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Active medicinal-food homologous complex nutrients enhance immune systems in zebrafish

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ABSTRACT

Background: Immunosuppression, whether caused by disease, stress, or medical treatments, compromises host defense by disrupting both innate and adaptive immune responses. Natural compounds such as Ganoderma lucidum, polygonatum, and mulberry leaves have long been used in traditional medicine for their immune-enhancing properties, yet their efficacy is often limited by low bioavailability. Biotransformation offers a promising approach to enhance the bioactivity of these medicinal-food homologous substances, but its potential in restoring immune function has not been fully explored.

Objective: The objective of this study was to investigate whether active medicinal-food homologous complex nutrients (AMCN) formulation containing Ganoderma lucidum, polygonatum, and mulberry leaf could restore immune function in an immunodeficient zebrafish model. Specifically, we aimed to evaluate its effects on both innate and adaptive immune cell populations, including neutrophils, macrophages, and T cells, as well as its ability to modulate key immune-related cytokines.

Methods: AMCN was prepared by culturing *Ganoderma lucidum* mycelia, followed by enzymatic biotransformation with polygonatum and mulberry leaf powders and subsequent fermentation with *Lactobacillus plantarum*. Transgenic zebrafish larvae were used as an *in vivo* model to evaluate immunomodulatory effects. Immune suppression was induced using cyclophosphamide, and larvae were exposed to AMCN to assess immune recovery. Changes in innate and adaptive

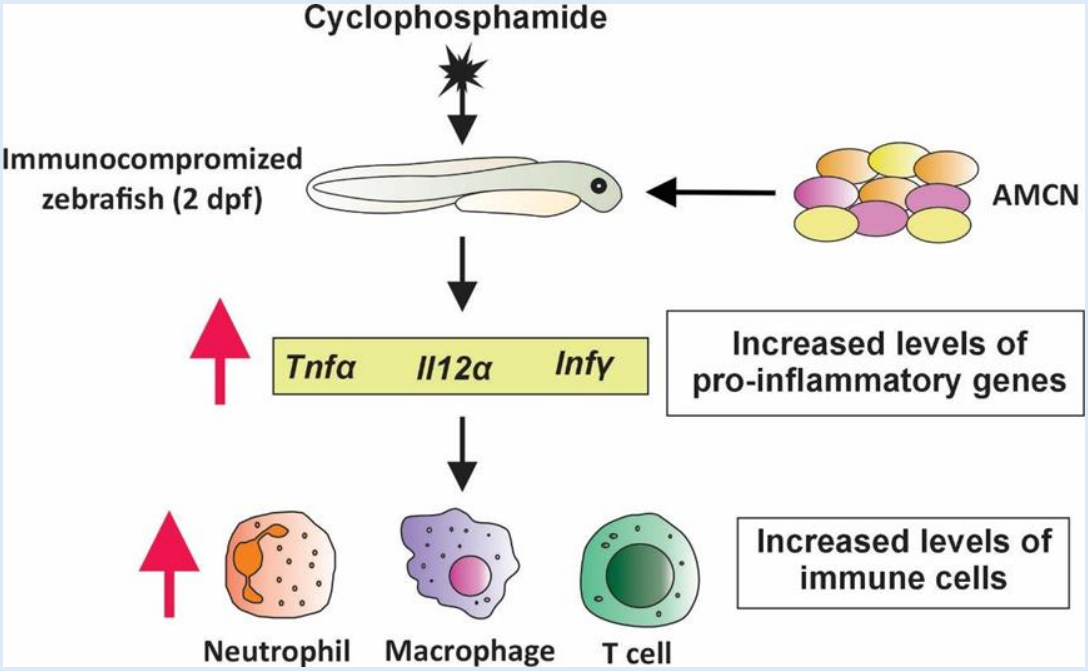
immune cell populations and key cytokine expression were analyzed to determine the efficacy of AMCN.

Results: AMCN treatment significantly restored immune cell populations in immunodeficient zebrafish, increasing neutrophils, macrophages, and T cells compared with the model group. It also upregulated key pro-inflammatory cytokines, including *tnf-α*, *il-12α*, and *ifn-γ*.

Conclusion: AMCN effectively restores both innate and adaptive immune cell populations in an immunodeficient zebrafish model, highlighting its potential as a natural immunomodulatory agent.

Novelty of the study: This study is the first to show that a biotransformed complex of AMCN can restore both innate and adaptive immune cell populations in an immunodeficient zebrafish model. AMCN exhibited broad immunostimulatory effects, upregulating key cytokines and highlighting biotransformation as a strategy to enhance the bioactivity of medicinal-food homologous substances for mitigating immunosuppression.

Key words: AMCN, biotransformation, immunomodulation, zebrafish model.



Graphical Abstract: Active medicinal-food homologous complex nutrients (AMCN) and their immunomodulatory effects in a zebrafish model.

INTRODUCTION

The immune system is essential for host defense, immune surveillance, and tissue homeostasis. In cancer patients, immune function is frequently impaired, either due to the tumor microenvironment or because of cytotoxic treatments such as chemotherapy and radiotherapy [1-2]. Immunosuppression in these patients can lead to increased risk of infection, slower recovery, and poor therapeutic outcomes [3]. There is thus an unmet clinical need for safe, effective agents that can

support or restore immune function—ideally from natural sources that offer both therapeutic benefit and favorable safety profiles.

