CHAPTER

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VALIDITY OF ZEBRAFISH AND MICE MODELS TO STUDY HYPERLIPIDEMIA AND ITS MITIGATION BY SEAWEED-DERIVED BIOACTIVE COMPOUNDS

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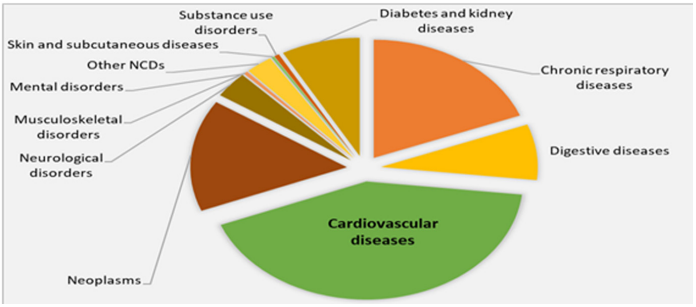
ABSTRACT

Cardiovascular diseases pose a significant global health challenge, claiming over 20 million lives annually. In India, cardiovascular diseases contribute to two-thirds of all recorded deaths, exhibiting a sharp increase over the years. One major risk factor for cardiovascular diseases is hyperlipidemia, characterized by elevated blood lipid levels. Seaweed-derived compounds, rich in polyphenols, sulfated polysaccharides, and other bioactive molecules, have demonstrated antihyperlipidemic effects through various mechanisms. This chapter explores the potential of seaweed-derived bioactive compounds in combating hyperlipidemia, focusing on their mechanisms of action. The use of animal models, especially mice and zebra fish in studying the bioactivity of seaweed-derived compounds is also discussed in detail. We provide an overview of the strengths and limitations of both models in studying the efficacy of seaweed-derived compounds in combating hyperlipidemia. The combined knowledge gained from studying mice and zebra fish models can help in the use of high-value compounds from seaweeds for the health and well-being of humans in the future.

Keywords: Animal models, Dyslipidemia, Hypercholesterolemia, Lipid profile, Macroalgae

CARDIOVASCULAR DISEASES AND THEIR PREVALENCE IN INDIA

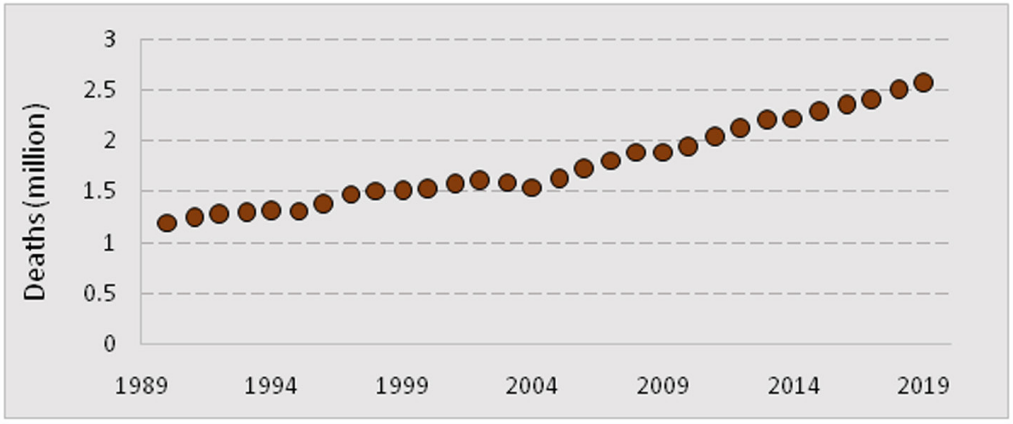
Cardiovascular diseases (CVDs) cause the death of more than 20 million people every year (Cesare et al. 2023), making it a global health issue. This staggering figure surpasses the combined mortality attributed to cancer, diabetes, and chronic respiratory diseases (Figure 1).



Pie chart of all major non-communicable disease-related deaths in 2019. Data were obtained from Global Burden of Disease Study 2019 Results (2020).

CVD is a unified term indicating numerous conditions affecting the heart and vascular system (Thiriet, 2018). There are several forms of CVDs, but the most lethal forms of the disease are ischemic heart disease and intracerebral hemorrhage together constituting approximately 85% of the overall global mortality attributable to CVDs (GBD, 2020). In recent decades, there has been a steep increase in the absolute number of deaths by CVDs from 12.1 million in 1990 to 20.5 million in 2021, representing about one-third of all recorded global deaths (Cesare et al. 2023). This escalation in CVD-related mortalities is attributed to the rise in the global population and an increased proportion of elderly individuals across most nations (Roth et al., 2015).

