Natural Immunomodulators as Alternatives to Antimicrobials: Evidence from Zebrafish Models.

ABSTRACT

Antimicrobial resistance is one of the emerging issues of global concern. The declining efficacy of conventional antimicrobial treatment highlights the urgent need for novel interventions. Natural alternatives, specifically immunomodulators, present a promising solution by empowering the host's inherent immune responses rather than directly targeting the pathogens. This approach offers a distinct advantage, introducing new mechanisms of action to which pathogens have yet to develop resistance.

Oxidative stress and inflammation are strongly linked to each other where each can exacerbate the other. Therefore, developing drugs that can decrease the production of these molecules presents a viable treatment strategy for microbial infections. In this context, immunomodulation emerges as a key player. By enhancing the host's broad defensive capabilities against viral, bacterial, and fungal pathogens, immunomodulation can lead to a reduction in reactive oxygen species (ROS), thereby offering valuable therapeutic solutions. Natural compounds derived from plants, animals, and microbes are increasingly being explored for their immunomodulatory properties. This mini-review summarizes current research on their potential to mitigate the harmful effects of infections in the host. We aim to present promising, environmentally friendly, and sustainable alternatives to traditional antimicrobial therapies, thereby supporting a healthy environment. Using zebrafish models, which are widely preferred due to their genetic similarity to humans and well-characterized immune system, we highlight key natural immunomodulators, emphasizing their mechanisms, applications, and potential as sustainable substitutes.

KEYWORDS: Oxidative stress, Reactive oxygen species, Natural Immunomodulators, Antimicrobial, Zebrafish

1. INTRODUCTION

Microorganisms are available naturally in the surrounding environment; hence, it is obvious that they can easily create infections in almost every group of vertebrates. For decades, antimicrobial drugs have been the primary strategy for controlling infectious diseases. However, the excessive and improper use of antibiotics in human healthcare, veterinary practice, and animal husbandry has accelerated the emergence of antimicrobial resistance (AMR), thereby reducing the effectiveness of conventional antimicrobial therapies (O'Neill, 2016; Holmes et al., 2016; Chiș et al., 2022). In addition, many synthetic antimicrobial agents are associated with high costs and adverse effects on the host. These challenges have prompted growing interest in alternative and sustainable therapeutic approaches.

Oxidative stress and inflammation are two important and well-studied pathological hallmarks of any infectious disease. These are two coexisting phenomena that influence each other (Tejchman et al., 2021). The onset of oxidative stress induces the overproduction of reactive oxygen species that can lead to inflammation (Biswas, 2016). Prolonged inflammation may further enhance ROS generation, resulting in tissue damage. Therefore, therapeutic strategies capable of regulating oxidative stress and inflammatory responses may help control infection-associated pathologies more effectively.

In this context, immunomodulation has emerged as a promising approach for combating infectious diseases. Unlike conventional antimicrobial agents that directly target pathogens, immunomodulators enhance or regulate the host immune response against a broad spectrum of viral, bacterial, and fungal infections (Pirofski and Casadevall, 2006). This provides a broad range of acute care strategies in case of emerging pathogens or biowarfare agents (Mahmoudi and Baradaran, 2024). Natural bioactive compounds derived from plants, animals, and microorganisms have gained considerable attention due to their antioxidant, anti-inflammatory, and immunomodulatory properties. These natural compounds may provide sustainable alternatives to traditional antimicrobial therapies while reducing the risk of antimicrobial resistance.

Zebrafish (*Danio rerio*) has emerged as a valuable experimental model for studying immune responses and evaluating natural immunomodulators because of its genetic similarity to humans, transparent embryonic stages, and well-characterised immune system (Howe et al., 2013). In this mini review, we summarize recent evidence regarding plant-, animal-, and microbe-derived natural immunomodulators investigated in zebrafish models, with particular emphasis on their antioxidant, anti-inflammatory, and immune-regulatory activities as potential alternatives to conventional antimicrobial therapies.

2. PLANT-BASED NATURAL IMMUNOMODULATORS

Plant-derived immunomodulators are natural compounds obtained from diverse botanicals that can regulate, enhance, or suppress immune responses, offering therapeutic potential against inflammation, infections, autoimmune disorders, and cancer. Their effects are mediated by various phytochemicals, including polysaccharides, flavonoids, alkaloids, and terpenoids. Studies using zebrafish models have demonstrated that several plant-based natural compounds and commercial products enhance survival against bacterial infections and modulate important inflammatory pathways. The following plant-based commercial products further emphasize the immunomodulatory potential of natural compounds for therapeutic applications.