In traditional Chinese medicine, certain natural substances are classified under the concept of "homology of medicine and food", meaning they can be used both as therapeutic agents and as part of the daily diet to promote health and prevent disease [4]. Among these, *Ganoderma lucidum* [5], polygonatum [6], and mulberry leaf [7] are well-known for their immune-supporting properties and long history of use in East Asian medicine. These natural substances are rich in polysaccharides, flavonoids, glycoproteins, and other bioactive compounds that have been shown to modulate immune responses, reduce inflammation, and provide antioxidant protection [4-7]. However, the natural forms of these materials often contain large, complex molecules that are difficult for the human body to absorb efficiently. For example, *Ganoderma lucidum* in its native form has a hard outer spore shell, which must be broken to release its bioactive content [8-9]. Polygonatum and mulberry leaf are traditional medicinal plants rich in polysaccharides, flavonoids, and other bioactive compounds. However, their oral bioavailability is limited due to the presence of high molecular weight polysaccharides, glycosylated flavonoids, and poor lipophilicity, which hinder intestinal absorption [10-11].

To overcome this limitation, we applied biotransformation—a process that uses microbial fermentation or enzymatic catalysis to break down macromolecules into smaller, more bioavailable compounds. This approach not only improves intestinal absorption but can also enhance the pharmacological activity of the resulting metabolites [12]. In the case of active medicinal-food homologous complex nutrients (AMCN), we hypothesize that the modified product exerts stronger immune-enhancing effects than its unprocessed form. A similar rationale applies to the biotransformed forms of *Polygonatum* and mulberry leaf used in our study.

To evaluate the immunomodulatory potential of these biotransformed compounds, we utilized a zebrafish model of immunosuppression, which mimics the features of hematopoietic dysfunction and immune cell depletion commonly observed in cancer patients [2, 13]. Zebrafish are increasingly recognized as a powerful model organism for studying vertebrate immunity due to their conserved hematopoietic pathways [14], optical transparency during early development [15], and availability of transgenic lines that express fluorescent markers in key immune cell populations [16-18]. The zebrafish immune system includes both innate components—such as macrophages and neutrophils—and adaptive components like T lymphocytes, allowing for comprehensive analysis of immune responses in vivo.

In this study, we first evaluated the in vivo toxicity of AMCN to confirm its safety. We then used transgenic zebrafish lines to quantify changes in macrophages, neutrophils, and T cells following treatment with AMCN. Finally, we investigated the molecular mechanisms underlying these immune enhancements through quantitative real-time PCR (qRT-PCR) analysis of immune-related genes.

MATERIALS AND METHODS

Production of AMCN: Single-clone isolates of Ganoderma lucidum (strain CICC 14021; China Center of Industrial Culture Collection, China) were initially cultured on potato dextrose agar (Huankai Microbial, Guangzhou, China) plates. Agar plugs containing actively growing mycelium were transferred to a medium containing 40 g/L of D-glucose (G116306, Aladdin Biochemical Technology, Shanghai, China) 5 g/L of peptone (P304955, Aladdin Biochemical Technology), 5 g/L of yeast extract (Y110517, Aladdin Biochemical Technology), 0.46 g/L of monopotassium phosphate (P113045, Aladdin Biochemical Technology) and 0.5 g/L of magnesium sulfate heptahydrate (M431166, Aladdin Biochemical Technology). A total of 25 mL of liquid

cultures were incubated in 250 mL Erlenmeyer flasks at 30 °C in the dark with agitation at 100 rpm.

β-Glucosidase activity in the culture supernatant was quantified using a β-glucosidase activity assay kit (β-glucosidase Assay Kit, Solarbio, Beijing, China). When enzymatic activity exceeded 100 mU/mL, cultures were centrifuged at 5000 rpm for 10 minutes to collect the supernatant. The supernatant was then supplemented with 1.5% (w/v) polygonatum powder (Shanghai All-Plus-One Health Technology, Shanghai, China), 1.5% (w/v) Ganoderma lucidum powder (Shanghai All-Plus-One Health Technology) and 1.5% (w/v) mulberry leaf powder (Shanghai All-Plus-One Health Technology). This mixture was incubated for an additional 24 hours under the same conditions to allow biotransformation. After biotransformation, cultures were centrifuged again at 5000 rpm for 10 minutes. The supernatant was inoculated with Lactobacillus plantarum at a final concentration of 1×10^7 CFU/mL to produce the AMCN. L. plantarum strain Vege-start 60 (MAT NO: 696265), a probiotic starter culture optimized for fermentation, was obtained from Chr. Hansen (No. 10 Jintong West Road, Chaoyang District, Beijing, China).

Subsequently, 10% (w/v) maltodextrin (M434571, Aladdin Biochemical Technology), 10% (w/v) mannitol (M108831, Aladdin Biochemical Technology), and 2% (w/v) D-(+)-trehalose dihydrate (D425084, Aladdin Biochemical Technology) were added as lyophilization protectants to the final supernatant. The mixture was stirred thoroughly for at least one hour to ensure uniform dispersion. Following mixing, the solution was subjected to freeze-drying using a laboratory freeze-dryer until a constant weight was achieved. Care was taken to ensure the resulting lyophilized powder was homogeneous and free of polysaccharide precipitation. All lyophilized powders were weighed and the weights recorded. The powders were then ground and sieved to obtain a particle size of approximately 100 mesh. The final product was designated as AMCN. The lyophilized AMCN powder was packaged in 1 kg aluminum foil bags and stored at room temperature, protected from direct sunlight.

Zebrafish husbandry and embryo collection: Zebrafish were maintained and bred as described in our previous study [19]. Briefly, adult zebrafish were housed in a recirculating water system at 28 °C under a 14 h light/10 h dark photoperiod and fed three times daily. For embryo collection, male and female zebrafish were paired at a 1:1 ratio in the evening and separated by a divider. The divider was removed the following morning to allow spawning within the first hour of the light cycle. Fertilized larvae were collected and maintained in 1 × E3 medium supplemented with methylene blue (0.3 ppm) (M196499, Aladdin Biochemical Technology) at 28.5 °C in an incubator until use. All zebrafish experiments conducted in our study were approved by Institutional Animal Care and Use Committee (MDL2025-03-04-03). Adult zebrafish and transgenic zebrafish larvae were bred and housed by Baihuan Biotechnology (Guangzhou, China).