In India, CVDs contribute to about 2/3rd of all recorded deaths (GBD, 2020) every year and likethe global trends, there has been a sharp year-on-year increase in CVD-related mortalities.



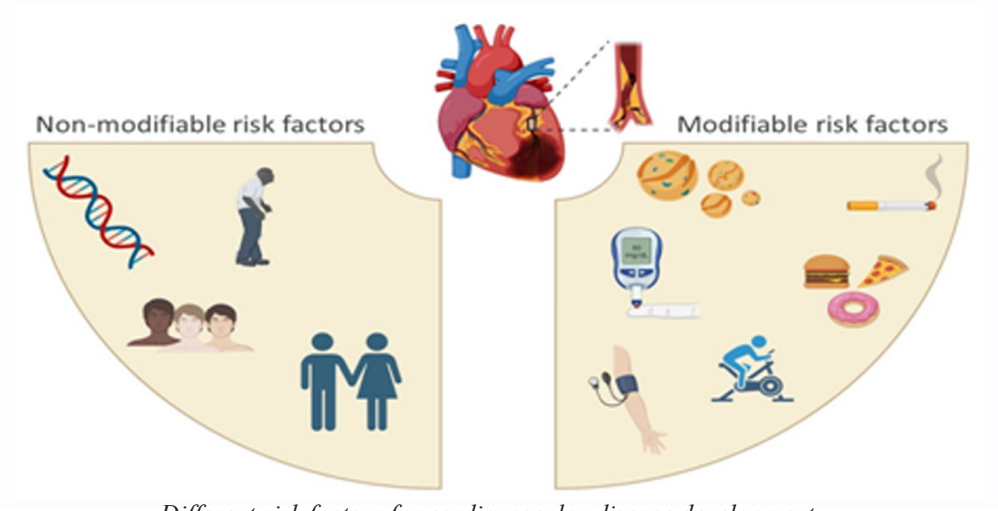
Year-on-year trend in the mortality caused by CVDs in India between 1990 and 2019.

HYPERLIPIDEMIA AS A RISK FACTOR FOR CARDIOVASCULAR DISEASES

In 1990, about 1.2 million people lost their lives due to CVDs, but this number has increased to more than 2.5 million in 2019 (GBD, 2020). Consequently, owing to the impact of CVDs on a global scale, concerted public health and research efforts are underway to formulate efficacious management strategies that can reduce the disease burden. A pivotal aspect in alleviating the burden of CVDs worldwide is the identification of risk factors that precipitate CVD development.

A risk factor constitutes a variable that can influence the likelihood of contracting a disease. Various risk factors associated with CVDs have been delineated, exhibiting diverse. In

contrast, classifications. Vaduganathan et al. (2022) organized CVD risk factors into distinct determinants, including metabolic conditions (e.g., elevated systolic blood pressure, kidney dysfunction, high body mass index), behavioral patterns (e.g., consumption of unhealthy/saturated lipid-rich diets, smoking, alcoholism), and environmental factors (such as exposure to ambient particulate matter and low temperatures). A recent classification proposed by Alves-Silva et al. (2021) distinguishes between non-modifiable and modifiable risk factors of CVDs. Modified risk factors comprise lifestyle choices such as the consumption of unhealthy/saturated lipid-rich diets, tobacco use, physical inactivity, and the presence of other metabolic disorders like high blood pressure, diabetes, and hyperlipidemia. Of note, hyperlipidemia emerges as a pivotal modifiable risk factor implicated in the development of CVDs.



Different risk factors for cardiovascular disease development. modified from Alves- Silva et al. (2021).

Hyperlipidemia is an umbrella term that represents a spectrum of conditions resulting in elevated blood lipid levels. Lipids exist in extremely complex forms in the blood, but the major proportionis found as lipoproteins in which lipids are attached to specific transport proteins called apolipoproteins (Jovandaric & Milenkovic, 2020). Depending upon the size, density, and tissue of origin, the lipoproteins are classified into chylomicrons, very low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs). In general, LDLs can promote CVDs whereas HDLs can prevent the disease (Sun et al. 2022).