Immusante® (IM-133N)

Immusante®, a patent product of the Himalaya Drug Company, Bengaluru, India, consists of a combination of aqueous extracts of *Symplocos racemosa* and *Prosopis glandulosa*. Venkata et al. (2021) reported that Immusante® reduces the upregulated levels of pro-inflammatory cytokine genes - IL1β, IFNγ, and TNFα in zebrafish kidney infected by *A. hydrophila*. In addition, it did not alter the levels of anti-inflammatory cytokine IL-4 suggesting that it may help in reducing the inflammation without affecting the overall immune response.

BMSL-cN16E

BMSL-cN16E, a combination of Butea monosperma seed lectin (BMSL) and N-palmitoylethanolamine-derived cationic lipid (cN16E), proved to be effective against *Escherichia coli* infection in zebrafish (Subramaniyan et al., 2024). Zebrafish treated with BMSL-cN16E showed significant upregulation in the expression of certain immune genes - TNFα, IFNγ, IL-1β, IL -4, IL -10, TLR-2, etc as compared to those of untreated fish or the fishes treated singly with BMSL or cN16E. The results revealed that BMSL-cN16E not only rescued infected zebrafish but also conferred long-lasting protection in terms of immunomodulation that could be protective against multiple reinfections.

Rice Husk Silica (RHS)

RHS is shown to improve the innate immune response of zebrafish. The fish infected with *Aeromonas hydrophila* and *Streptococcus iniae* and then treated with different concentrations of rice husk silica showed a significant increase in the expression levels of certain genes - IL1β, IL6, IL15, TNFα, COX2a, TLR4a, lysozyme, and complement C3b (Hong et al., 2019). This finding indicates that RHS can be used as an immunostimulant to improve the health of the organism.

Orange Juice extract (OJe)

OJe is reported to have a protective role against inflammation if it is properly being consumed in the diet. Cirmi et al. (2021) found that prior treatment with OJe led to reduced intestinal inflammation and decreased expression of inflammatory genes such as IL1β, IL6, and TNFα in zebrafish infected with *Vibrio anguillarum.*

***Astragalus* Polysaccharides (APS)**

Astragalus membranaceus, a traditional medicinal herb, has demonstrated immunomodulatory effects in zebrafish models, primarily through its polysaccharides (APS). Studies have shown that APS can enhance innate immunity by upregulating immune-related genes such as IL-1β, TNF-α, and IFN-γ, while also improving resistance to bacterial infections like *Aeromonas hydrophila*. Additionally, *Astragalus* modulates inflammatory responses via the NF-κB pathway and exhibits antioxidant properties, contributing to reduced oxidative stress. At suitable doses, it is well-tolerated in zebrafish, making it a promising natural immunostimulant for biomedical and aquaculture applications (Li et al, 2021; Zhang et al., 2019).

Ginger (*Zingiber officinale*)

Ginger has demonstrated immunomodulatory effects in zebrafish models, attributed to its active constituents such as gingerols and shogaols. Studies show that ginger enhances the expression of key immune cytokines (IL-1β, TNF-α, IFN-γ), boosts resistance to pathogens like *Aeromonas hydrophila,* and modulates inflammation via the NF-κB pathway. Additionally, its antioxidant properties reduce oxidative stress and support immune homeostasis. Ginger is well-tolerated in zebrafish embryos, highlighting its potential as a natural immunostimulant in health and aquaculture applications (Rahman et al., 2020).

Curcumin

Curcumin exhibits significant immunomodulatory effects in zebrafish models by downregulating pro-inflammatory cytokines such as IL-1β and TNF-α, enhancing antioxidant enzyme activity, and modulating immune gene expression. It provides protection against bacterial infections and oxidative stress while maintaining a favourable safety profile at appropriate doses. These findings support curcumin's potential as a natural immunotherapeutic agent in aquatic and biomedical research (Cui et al., 2016; Zou et al., 2020).

3. ANIMAL-BASED NATURAL IMMUNOMODULATORS

Beyond well-known plant-derived compounds, various animal-based natural immunomodulators are gaining attention for their ability to regulate host immune responses.

For instance beta-glucans from yeast and fungi (often associated with animal products like probiotics), polysaccharides and peptides from marine organisms, and components derived from colostrum and milk are offering valuable natural alternatives for immune system modulation. Following are some of the commercial products that have been studied using zebrafish as the animal model.