Immunomodulatory Activity Assessment

Analysis on neutrophil number in zebrafish model: Meloperoxidase (mpo) is a well-known neutrophil marker in zebrafish [18]. Tg(mpo:EGFP) zebrafish are a transgenic line that expresses enhanced green fluorescent protein (EGFP) under the control of the mpo promoter, allowing for the specific labeling and in vivo visualization of neutrophils (18). Tg(mpo:EGFP) zebrafish larvae (2 dpf) were randomly distributed into 6-well plates, with 30 larvae per well. AMCN was administered at concentrations of 12.5, 25, and 50 μg/mL. Three experimental groups included normal control, model, and treatment groups. All groups except the normal control group were exposed to 20 μg/mL of cyclophosphamide to induce immune suppression. All treatments were conducted at 28 °C for 48 hours. After treatment, 10 larvae per group were randomly selected and imaged under a fluorescence microscope (MZX81,

Mingmei shot, Guangzhou, China). The number of neutrophils in the caudal vein was quantified using NIS-Elements software.

Analysis on macrophage levels in zebrafish model: Macrophage expressed gene 1 (mpeg1) is a macrophage-specific gene in zebrafish [17]. Tg(mpeg1:EGFP) zebrafish are a transgenic line in which mpeg1 promoter drives the expression of EGFP, allowing specific visualization of macrophages in vivo [17]. Thirty Tg(mpeg1:EGFP) zebrafish larvae (2 dpf) were randomly distributed into 6-well plates under the same conditions as described above. Following the same treatment scheme and exposure to AMCN (12.5, 25, and 50 µg/mL) in the presence or absence of cyclophosphamide as described above, the larvae were incubated at 28 °C for 48 hours. Subsequently, ten larvae from each group were randomly selected and imaged under the MZX81 fluorescent microscope. The levels of fluorescent intensity of macrophages in the caudal vein was quantified using NIS-Elements software.

Analysis on T-cell levels in zebrafish in zebrafish model: Recombination activating gene 2 (rag2) is a well-known T lymphocyte-specific marker in thymus, which is highly conserved across vertebrates, including mice, humans and zebrafish [20]. Tg(rag2: dsRed) zebrafish are a transgenic line in which the rag2 promoter drives expression of Discosoma red fluorescent protein (dsRed), allowing enabling specific detection of lymphoid progenitor cells, particularly T cells in the thymus [16]. Thirty Tg(rag2:dsRed) zebrafish (2dpf) larvae were randomly distributed into 6-well plates under the same conditions, followed by treatment with AMCN (12.5, 25, and 50 µg/mL) in the presence or absence of cyclophosphamide as described above. Following a 48-hour incubation at 28°C, 10 larvae per group were randomly selected for imaging, and the levels of fluorescence intensity of T-cells were quantified in the caudal vein using NIS-Elements software.

Analysis of gene expression Green, fluorescent neutrophil transgenic zebrafish larvae (2 dpf) were used to assess immune gene expression. The larvae were treated as in section 3.1 and incubated at 28 °C for 48 hours. Subsequently, total RNA was extracted from the larvae in each group using the RNeasy Universal RNA Extraction kit (Qiagen, MD, USA) according to the manufacturer's protocol. RNA concentration and purity were assessed by UV-Vis spectrophotometry. A total of 2 µg of RNA was reverse transcribed into cDNA using a First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, MA, USA) in a final volume of 20.0µL. Quantitative real-time PCR (qRT-PCR) was performed to measure the relative expression levels of the pro-inflammatory genes, tumour necrosis factor-α (*tnf-α*), interleukin-12α (*il-12α*), and interferon-gamma (*ifn-γ)*, with *β-actin* serving as an endogenous control. Each experiment was conducted in triplicate. The primers used for this assay are listed in Table 1.

Table 1. The sequence of primers used in qPCR.

Gene	Primer Sequence (5' to 3')	
β-actin	Forward	TCGAGCAGGAGATGGGAACC
	Reverse	CTCGTGGATACCGCAAGATTC
tnf-α	Forward	GCGCTTTTCTGAATCCTACG
	Reverse	TGCCCAGTCTGTCTCCTTCT
il-12α	Forward	AACTCCTACAAGCCCAGCAC
	Reverse	ACACTCGGTCGTCAAACGAA
Ifn-γ	Forward	CTTTCCAGGCAAGAGTGCAGA
	Reverse	TCAGCTCAAACAAAGCCTTTCG

Statistical Analysis: Statistical analysis was performed using one-way ANOVA followed by Dunnett's post hoc test to compare the normal control and AMCN treatment groups against the model group. All quantitative data are presented as mean ± standard error (SE). Analyses were conducted using GraphPad Prism software (version 8.0.2, GraphPad Software Inc., CA, USA) in a blinded manner. A p-value < 0.05 was considered statistically significant.

RESULTS

AMCN increases the number of neutrophils in zebrafish model:

To determine whether AMCN modulates the immune system in our immunodeficient zebrafish model, we first examined its effect on neutrophils—key effectors of innate immunity [21]—using Tg(mpo:EGFP) larvae. Compared with the normal control group, the model group exhibited a ~30% reduction in neutrophil numbers (Fig. 1A&B), confirming successful establishment of the immunodeficient model. Importantly, AMCN treatment significantly increased neutrophil numbers by ~14% relative to the model group.