Another aspect of hyperlipidemia is an increase in levels of total cholesterol and total triglycerides in the blood. Total cholesterol is the sum of free and lipoprotein-bound

cholesterol in the blood—often the primary indicator of hyperlipidemia. Additionally, the lipid composition of lipoproteins, particularly VLDL and LDL, prominently includes triacylglycerols, correlating elevated triglyceride levels with hyperlipidemia. Therefore, hyperlipidemia is a nuanced and complex condition characterized by altered levels of different speciesof lipoproteins (LDLs and HDLs) and molecular classes (cholesterol, triacylglycerols) of lipids in the blood (Table 1).

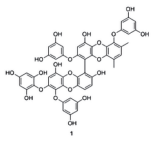
Table 1: Normal and hyperlipidemic blood lipid profile of humans (Lee and Siddiqui, 2019).

	TC	TG	LDL-C	HDL-C	LDL-C/HDL-C
Normal	<190 (optimal 150)	<150	70-100	40 to 60	1.6-2.5
Hyperlipidemia	>190	>150	>100	>60	>3

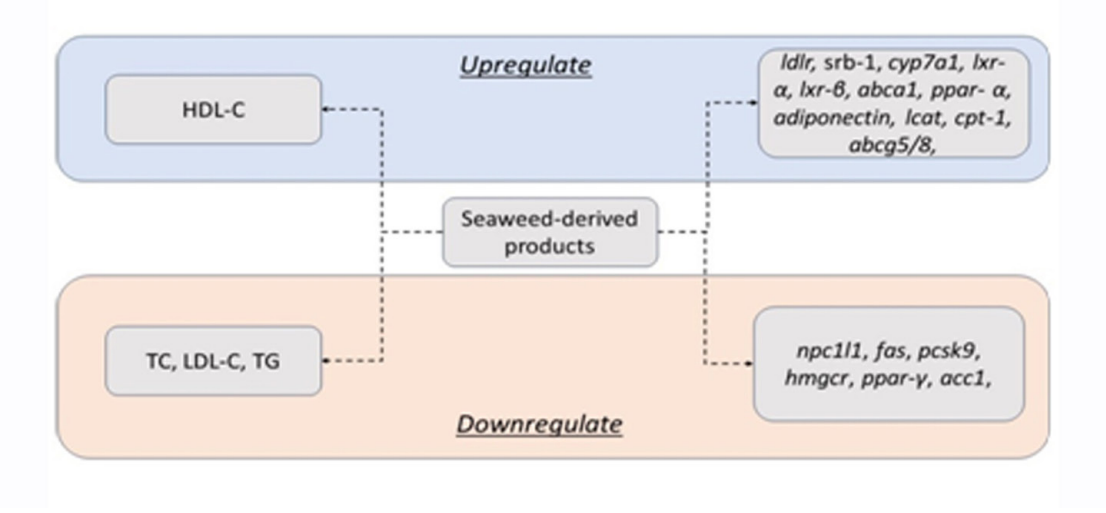
Management of hyperlipidemia typically involves lifestyle modifications such as adopting a healthy diet, regular physical activity, and, when deemed necessary, pharmacological interventions. There are many drugs to prevent and treat hyperlipidemia. The primary emphasis of drug-based treatments is to lower the circulating LDL cholesterol and triacylglycerol content. These drugs have differentmodes of action which include, for instance, suppression of endogenous cholesterol biosynthesis in the liver or restriction of absorption of dietary cholesterol or triglycerides in the intestine (Arya et al. 2014). Though these drugs are efficient in mitigating hyperlipidemia, they do not come without side effects such as steatorrhea, vitamin deficiency (Tak and Lee, 2021), myalgia, hepatotoxicity (Zhou et al., 2017), elevated hepatic transaminase activity, livertoxicity (Florentin et al., 2008), injection-site erythema and muscle pain (Stoekenbroeket al., 2018). These mild to severe side effects of the different antihyperlipidemic drugs have necessitated the search for alternative approaches to help alleviate hyperlipidemia.

