimental groups following the administration of wood ear mushroom extract in male Wistar rats. The overlap in the 95% confidence intervals (CI) and non-specific inter-group variation further demonstrate the lack of significant variance between the treatment and control groups. Subsequently, post-hoc analysis was conducted using Duncan's Multiple Range Test (DMRT), the results of which are detailed in Table 3.

Table 3. Analysis of total leukocyte counts

Group	Mean Leukocyte (cells/mm^3)/ Duncan Subset ($\alpha = 0.05$)
Dose 1	4.1381^a
Dose 2	4.0882^a
Dose 3	4.0375 ^{a}
Positive Control	4.1627^a
Negative Control	3.9858^a

Note: Post-hoc test; different superscript letters within the same column indicate statistically significant differences between groups based on DMRT (p < 0.05)

As presented in Table 3, the post-hoc DMRT yielded a single homogeneous subset denoted by the superscript letter 'a'. This statistical analysis confirms that there were no significant differences in total leukocyte counts among the treatment groups. Nevertheless, based on the observed absolute values, Dose 1 was identified as the optimal dosage, yielding results closest to the positive control group.

Administration of wood ear mushroom extract did not significantly alter total leukocyte counts (p > 0.05), despite presenting higher mean values compared to the negative control. This suggests that immunomodulatory effects do not necessarily manifest as an elevation in total systemic leukocytes; rather, they may specifically target immune cell subpopulations, such as lymphocytes, via the activation of Dectin-1 and Toll-like receptors (TLRs) mediated by *Auricularia* polysaccharides and β-glucans (Steenwijk et al., 2021). Consistent with this mechanism, previous findings by Ibe et al. (2020) emonstrated selective increases in lymphocyte and neutrophil counts following A. *auricula-judae* administration, supporting the concept that immunomodulating responses can occur independently of total leukocyte expansion.

Furthermore, the observed physiological effects did not display a linear dose-dependent increase. At lower concentrations, β-glucans typically enhance leukocyte activity and recruitment; however, higher doses may trigger negative feedback regulatory mechanisms to prevent hyperactivation and maintain immune homeostasis. Consequently, while the immunomodulatory activity of medicinal mushrooms exhibits dose dependency, it is non-linear due to endogenous homeostatic regulation (Zhao et al., 2020).

A previous study conducted by Erawati (2020) valuating various concentrations of β-glucan on immune cytokine production revealed that different doses elicited distinct biological responses. Specifically, a concentration of 5 μg/mL induced the highest level of interleukin-12 (IL-12), whereas the peak production of interferon-gamma (IFN-γ) was achieved at 10 μg/mL among the evaluated doses (1, 5, 10, 20 and 50 μg/mL. Notably, maximal cytokine responses were concentrated at low-to-moderate doses, whereas higher concentrations yielded diminished responses. This pattern aligns with the findings of the present study, wherein rats receiving higher dosages exhibited a paradoxical reduction in total leukocyte counts. These observations indicate the existence of an optimal threshold for specific immunological parameters—such as leukocyte counts—rather than a linear, dose-dependent increase. Therefore, the immunostimulatory response is contingent upon dosage, as excessively high or low concentrations fail to provide optimal stimulation for specific cytokine production.

Table 4. Analysis of lymphocyte percentages among experimental groups

Variable	n	Mean ± SD (%)	95% CI	p-value
D1	5	46.80 ± 7.596	37.37 – 56.23	
D2	5	60.60 ± 8.961	49.47 – 71.73	
D3	5	70.00 ± 7.416	60.79 – 79.21	0.0005
K(+)	5	56.00 ± 5.099	49.67 – 62.33	
K(−)	5	67.80 ± 5.357	61.15 – 74.45	

Note: One-way ANOVA was used to assess differences among experimental group; D1: Group administered wood ear mushroom extract at dose 1 (300 mg/kg bw); D2: Group administered wood ear mushroom extract at dose 2 (400 mg/kg bw); D3: Group administered wood ear mushroom extract at dose 3 (500 mg/kg bw); K(+): Positive control group administered an immune booster supplement; K(-): Negative control group (untreated). n: number of animals per group; SD: standard deviation; CI: confidence interval.

Based on the one-way ANOVA results presented in Table 4, administration of wood ear mushroom extract exerted a statistically significant effect on lymphocyte percentage ($p = 0.0005$). The mean values closest to the positive control group (56.00 ± 5.099%) were observed in male Wistar rats treated with Dose 1 (46.80 ± 7.596%) and Dose 2 (60.60 ± 8.961%). Bonferroni post-hoc analysis further revealed significant differences between Dose 1 and Dose 3 ($p < 0.05$), as well as between Dose 1 and the negative control group ($p < 0.05$).