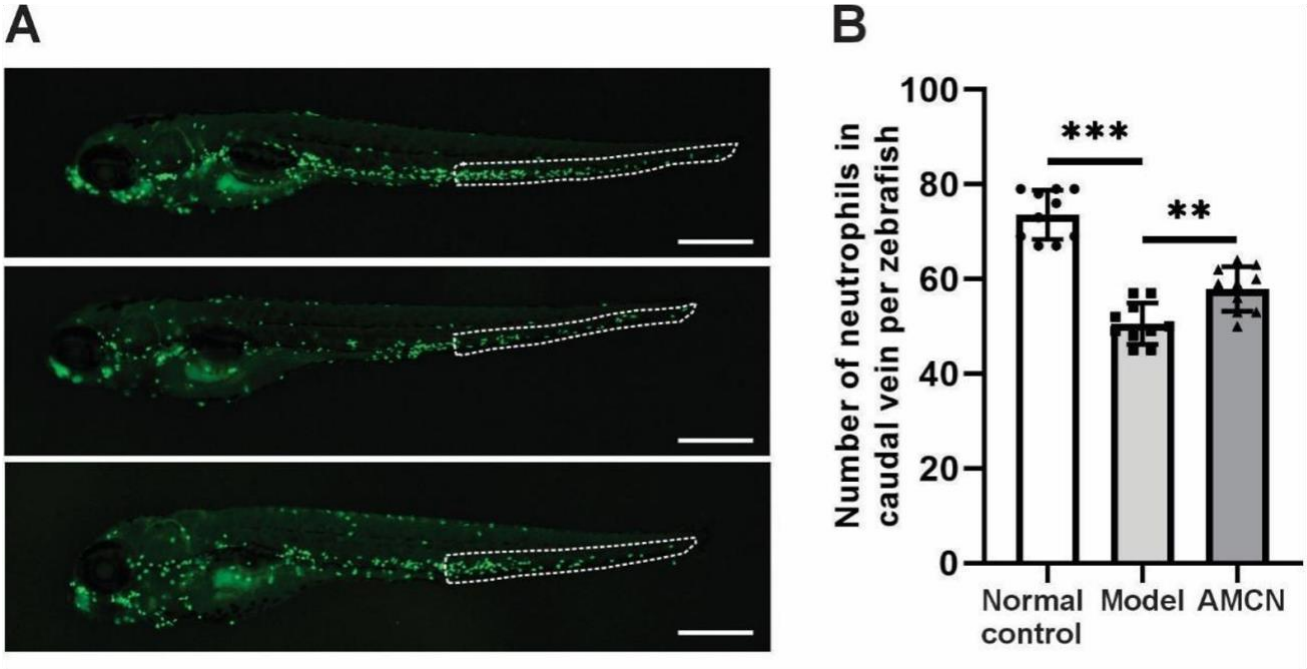


Figure 1. Effect of AMCN on neutrophil counts in the zebrafish larvae. (A) Representative images of Tg(mpo:EGFP) zebrafish larvae (4 dfp) after 48 h of AMCN treatment. White dotted areas indicate the regions used for quantification. Scale bar = 100 µm. (B) Quantification of neutrophil numbers in the caudal vein. Data are presented as mean ± SD (n=10 per group), ** p<0.01 and *** p<0.001 vs. Model group (one-way ANOVA with Dunnett's post hoc test).

AMCN increases the number of macrophages in zebrafish model:

We next evaluated the effect of AMCN on macrophages, another key cell type responsible for phagocytosis of cellular debris in zebrafish [22], using Tg(mpeg1:EGFP) larvae. In the normal control group, macrophage numbers were highest, representing baseline levels in zebrafish larvae (Fig. 2A & B). Compared with the normal control, macrophage numbers in the model group were reduced by ~54%. Strikingly, AMCN treatment significantly restored macrophage abundance, leading to a ~57% increase relative to the model group.

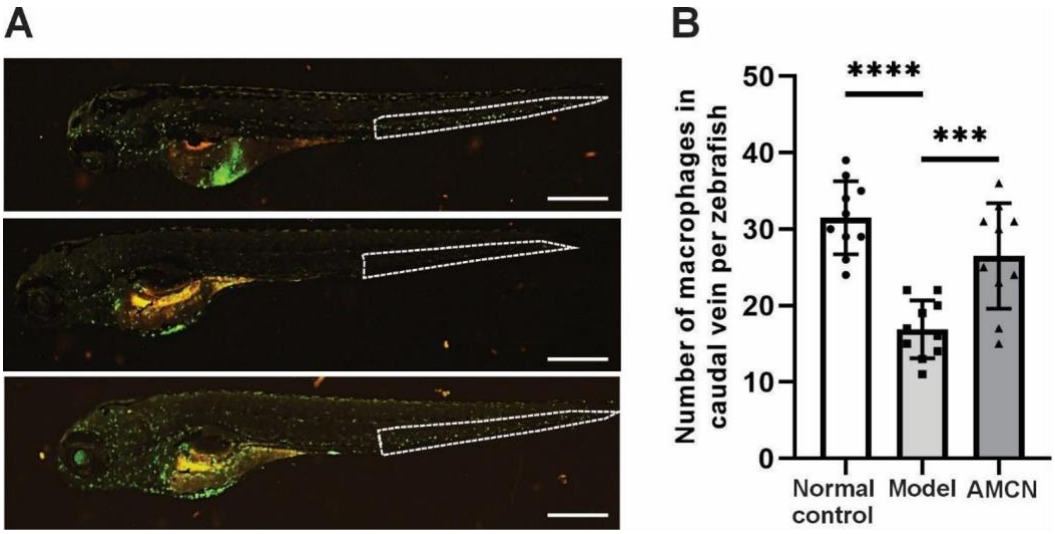


Figure 2. Effect of AMCN on macrophage levels in the zebrafish larvae. (A) Representative images of Tg(mpeg1:EGFP) zebrafish larvae (4 dfp) after 48 h of AMCN treatment. White dotted areas indicate the regions used for quantification. Scale bar = 100 µm. (B) Quantification of macrophage levels by fluorescence intensity in the caudal vein. Data are presented as mean ± SD (n=10 per group), *** p<0.001 and **** p<0.0001 vs. Model group (one-way ANOVA with Dunnett's post hoc test).