SEAWEED-DERIVED BIOACTIVE COMPOUND AGAINST HYPERLIPIDEMIA

The advancement of drug development is closely tied to the discovery of novel bioactive compounds, with marine organisms standing out as particularly valuable reservoirs of these bioactive substances (Hamed et al. 2015). Several epidemiological studies have indicated a lower occurrence of obesity-related diseases among individuals who consume marine products, suggesting that these foods may possess specific health benefits. Within the array of marine organisms, seaweeds emerge as crucial reservoirs of bioactive compounds boasting



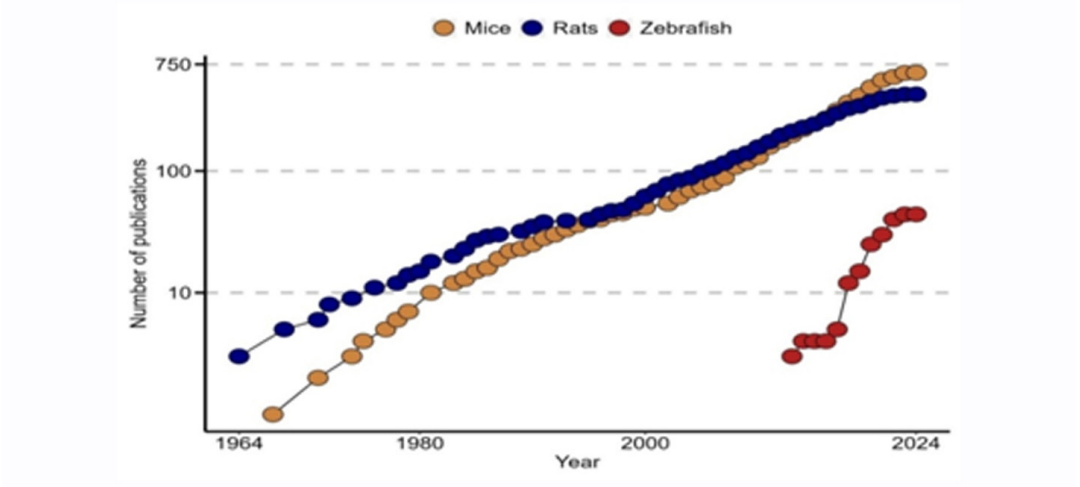
diverse structures and bioactivities. The main mechanisms of action involve the lowering of total cholesterol, LDL cholesterol, and triglyceride levels in the circulation (Yokota et al. 2016). Mitigation of hyperlipidemia is also facilitated by seaweed products through the increase in circulating levels of HDL cholesterol (Kim et al. 2008) which is crucial for reverse-cholesterol transport (i.e., transport of excess cholesterol from peripheral tissues to the liver to be excreted through bile). Several seaweed-derived compounds also impart their effects on the signaling pathways that regulate the development of hyperlipidemia. For instance, it has been well recognized that the consumption of seaweed products stimulates the β-oxidation of lipids through the upregulated expression of carnitine palmitoyl transferase 1Aa(cpt1a) (Gómez-Zorita et al. 2020). Seaweeds are rich in various nutrients and health-promoting compounds such as polyphenols, alginates, fucose-containing sulfated polysaccharides, laminarins, terpenoids, carotenoids and sterols (Cardoso et al. 2015) which can reduce the risk of hyperlipidemia. On the other hand, seaweed-derived compounds can downregulate the expression of genes that are connected to lipid biosynthesis, cholesterol absorption and endogenous biosynthesis of cholesterol (Zhao et al. 2020). These investigations suggest that the inclusion of seaweed in the diet can favorably influence lipid metabolism. However, it is crucial to elucidate these effects at the molecular level to understand their impact on specific signaling pathways. To clarify the precise effects of dietary seaweed products on the lipid metabolism of vertebrates, several animal models have been used. There are four general mechanisms of action by which seaweed-derived compounds can impart antihyperlipidemic effects in vertebrates.



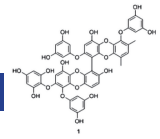
Mechanisms of hypolipidemic effects of seaweed-derived products.