In contrast to the total leukocyte counts, the evaluation of lymphocyte percentages demonstrated significant inter-group variability ($p = 0.0005$). Notably, the increase observed in the 500 mg/kg bw group was significantly higher than that in the 300 mg/kg bw group and the negative control. These findings indicate that wood ear mushroom extract effectively stimulates lymphocyte-mediated immune responses.

This outcome aligns with the findings reported by Elhusseiny et al. (2022), who demonstrated that specific edible mushrooms significantly increase lymphocyte counts and enhance overall immune system activation in male Wistar rats. The observed elevation in lymphocyte counts and immune function is attributed to bioactive compounds present in mushrooms, particularly polysaccharides such as β-glucans and lentinan, which function as potent immunomodulators. β-glucans derived from mushrooms are also known to stimulate lymphocyte proliferation within lymphoid organs, including the spleen and thymus. This proliferative activity accelerates lymphocyte production and circulation in the bloodstream, thereby elevating absolute systemic counts.

The evaluation of lymphocyte percentages was further analyzed using Duncan's Multiple Range Test (DMRT) to identify specific inter-group differences. The resulting post-hoc DMRT data are presented in Table 5:

Table 5. Analysis of lymphocyte percentages

Group	Mean Lymphocyte (%)/ Duncan Subset ($\alpha = 0.05$)
Dose 1	46.80^{a}
Dose 2	$60.60^{b,c}$
Dose 3	70.00^{c}
Positive Control	$56.00^{a,b}$
Negative Conttrol	67.80^{c}

Note: Post-hoc test; different superscript letters within the same column indicate statistically significant differences between groups based on DMRT ($p < 0.05$)

Overall, the post-hoc DMRT demonstrated a dose-dependent increase in lymphocyte percentage with increasing concentrations of wood ear mushroom extract. The highest lymphocyte percentage was observed at Dose 3 (500 mg/kg bw); however, Dose 3 shared a homogeneous subset with Dose 2 (400 mg/kg bw), indicating no statistically significant difference between these two treatments. Furthermore, Dose 2 shared a subset with the positive control group. This suggests that Dose 2 (400 mg/kg bw) yielded a mean value most comparable to the positive control, representing the optimal dosage for elevating circulating lymphocyte percentages.

Intriguingly, the positive control group treated with the commercial immune booster supplement exhibited lower lymphocyte percentages compared to most treatment groups. This response can be attributed to the regulatory nature of immunomodulatory agents, which fine-tune the balance of immune cell activation rather than inducing continuous expansion. In many instances, immunomodulatory compounds trigger shifts in immune dynamics, including the suppression of cell proliferation or specific cell subset populations once an initial immune threshold is attained or when alternative regulatory pathways are engaged. This mechanism reflects the physiological endeavor to maintain immune homeostasis and prevent detrimental hyperactivation. Consequently, the relative decrease in lymphocyte percentage within the positive control group likely does not represent an adverse effect, but rather reflects a complex systemic regulatory response. This may involve tissue-specific lymphocyte redistribution, activation of negative feedback control mechanisms (suppressive pathways), or shifts in lymphocyte subset distribution, as the immune system actively preserves equilibrium to prevent excessive reactivities (Strzelec et al., 2023).

Evaluation of total leukocyte and lymphocyte counts is not only conducted in clinical human studies but is also routinely performed in animal models, particularly rats. Standard physiological values for total leukocyte counts in rats typically range from 4 $x10^{3}$ sel/µL to 11 $x10^{3}$ sel/µL, while normal lymphocyte percentages range between 55% and 85% (W.H Nugrahaningsih et al., 2021; Sulistiyowati et al., 2024).

The present study revealed that administration of wood ear mushroom extract exerted no significant effect on total leukocyte counts, yet it significantly increased lymphocyte percentages. This outcome contrasts with findings reported by Elhusseiny et al. (2022), who observed that high-dose administration of extracts from *Lentinula edodes*, *Agaricus bisporus*, *Pleurotus ostreatus*, *Pleurotus columbinus*, and *Pleurotus sajor-caju* in male albino Wistar rats led to a significant increase in both total white blood cell (leukocyte) and lymphocyte counts. Such elevations represent active immune cell stimulation within the host system. The discrepancy in results may be attributed to variations in mushroom species, extraction protocols, dosage regimens, and the duration of extract administration. In alignment with this observation, studies by Zhao et al. (2020) and Roszczyk et al. (2022) demonstrated that mushroom-derived polysaccharides can activate immune functions without triggering an overall rise in systemic leukocyte counts. These theoretical divergences are likely driven by structural variations in polysaccharides among mushroom species, which fundamentally alter their immunomodulatory pathways. Furthermore, treatment duration plays a crucial role in optimizing immune system activation, potentially bypassing systemic total leukocyte expansion while still modulating specific immune cell subsets, such as lymphocytes.