AMCN increases the levels of T cells in zebrafish model: We next examined the effect of AMCN on T cells using Tg(rag2:dsRed) zebrafish larvae. Because T cells within the thymus were tightly clustered and individual cell counts could not be reliably obtained, fluorescent intensity was used as a surrogate measure of T cell abundance. Compared with the normal control group, the model group exhibited a ~45% reduction in thymic fluorescence intensity, indicating a marked decrease in T cell levels (Figure 3A & B). Notably, AMCN treatment significantly increased fluorescent intensity by 61% relative to the model group, suggesting a robust restoration of T cell populations.

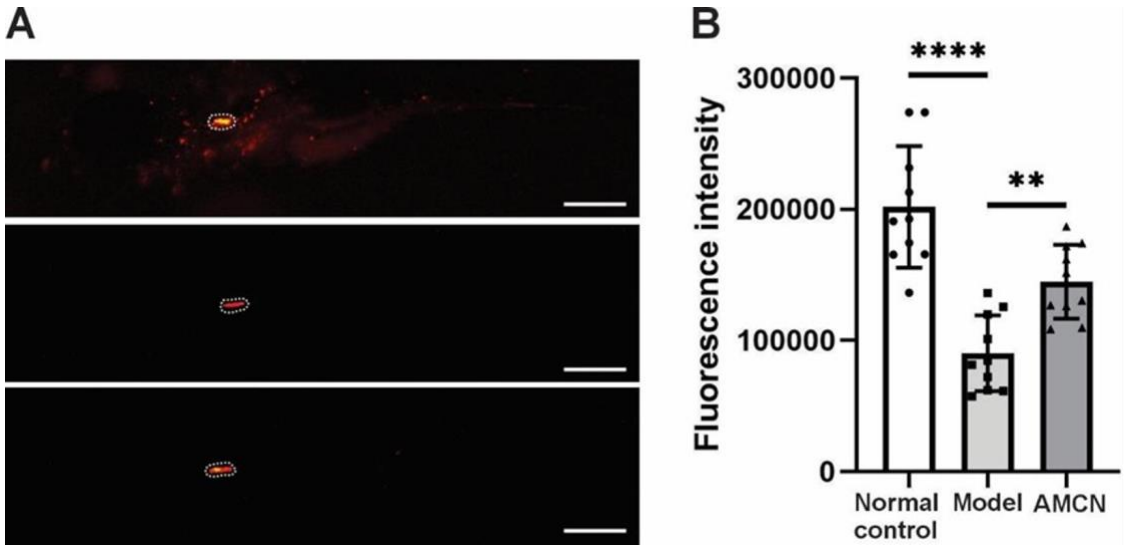


Figure 3. Effect of AMCN on T cell levels in the zebrafish larvae. (A) Representative images of Tg(rag2: dsRed) zebrafish larvae (4 dpf) after 48 h of AMCN treatment. White dotted areas indicate the regions used for quantification in the thymus. Scale bar = 100 µm. (B) Quantification of T cell levels by fluorescence intensity in the thymus. Data are presented as mean ± SD (n=10 per group), ** p < 0.01 and **** p < 0.0001 vs. Model group (one-way ANOVA with Dunnett's post hoc test).

AMCN increases pro-inflammatory genes in zebrafish model: Given that AMCN treatment could elevate the levels of key immune cell types in the zebrafish model, we further investigated the mechanisms by which AMCN influences the immune system by assessing several pro-inflammatory cytokine genes known to be involved in the regulation of innate immunity- namely *tnf-α*, *il-12α* and *ifn-γ* [21-23].

Our qRT-PCR demonstrated that the expression of *tnf-α* was markedly reduced in the model group compared to the normal control, whereas the AMCN treatment significantly increased the expression of *tnf-α* e, as compared to the model (Figure 4, left panel). Similar to *tnf-α*, the expression levels of *il-12α* and *ifn-γ* were also significantly lower in the model group as compared to the normal control, and the AMCN treatment led to statistically significant increases in the levels of expression of these genes (Figure 4, middle and right panels). These findings suggest that AMCN upregulates the expression of key pro-inflammatory cytokine genes.

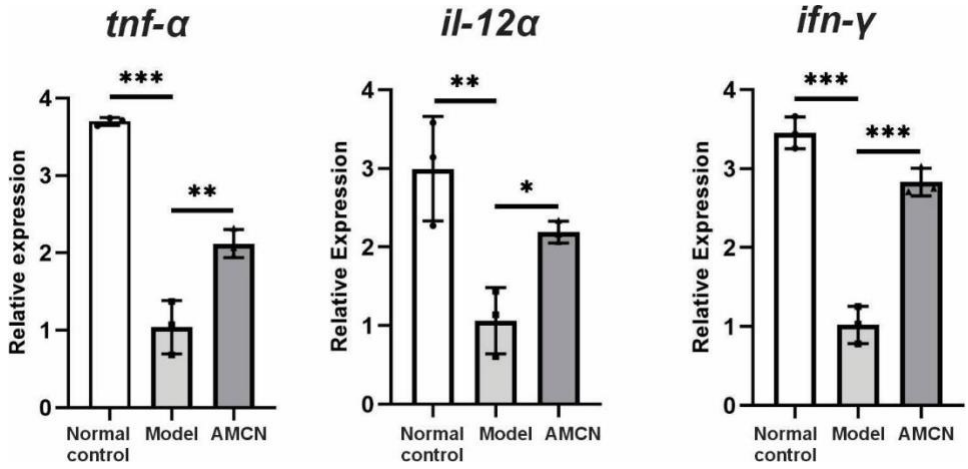


Figure 4. Effect of AMCN on relative gene expression of *tnf-α*, *il-12α* and *ifn-γ* in the zebrafish larvae assessed by qRT-PCR Data are presented as mean ± SD (n=3 per group), * p<0.05, ** p<0.01, *** p<0.001 and **** p<0.0001 vs. Model group (one-way ANOVA with Dunnett's post hoc test).