DEVELOPING SEAWEED-BASED THERAPEUTICS FOR HYPERLIPIDEMIA USING ANIMAL MODELS

Animal models play a crucial role in studying lipid metabolism and hyperlipidemia, providing valuable insights into the underlying mechanisms of seaweed-based therapeutic strategies. Since the latter part of the 20th century, there have been notable advancements in hyperlipidemia research and associated metabolic disorders through the utilization of animal models. These models have allowed researchers to delve into the mechanisms of hyperlipidemia and evaluate the effectiveness of various therapeutic approaches in alleviating the condition. Animal models for researching diseases are typically classified into spontaneous and induced categories. Spontaneous models of hyperlipidemia usually bear a natural genetic aberration that causes a natural hyperlipidemic condition. Watanabe heritable hyperlipidemic rabbit is an example of a spontaneous model of hyperlipidemia that bears a defect on the low-density lipoprotein receptor. As a result, the animal sexhibit unprovoked hyperlipidemia, with increased levels of LDL-cholesterol and extended atherosclerotic lesions (Fan and Watanabe, 2000). On the other hand, induced models of hyperlipidemia are more predominantly used by researchers. In the induced models, modifications to the blood lipid profile of the animal are induced through genetic manipulation, or dietary interventions. Rodent models, mice, and rats have been in use for many decades for hyperlipidemia research whereas zebrafish is an emerging model because of the unique features like ex-vivo fertilization, optical transparency during embryonic and larval stages and small size.



Increase in the number of publications on seaweed.



VALIDITY OF MICE MODELS TO INVESTIGATE ANTIHYPERLIPIDEMIC SEAWEED-DERIVED COMPOUNDS

Like hyperlipidemia research, a great proportion of our understanding of the bioactive potential of seaweed-derived compounds comes from animal models. Rodents are the most comprehensively used animal species for seaweed research. Mice are widely used as models in not only seaweed research but also in other fields of biomedical sciences for several reasons. The most important feature of the mice models is that they share a high level of phylogenetic similarity with humans which makes it possible to translate the results obtained in the models to humans (Breschi et al. 2017). Mice share similarities in organ systems, immune responses, and basic physiology with human. Mice also have short reproductive cycles and can produce multiple generations of offspring in a few months. Their small size makes them easy and cost-effective to maintain. Since the previous decades, researchers have developed dedicated genetic modelsof mice for hyperlipidemia (Andreadou et al. 2020). This feature of the mice models further enables the researchers to capture the key aspects of human disease for more specific investigations. Mice models have revealed several mechanisms of action of different seaweed-derived compounds against hyperlipidemia. These seaweed-derived compounds include fucoidan, alginates, ulvan, carrageenan, and fucoxanthin.

Studies on hyperlipidemic mice indicated that fucoidan derived from different seaweed species impart different effects on lipid metabolism. Fucoidan derived from *Ascophyllum nodosum* reduced the total cholesterol and triglyceride levels in hyperlipidemic mice likely through enhancing the expression of genes and proteins reverse-cholesterol transport (Yang et al. 2019). On the other hand, when fucoidan from *Sargassum wightii* is fed to hyperlipidemic mice, it leads to a reduction of total cholesterol by inhibiting the endogenous biosynthesis of cholesterol and improving HDL metabolism (Chen et al. 2017). Another study indicated that dietary fucoidan from Fucus vesiculosis can reduce the total cholesterol and triglyceride levels in circulation by regulating the expression of key enzymes related to cholesterol and triglyceride synthesis (Park et al. 2016).

Alginates are one of the most abundant carbohydrates in brown algae and have been widely used in the food and pharmaceutical industries. In nature, alginate is a linear hetero-polychronic acid polymer, composed of β-d-mannuronate (M) and its C5 epimer α-l-guluronate (G). Alginate imparts an antihyperlipidemic effect by reducing the absorption of lipids from the digestive tract. The restricted absorption of lipids in the gut leads to a reduction in circulating triacylglycerol, total cholesterol, and serum alanine aminotransferase and aspartate aminotransferase levels in high-fat diet mice. It also activates the (AMP-activated protein kinase) AMPK signaling pathway in the adipose tissue by reversing the high-fat diet-induced

reduction in the protein expression levels of phospho-acetyl-CoA Carboxylase (p-ACC) and p-AMPK in the adipose tissues (Li et al. 2019). It has also been reported that dietary alginates can reduce LDL cholesterol by about 83% in high-fat diet-fed mice (Back et al. 2014).