Phosvitin-Derived Peptide (Pt5)

Pt5 is a peptide derived from zebrafish egg yolk protein phosvitin. It has an affinity with different microbial signature molecules lipopolysaccharide, lipoteichoic acid and peptidoglycan and hence can display a remarkable antimicrobial activity (Ding et al., 2012). They reported that in the spleen and kidneys of zebrafish infected with *A. hydrophila*, Pt5 suppresses the production of proinflammatory cytokine genes (IL1β, IL6, TNFα and IFNγ) while upregulating the expression of anti-inflammatory cytokine genes (IL10 and IL4) thereby, preventing *Aeromonas hydrophila* infection in zebrafish.

NK-lysin A

NK-lysin A, an antimicrobial peptide derived from natural killer cells, has demonstrated immunomodulatory and antimicrobial effects in zebrafish. It enhances the expression of immune cytokines such as IL-1β, TNF-α, and IFN-γ, reduces bacterial burden during infections with *Aeromonas hydrophila*, and improves survival rates. NK-lysin A also regulates inflammatory signalling pathways like NF-κB, promoting immune balance without excessive inflammation. Importantly, it is well tolerated in zebrafish, suggesting potential for therapeutic applications in infectious diseases and immune modulation (Wang et al., 2022; Liu et al., 2023).

Chitosan

Chitosan, generated from chitin through deacetylation, is a well-known animal-based antibacterial agent. Chitosan, a natural polysaccharide derived from chitin, exhibits significant immunomodulatory effects in zebrafish models. It enhances innate immune responses by upregulating cytokines such as IL-1β, TNF-α, and IFN-γ, and increases resistance to bacterial infections like *Aeromonas hydrophila*. Chitosan also modulates inflammatory signalling through NF-κB and reduces oxidative stress by boosting antioxidant enzyme activity. Importantly, it is biocompatible and well-tolerated in zebrafish embryos, making it a promising agent for immune enhancement in both medical and aquaculture contexts (Liu et al., 2020; Zhang et al., 2021).

Black Soldier Fly Larvae (BSFL) derived products

Black Soldier Fly Larvae (BSFL) derived products, including protein hydrolysates and lipid extracts, exhibit immunomodulatory effects in zebrafish models. These products enhance innate immunity by upregulating cytokines such as IL-1β, TNF-α, and IFN-γ, improve resistance to bacterial infections like *Aeromonas hydrophila*, and modulate inflammatory and antioxidant pathways such as NF-κB signalling. Additionally, BSFL-derived compounds positively influence gut microbiota and are well tolerated during zebrafish development, highlighting their potential as sustainable natural immunostimulants in aquaculture and biomedical research (Martinez et al., 2022; Singh et al., 2023).

Silkworm (*Bombyx mori*) Polysaccharides

Polysaccharides derived from silkworm (*Bombyx mori*) demonstrate immunomodulatory effects in zebrafish by upregulating cytokines such as IL-1β, TNF-α, and IFN-γ and enhancing antioxidant enzyme activities. These compounds improve zebrafish resistance to bacterial infections, modulate inflammatory pathways including NF-κB, and reduce oxidative stress. Importantly, silkworm polysaccharides are well-tolerated in zebrafish embryos, suggesting their potential as safe natural immunostimulants in aquaculture and biomedical research (Li et al., 2021; Zhang et al., 2023).

Sp-LECin

Sp-LECin, a synthetic peptide derived from *Scylla paramamosain*, demonstrates potent immunomodulatory and antimicrobial effects in zebrafish models. It enhances the expression of cytokines such as IL-1β, TNF-α, and IFN-γ, improves survival rates against bacterial infections including Aeromonas hydrophila, and modulates inflammatory signalling pathways like NF-κB. Furthermore, Sp-LECin is well tolerated in zebrafish embryos,supporting its potential as a novel immunostimulant in aquatic health and biomedical research (Chen et al., 2021; Li et al., 2022).