DISCUSSION

In this study, we investigated the immunomodulatory effects of AMCN, a biotransformed complex of *Ganoderma lucidum*, polygonatum, and mulberry leaf, in an immunodeficient zebrafish model. Our findings provide compelling evidence that AMCN promotes recovery of innate and adaptive immune functions by restoring neutrophil, macrophage, and T cell populations, while simultaneously enhancing the expression of key pro-inflammatory cytokine genes, including tnf-α, il-12a, and ifn-γ. These results highlight AMCN as a promising natural agent with the capacity to counteract immunosuppression, a common and clinically significant complication in cancer and other chronic diseases.

Innate immune cells such as neutrophils and macrophages form the first line of defense against pathogens, clearing cellular debris and providing signals that shape adaptive responses [21]. In our immunodeficient zebrafish model, the number of neutrophils and macrophages was markedly reduced, consistent with immune suppression. Treatment with AMCN significantly increased the abundance of both cell types, suggesting that AMCN helps restore innate immune competence. Neutrophils are particularly critical for acute antibacterial defense [24], and their depletion is strongly associated with heightened infection risk in cancer patients undergoing chemotherapy [25]. By partially restoring neutrophil levels, AMCN may help mitigate neutropenia-related complications. Similarly,

macrophages contribute to tissue repair and antigen presentation [26]. The ability of AMCN to recover macrophage numbers suggests a broader role in maintaining immune homeostasis and facilitating immune recovery following cytotoxic stress. The concurrent increase in *tnf-α* and *il-12a* expression further supports this interpretation. TNF-α, produced by activated macrophages and other cells, promotes inflammation, enhances phagocytosis, and recruits additional immune effectors [27-28]. IL-12a is a pivotal cytokine linking innate and adaptive immunity, as it drives the differentiation of naïve T cells into Th1 effector cells [29]. The upregulation of these cytokines by AMCN indicates that its immunostimulatory effects are not limited to the numerical recovery of cells, but extend to the functional activation of innate immune pathways.

The adaptive immune system, particularly T cells, plays a central role in long-term immune protection and anti-tumor surveillance [30]. In our zebrafish model, thymic T cell levels were substantially reduced under immunodeficient conditions, consistent with impaired adaptive immunity. Remarkably, AMCN treatment restored T cell levels, as evidenced by a significant increase in thymic fluorescence intensity in Tg(rag2: dsRed) larvae. This finding is noteworthy because recovery of adaptive immunity is often slow and incomplete in immunocompromised states following chemotherapy [31]. The increase in *ifn-γ* expression provides additional mechanistic support. IFN-γ, primarily secreted by T cells and natural killer cells, enhances antigen presentation, activates macrophages, and contributes to anti-viral and anti-tumor defenses [32]. Elevated ifn-γ levels in AMCN-treated zebrafish suggest that not only are T cells numerically restored, but their functional activity may also be enhanced, although further functional studies are required. Together, these results demonstrate that AMCN could exert immunomodulatory effects that bridge both innate and adaptive arms of the immune system in zebrafish.

Biotransformation using microbial or enzymatic processes can break down macromolecules into smaller, more bioavailable metabolites, while sometimes generating novel compounds with enhanced pharmacological activity [33]. A distinctive feature of AMCN is the use of biotransformation to process *Ganoderma lucidum*, polygonatum, and mulberry leaf. Natural forms of these materials are rich in bioactive compounds but often suffer from poor oral bioavailability due to high molecular weight polysaccharides and complex glycosylated structures [8-11]. Previous studies have shown that biotransformed *Ganoderma lucidum* polysaccharides exhibit stronger immunostimulatory and anti-tumor effects compared with their unprocessed counterparts [34-35]. Similarly, fermentation-derived flavonoid metabolites from mulberry leaf and polygonatum display improved antioxidant and immune-supporting activities [36-37]. Our findings suggest that AMCN benefits from these advantages, providing improved immune restoration compared with what might be expected from the unprocessed materials.

Immunosuppression remains a major barrier to effective cancer therapy. Chemotherapy-induced immunodeficiency, including neutropenia and lymphopenia, is often associated with increased risk of infections, delayed recovery, and reduced responsiveness to immunotherapies [38]. Current clinical approaches to address immunosuppression, such as granulocyte colony-stimulating factor or chimeric antigen receptors-T cell therapy, can be effective but are associated with significant cost and adverse effects, including bone pain, fatigue, or excessive inflammatory responses [39-41]. Nutritional immunomodulators derived from medicinal-food homologous substances, such as AMCN, may potentially provide safer and more accessible adjunctive strategy. Their dual status as both food and medicine make them attractive candidates for long-term use in vulnerable patient populations,

including cancer survivors and the elderly, where safety and tolerability are paramount.

While our study provides important insights, several limitations must be acknowledged. First, zebrafish serve as an excellent vertebrate model due to conserved hematopoietic pathways [14], but differences from mammalian immunity necessitate validation in mouse and human systems. Moreover, our analysis focused on three immune cell populations and cytokines. Future studies using single-cell RNA sequencing or proteomics would provide a more comprehensive understanding of AMCN's effects on immune networks.

CONCLUSION

AMCN, a biotransformed complex of *Ganoderma lucidum*, polygonatum, and mulberry leaf, effectively restored key innate and adaptive immune cells and enhanced pro-inflammatory cytokine expression in an immunodeficient zebrafish model. These results highlight its potential as a safe and natural agent to support immune function in immunocompromised conditions.

Authors' Contributions: Conceptualization: Y.Y., and L. Y.; Methodology: Y.Y., F. X., and H. L; Funding acquisition: X. R.; Supervision: L. Y**.** Writing – original draft: Y.Y. ; Writing – review and editing: Y.Y., F. X., H. L., X. R and L. Y.; All the authors read and approved the manuscript.

Competing Interests: There are no conflicts of interest to declare.

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List of Abbreviations: AMCN: active medicinal-food homologous complex nutrients, dpf: days post-fertilization, tnf-α: tumor necrosis factor-alpha, il-12a, interleukin-12 alpha, ifn-γ: interferon-gamma.