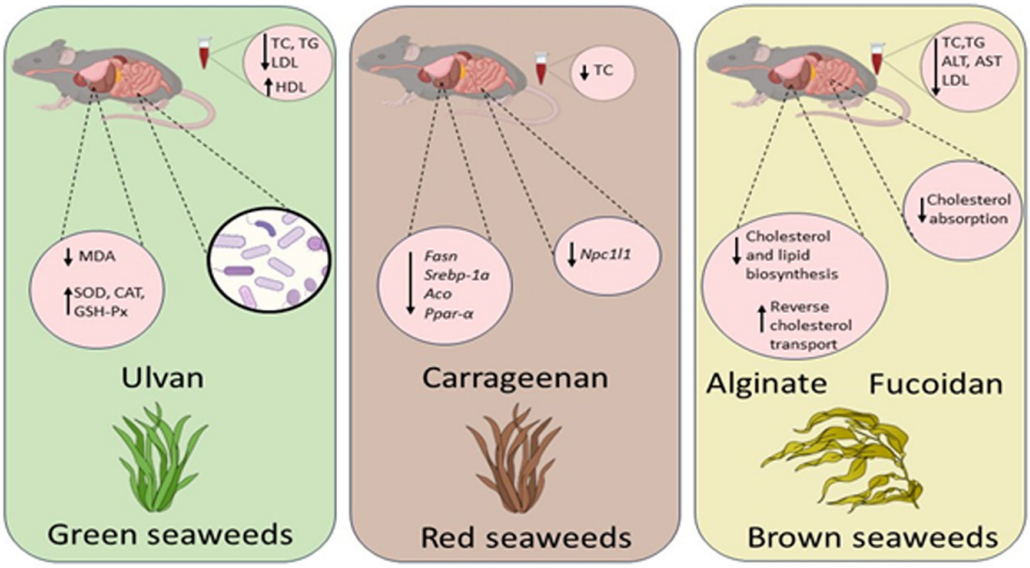
Ulvans obtained from green seaweed like *Ulva pertus* aand belongs to a group of sulfated heteropolysaccharides with [β-D-GlcpA-(1 → 4)-α-L-Rhap3s] and [α-L-IdopA-(1 → 4)-α-L-Rhap3s] as main disaccharide units (Pengzhanel al. 2003). Several studies have reported that ulvans exhibit substantial antihyperlipidemic activities, however, the effect of monosaccharide components and physicochemical properties on the antihyperlipidemic effect remained unknown. Recently, a study used a Kunming mice model to decipher the effect of the physicochemical properties of ulvans on antihyperlipidemic bioactivity (Li et al. 2018). The authors confirmed that the fraction with the highest monosaccharide and sulfate contents had the highest antihyperlipidemic effect in the mice.

Using the same mice model, a study revealed that phosphorylation of *ulva* heteropolysaccharide significantly altered its ability to reduce the total cholesterol and triglyceride levels in hyperlipidemic mice (Jiang et al. 2020). The study also showed that the antioxidant status of the liver was improved when the mice were fed with phosphorylated ulva polysaccharides compared to non-phosphorylated controls.

Carrageenan is a high molecular weight polysaccharide extracted from red seaweeds, composed of D-galactose residues linked in β-1,4 and α-1,3 galactose-galactose bond, widely used as a food additive in processed foods for its properties as a thickener, gelling agent, emulsifier, and stabilizer (Borsaniel al. 2021). In C57BL/6J hyperlipidemic mice the consumption of carrageenan reduces the total cholesterol levels by downregulating the expression of a gene connected to cholesterol absorption in the small intestine Niemann-pick c1-like 1(Npc111) and downregulating the hepatic expression of genes connected to lipid biosynthesis (Chin el al. 2019).

Fucoxanthin is a xanthophyll carotenoid that is found in the chloroplasts of brown seaweeds. To clarify the antihyperlipidemic effects of fucoxanthin, diabetic mutant mice (C57BL/KsJ-db/db) were used in a study and provided with methanol-extracted fucoxanthin from *Laminaria japonica.* The study revealed that dietary fucoxanthin can significantly reduce the total cholesterol and regulate the expression of Insulin receptor substrate 1 (IRS-1)/Phosphoinositide 3-kinases (PI3K)/Protein kinase B (AKT) and AMPK signaling pathway in the liver. The regulation of hepatic IRS-1/PI3K/AKT and AMPK signaling pathways by fucoxanthin indicated that dietary fucoxanthin plays a key role in modulating glucose metabolism, and its activation is essential to the regulation of insulin receptor signaling

transduction (Zhang et al. 2018). The collective findings from diverse seaweed-derived compounds and their effects on hyperlipidemic mice underscore the potential of these mice models to reveal the precise molecular effects of marine resources for therapeutic applications against hyperlipidemia.