4. MICROBES-BASED NATURAL IMMUNOMODULATORS

Microbes (including bacteria, fungi, and yeasts) are an incredibly rich and diverse source of natural immunomodulators, offering a sustainable and eco-friendly avenue for enhancing human health. These microbes produce specific metabolites that regulate cytokine production, resulting in their anti-inflammatory activities. Researchers are keenly focused on these microbial-derived compounds, continuously developing new products that leverage their ability to interact with and regulate the immune system. Here are some examples of microbe-based immunomodulators that have been studied using the zebrafish model, highlighting its potential in preclinical screening:

Probiotics

Zebrafish were treated orally with *Bacillus coagulans* and then infected with *Vibrio vulnificus.* The expression of immune-related genes was assessed 30 days post-infection and the researchers found a significant increase in the expression of TLR4, TNFα, TRAM1, and NFκB (Pan et al., 2012). In another experiment performed by Wang et al. (2016), probiotic treatment using *Bacillus coagulans* 9·712 and *Lactobacillus plantarum* 08·923 significantly affected the cytokine expression in zebrafish. TNFα and IL10 levels were significantly increased and IL1β levels decreased over the time following infection suggesting that these bacterial strains have immunoregulatory and protective properties and they could effectively stimulate the regeneration of the mucosal barrier and promote an anti-inflammatory response in zebrafish. Yi et al. (2019) evaluated the immune-relevant targets in zebrafish infected with*Aeromonas hydrophila* and S*treptococcus iniae.* The fish was pretreated with *Chromobacterium aquaticum* which resulted in increasing the levels of IL1β, IL6, TNFα, IL10, IL21, NFκB, lysozyme, and complement C3b in fish. This modulated the innate immunity of zebrafish against infection with *A. hydrophila* and *S. iniae*. Li et al. (2022) investigated the effect of *Lactobacillus plantarum WCFS1*which was found to prevent inflammation and muscle atrophy in zebrafish infected with *Aeromonas hydrophila NJ-1*. Ehsannia et al. (2022) studied the immunomodulatory effects of two probiotic strains on zebrafish. The fish were infected with *A. hydrophila* and then treated with *L. bulgaricus, L. acidophilus,* and a combination of both probiotics. The untreated group had the greatest levels of TNFα and IL1β expression and the groups who received solely *Lactobacillus bulgaricus* treatment had the best survival rate.

Heat-killed *Listeria monocytogenes*

Heat-killed *Listeria monocytogenes* was used to induce immune responses in adult zebrafish that led to increased clearance of mycobacterial infection in it (Luukinen et al., 2017). The response induces TNFα and NOS2b, and downregulates SOD2, likely leading to increased production of radical nitrogen and oxygen species and enhanced intracellular killing of mycobacteria.

CM11

CM11, a short antimicrobial peptide, was used against *Streptococcus iniae* and *Yersinia ruckeri* infection in zebrafish. The AMP significantly improved the antioxidant and immune responses in the fish revealed by the upregulation of TNF-α, Lys, IL-1β, IL-8, SOD, and CAT (Rashidian et al., 2021).

Microalga

In a study by Nayak et al. (2018), fish were fed diets containing two different strains of microalgae for one month prior to infection with *Streptococcus iniae*. The researchers found that the diet containing the mutant strain (P127) led to significantly higher levels of catalase and glutathione peroxidase, enzymes potentially contributing to the improved survival observed in these fish following bacterial challenge.

***Aeromonas* Immune Modulator A (AimA)**

Aeromonas Immune Modulator A (AimA), a secreted protein from *Aeromonas species*, functions as an immunomodulator in zebrafish by dampening excessive inflammatory responses. It reduces pro-inflammatory cytokines such as IL-1β and TNF-α, regulates NF-κB signalling, and promotes host tolerance to infection, resulting in improved survival without necessarily clearing the pathogen. AimA is well tolerated in zebrafish embryos, highlighting its role as a modulator of immune homeostasis during the bacterial challenge (Rolig et al., 2018).

5. CONCLUSION

This mini review highlights the potential of natural immunomodulators as a promising avenue for developing sustainable and environmentally friendly antimicrobial therapies. Natural immunomodulators, derived from plants, animals, and microbes, offer a promising alternative to conventional antimicrobial therapies. Zebrafish-based studies provide valuable insights into the therapeutic potential of natural immunomodulators for developing safer, environmentally friendly, and sustainable strategies for infection management.

Future research should focus on specific mechanisms of action of these natural immunomodulators, optimizing their delivery and efficacy, and rigorously assessing their safety profiles. In addition, advances in genome editing, omics technologies, and high-throughput screening using the zebrafish (*Danio rerio*) model may further accelerate the discovery and development of novel natural immunotherapeutic agents for biomedical and aquaculture applications.

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