REFERENCES

1. Yürekli A, Erbas O. Cancer and immunosuppression. Journal of Experimental and Basic Medical Sciences. 2021;2(2):116–21. DOI: http://10.5606/jebms.2021.75646.
2. Plackoska V, Shaban D, Nijnik A. Hematologic dysfunction in cancer: Mechanisms, effects on antitumor immunity, and roles in disease progression. Front Immunol. 2022; 13:1041010. DOI: http://10.3389/fimmu.2022.1041010.
3. Wozniak H, Tabah A, Barbier F, Ruckly S, Loiodice A, Akova M, et al. Hospital-acquired bloodstream infections in critically ill cirrhotic patients: a post-hoc analysis of the EUROBACT-2 international cohort study. Ann Intensive Care. 2024;14(1):70. DOI: http://10.1186/s13613-024-01299-x.
4. Ng CYJ, Bun HH, Zhao Y, Zhong LLD. TCM "medicine and food homology" in the management of post-COVID disorders. Front Immunol. 2023; 14:1234307. DOI: http://10.3389/fimmu.2023.1234307.
5. Wang X, Lin Z. Immunomodulating Effect of Ganoderma (Lingzhi) and Possible Mechanism. Adv Exp Med Biol. 2019; 1182:1–37. DOI: http://10.1007/978-981-32-9421-9_1.
6. He Y, Huang L, Jiang P, Xu G, Sun T. Immunological regulation of the active fraction from Polygonatum sibiricum F. Delaroche based on improvement of intestinal microflora and activation of RAW264.7 cells. J Ethnopharmacol. 2022; 293:115240. DOI: http://10.1016/j.jep.2022.115240.
7. Abbas Z, Tong Y, Wang J, Zhang J, Wei X, Si D, et al. Potential Role and Mechanism of Mulberry Extract in Immune Modulation: Focus on Chemical Compositions, Mechanistic Insights, and Extraction Techniques. Int J Mol Sci. 2024;25(10). DOI: http://10.3390/ijms25105333.
8. Plosca MP, Chis MS, Farcas AC, Paucean A. Ganoderma lucidum-From Ancient Remedies to Modern Applications: Chemistry, Benefits, and Safety. Antioxidants (Basel). 2025;14(5). DOI: http://10.3390/antiox14050513.
9. Ming C, Hua M, Haoyong X, Zhenhao L. In vitro gastrointestinal digestion and fermentation properties of Ganoderma lucidum spore powders and their extracts. LWT. 2021;135(4). DOI: http://10.1016/j.lwt.2020.110235.
10. Wu W, Wang Y, Yi P, Su X, Mi Y, Wu L, et al. Various steaming durations alter digestion, absorption, and fermentation by human gut microbiota outcomes of Polygonatum cyrtonema Hua polysaccharides. Front Nutr. 2024; 11:1466781. DOI: http://10.3389/fnut.2024.1466781.
11. Chen R, Zhou X, Deng Q, Yang M, Li S, Zhang Q, et al. Extraction, structural characterization and biological activities of polysaccharides from mulberry leaves: A review. Int J Biol Macromol. 2024;257(Pt 2):128669. DOI: http://10.1016/j.ijbiomac.2023.128669.