Wood ear mushroom, rich in bioactive polysaccharides—predominantly β-glucans—is widely recognized for its immunomodulatory properties. These polysaccharides enhance immune cell functionality via complex signaling cascades rather than merely expanding the total leukocyte pool. Preclinical animal studies further support that wood ear mushroom polysaccharides enhance immune responsiveness by augmenting immune organ indices and modulating cytokine expression under immunosuppressed conditions, even in the absence of a direct elevation in total leukocytes. This indicates that in healthy experimental animals, immunomodulatory effects manifest primarily as qualitative shifts in functional immune parameters rather than quantitative changes in total leukocyte counts, as the baseline immune system is already in an optimal homeostatic state, thereby preventing dramatic shifts in absolute leukocyte populations (Kong et al., 2020).

The elevation in lymphocyte counts in male Wistar rats treated with wood ear mushroom extract is attributable to the presence of polysaccharides, which modulate the host immune system, specifically target lymphocyte populations. The underlying mechanism of wood ear mushroom polysaccharides involves stimulating B cells, T cells, natural killer (NK) cells, and macrophage-dependent immune responses via receptor upregulation. Furthermore, bioactive constituents such as lentinan and β-glucans induce lymphocyte proliferation, thereby expanding circulating lymphocyte pools in rats following activation by mushroom-derived components (Maity et al., 2021).

The absence of a statistically significant increase in total leukocyte counts does not imply a lack of immunomodulatory activity. Mushroom-derived polysaccharides, particularly those operating within immune pathways, exert targeted biological effects. Rather than inducing an unselective expansion of overall white blood cell populations, these polysaccharides selectively enhance the activity and function of specific immune sub-populations, such as lymphocytes involved in adaptive responses, phagocytic macrophages, and natural killer (NK) cells. Mechanistically, this occurs through specific binding to immune cell surface receptors. Upon ligand-receptor interaction, immune cells are activated to produce signaling molecules (cytokines) and clear pathogens. Importantly, this targeted activation proceeds without triggering generalized systemic leukocyte expansion, a physiological pattern typically observed during inflammatory states (Zhao et al., 2020).

Polysaccharides and polypeptides derived from wood ear mushrooms exhibit therapeutic potential in modulating leukocyte counts in blood circulation. These macromolecules upregulate key immune cytokines, including interleukin-4 (IL-4) and tumor necrosis factor-alpha (TNF-α which play pivotal roles in activating and regulating immune responses. Both IL-4 and TNF-α are intrinsically linked to leukocyte dynamics, as they are synthesized by and act upon leukocyte subsets within the immune network. Mushroom bioactive compounds potentially elevate leukocyte counts by promoting immune cell differentiation and functional activation. Upon activation, immune cells upregulate the secretion of regulatory mediators, such as cytokines and antibodies. These properties underscore the potential of medicinal mushrooms as natural immunostimulants that facilitate host immune system activation and optimize overall immunological function (Zhao et al., 2025).

A limitation of the present study is the absence of baseline measurements for leukocyte and lymphocyte counts prior to treatment, as well as the lack of active compound profiling. Consequently, longitudinal shifts from baseline conditions and the precise bioactive components responsible for the observed immunomodulatory effects remain to be fully characterized.

CONCLUSIONS

Administration of wood ear mushroom (*Auricularia auricula*) extract significantly affected the lymphocyte percentage but showed no significant effect on the total leukocyte count in male Wistar rats. Based on Duncan's Multiple Range Test (DMRT) post hoc analysis, a dose of 400 mg/kg BW was identified as the optimal dosage for increasing lymphocyte percentage. Thus, wood ear mushroom extract exhibits strong potential as a natural immunomodulator that modulates the adaptive immune response without elevating overall leukocyte levels. Future studies are recommended to evaluate immune parameters pre- and post-treatment, as well as to isolate and quantify specific bioactive compounds—particularly polysaccharides or β-glucans—to elucidate the precise immunomodulatory mechanisms of wood ear mushroom extract.

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