12. Liu Y, Yang L. Early metabolism evaluation making traditional Chinese medicine effective and safe therapeutics. J Zhejiang Univ Sci B. 2006;7(2):99–106. DOI: http://10.1631/jzus.2006.B0099.
13. Pich O, Cortes-Bullich A, Muinos F, Pratcorona M, Gonzalez-Perez A, Lopez-Bigas N. The evolution of hematopoietic cells under cancer therapy. Nat Commun. 2021;12(1):4803. DOI: http://10.1038/s41467-021-24858-3.
14. Franza M, Varricchio R, Alloisio G, De Simone G, Di Bella S, Ascenzi P, et al. Zebrafish (Danio rerio) as a Model System to Investigate the Role of the Innate Immune Response in Human Infectious Diseases. Int J Mol Sci. 2024;25(22). DOI: http://10.3390/ijms252212008.
15. Lopez Nadal A, Ikeda-Ohtsubo W, Sipkema D, Peggs D, McGurk C, Forlenza M, et al. Feed, Microbiota, and Gut Immunity: Using the Zebrafish Model to Understand Fish Health. Front Immunol. 2020; 11:114. DOI: http://10.3389/fimmu.2020.00114.
16. Tang Q, Abdelfattah NS, Blackburn JS, Moore JC, Martinez SA, Moore FE, et al. Optimized cell transplantation using adult rag2 mutant zebrafish. Nat Methods. 2014;11(8):821–4. DOI: http://10.1038/nmeth.3031.
17. Ellett F, Pase L, Hayman JW, Andrianopoulos A, Lieschke GJ. mpeg1 promoter transgenes direct macrophage-lineage expression in zebrafish. Blood. 2011;117(4):e49–56. DOI: http://10.1182/blood-2010-10-314120.
18. Buchan KD, Prajsnar TK, Ogryzko NV, de Jong NWM, van Gent M, Kolata J, et al. A transgenic zebrafish line for in vivo visualisation of neutrophil myeloperoxidase. PLoS One. 2019;14(4):e0215592. DOI: http://10.1371/journal.pone.0215592.
19. Yuan L, Davis PF. Regetting-18 complex powder (R-18CP) - A formulation of components from food-medicine and its anti-aging effects. Functional Foods in Health and Disease. 2025;15(7):369–79. DOI: https://doi.org/10.31989/ffhd.v15i7.1647.
20. Xu K, Liu H, Shi Z, Song G, Zhu X, Jiang Y, et al. Disruption of the RAG2 zinc finger motif impairs protein stability and causes immunodeficiency. Eur J Immunol. 2016;46(4):1011–9. DOI: http://10.1002/eji.201545896.
21. Rosowski EE. Determining macrophage versus neutrophil contributions to innate immunity using larval zebrafish. Dis Model Mech. 2020;13(1). DOI: http://10.1242/dmm.041889.
22. Sakkas LI, Johanson NA, Scanzello CR, Platsoucas CD. Interleukin-12 is expressed by infiltrating macrophages and synovial lining cells in rheumatoid arthritis and osteoarthritis. Cell Immunol. 1998;188(2):105–10. DOI: http://10.1006/cimm.1998.1363.
23. Hu X, Chakravarty SD, Ivashkiv LB. Regulation of interferon and Toll-like receptor signaling during macrophage activation by opposing feedforward and feedback inhibition mechanisms. Immunol Rev. 2008; 226:41–56. DOI: http://10.1111/j.1600-065X.2008.00707.x.
24. Skerniskyte J, Barroso MV, Chicher J, Hammann P, Demais V, Wright K, et al. Neutrophils display antibacterial defense via non-canonical LC3 decoration of extracellular bacteria. Microbes Infect. 2025:105545. DOI: http://10.1016/j.micinf.2025.105545.
25. Mazzaro RT, Vaz MV, Badin RC, Bernardina ED, Manacas LRA. Management of chemotherapy-induced febrile neutropenia and use of granulocyte colony-stimulating factor in patients with soft tissue or bone sarcoma. J Oncol Pharm Pract. 2023;29(6):1428–36. DOI: http://10.1177/10781552221131901.
26. Brancewicz J, Wojcik N, Sarnowska Z, Robak J, Krol M. The Multifaceted Role of Macrophages in Biology and Diseases. Int J Mol Sci. 2025;26(5). DOI: http://10.3390/ijms26052107.
27. Parameswaran N, Patial S. Tumor necrosis factor-alpha signaling in macrophages. Crit Rev Eukaryot Gene Expr. 2010;20(2):87–103. DOI: http://10.1615/critreveukargeneexpr.v20.i2.10.
28. Vieira SM, Lemos HP, Grespan R, Napimoga MH, Dal-Secco D, Freitas A, et al. A crucial role for TNF-alpha in mediating neutrophil influx induced by endogenously generated or exogenous chemokines, KC/CXCL1 and LIX/CXCL5. Br J Pharmacol. 2009;158(3):779–89. DOI: http://10.1111/j.1476-5381.2009.00367.x.
29. Xue D, Moon B, Liao J, Guo J, Zou Z, Han Y, et al. A tumor-specific pro-IL-12 activates preexisting cytotoxic T cells to control established tumors. Sci Immunol. 2022;7(67): eabi6899. DOI: http://10.1126/sciimmunol.abi6899.
30. Montauti E, Oh DY, Fong L. CD4(+) T cells in antitumor immunity. Trends Cancer. 2024;10(10):969–85. DOI: http://10.1016/j.trecan.2024.07.009.
31. Verma R, Foster RE, Horgan K, Mounsey K, Nixon H, Smalle N, et al. Lymphocyte depletion and repopulation after chemotherapy for primary breast cancer. Breast Cancer Res. 2016;18(1):10. DOI: http://10.1186/s13058-015-0669-x.

32. Tau G, Rothman P. Biologic functions of the IFN-gamma receptors. Allergy. 1999;54(12):1233–51. DOI: http://10.1034/j.1398-9995.1999.00099.x.
33. Li X, Zheng Y. Biotransformation of lignin: Mechanisms, applications and future work. Biotechnol Prog. 2020;36(1):e2922. DOI: http://10.1002/btpr.2922.
34. Liu G, Zhang J, Kan Q, Song M, Hou T, An S, et al. Extraction, Structural Characterization, and Immunomodulatory Activity of a High Molecular Weight Polysaccharide from Ganoderma lucidum. Front Nutr. 2022; 9:846080. DOI: http://10.3389/fnut.2022.846080.
35. Ye T, Ge Y, Jiang X, Song H, Peng C, Liu B. A review of anti-tumour effects of Ganoderma lucidum in gastrointestinal cancer. Chin Med. 2023;18(1):107. DOI: http://10.1186/s13020-023-00811-y.
36. Chen Y, Liu C, Yang F, Chen H, Yang C, Fan Z, et al. UPLC–QQQ–MS/MS-based widely targeted metabolomic analysis, antioxidant and α-glucosidase inhibitory activities of mulberry leaves processed by solid-state fermentation. LWT. 2023;188(115351). DOI: https://doi.org/10.1016/j.lwt.2023.115351
37. Li J, Li G, Zhang H, Yang T, Abbas Z, Jiang X, et al. The Fermentation Quality, Antioxidant Activity, and Bacterial Community of Mulberry Leaf Silage with Pediococcus, Bacillus, and Wheat Bran. Fermentation. 2024;10(4). DOI: https://doi.org/10.3390/fermentation10040214.
38. Goodman M. Managing the side effects of chemotherapy. Semin Oncol Nurs. 1989;5(2 Suppl 1):29–52. DOI: http://10.1016/0749-2081(89)90080-6.
39. Mucenski JW, Shogan JE. Maximizing the outcomes in cancer patients receiving chemotherapy through optimal use of colony-stimulating factor. J Manag Care Pharm. 2003;9(2 Suppl):10–4. DOI: http://10.18553/jmcp.2003.9.s2.10.
40. Turhan AB, Binay C, Bor O, Simsek E. The effects of short-term use of granulocyte colony-stimulating factor on bone metabolism in child cancer patients. North Clin Istanb. 2018;5(4):277–81. DOI: http://10.14744/nci.2017.59320.
41. Frey N, Porter D. Cytokine Release Syndrome with Chimeric Antigen Receptor T Cell Therapy. Biol Blood Marrow Transplant. 2019;25(4):e123–e7. DOI: http://10.1016/j.bbmt.2018.12.756.