Different molecular effects of dietary seaweed-based compounds against hyperlipidemia in mice

Important as mice models are, they suffer from certain inherent limitations that hamper their use for hyperlipidemia research. These limitations include differences in the lipoprotein and bile profile of mice compared to humans (Takahashi el al. 2022) and resistance to developing cardiovascular diseases even after feeding lipid-rich diets for long periods (Zhu et al. 2022). These issues necessitate the development of other vertebrate models to understand the efficacy of seaweed-derived compounds against hyperlipidemia.

VALIDITY OF ZEBRAFISH MODELS TO EXPLORE BIOACTIVITY OF SEAWEED-DERIVED COMPOUNDS AGAINST HYPERLIPIDEMIA

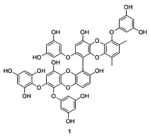
Zebrafish have become a popular model organism in scientific research due to their genetic and physiological similarities to humans, rapid development, and naturally transparent embryos (a feature that is missing in mice models). Zebrafish models have been employed in various biomedical studies, including research on hyperlipidemia (Gora et al. 2022). Unlike

mice, zebrafish can be exposed to high-fat diets to raisethe LDL/HDL ratio in the blood, an important driver of cardiovascular disease development (Gora et al. 2023). By feeding zebrafish with diets rich in cholesterol or saturated fats, researchers can study the effects on lipid metabolism and the development of hyperlipidemia. Transparent zebrafish embryos allow researchers to easily observe and analyze the development of hyperlipidemia-related phenotypes, particularly the initial stages of atherosclerotic development. Owing to these advantages, zebrafish has emerged recently as a model to investigate the efficacy and mode of action of several seaweed-derived compounds against hyperlipidemia.

Recently, a study was conducted to investigate the anti-obesity activity Palmaria mollis, a red seaweed,using zebrafish (Nakayama et al. 2018). Obesity was induced in the fish by overfeeding freshly newly hatched *Artemia nauplii* at aration of approximately 150 cal/day for six weeks. The overfeeding caused an increase in the plasma triglyceride and glucoselevels, upregulated the peroxisome proliferator-activated receptor gamma (pparg) and downregulated the acyl-CoA oxidase 1 (acox1) gene expression. The altered expression of genes likely indicated a tendency towards increased lipid depositionand reduced lipid β-oxidation in the liver, induced by overfeeding *Artemia*. However, feeding *Palmaria mollis* granules for 4 weeks induced a restoration of blood lipid values and hepatic expression of genes that were disrupted by the *Artemia nauplii* overfeeding.

A major advantage over the mice model is the small size of the zebrafish, especially during the larval stages which allows for high throughput screening of many therapeutic strategies/ drugs in a relatively shorter time (Clifton et al. 2010). Combining this feature with the ease of transgenic reporter line development makes zebrafish a unique vertebrate model to discover novel seaweed-derived therapeutic strategies against hyperlipidemia. For instance, Cha et al. (2018) evaluated about 50 different seaweed extracts against insulin resistance using transgenic zebrafish larvae expressing an enhanced green fluorescentprotein under the control of the insulin promoter Tg (ins-egfp). They observed that *Polysiphonia japonica* extract was able to stimulate insulin secretion and protect against palmitate-induced pancreatic β-cell dysfunction in zebrafish.

Even though zebrafish are emerging as preferred models for research of bioactive compounds against hyperlipidemia, these models suffer from certain drawbacks. Zebrafish is a poikilothermic fish; the patterns of vascularization are drastically different from that of humans, and it has been reported that the zebrafish does not present the advanced stages of atherosclerosis which are observed in genetic mice models (Vedder et al. 2020).



CONCLUSION

The rising global prevalence of cardiovascular diseases underscores the need for a deeper understanding of risk factors and innovative therapeutic approaches. Hyperlipidemia, a critical modifiable factor, significantly contributes to cardiovascular disease development. Conventional drug-based treatments, while efficacious, are not devoid of side effects, prompting the exploration of alternative approaches. Herein lies the significance of seaweed-derived bioactive compounds, which have shown promise in mitigating hyperlipidemia through various mechanisms, such as regulation of lipid metabolism and modulation of signaling pathways. To evaluate the bioactive potential of seaweed-derived compounds against hyperlipidemia, animal models offer important insights. Mice and zebrafish models are equipped with unique features that can help provide insights to complement the information generated by mice models. Combining insights from both models presents a robust platform for rapid screening of seaweed-derived molecules and eventual pre-clinical testing of their antihyperlipidemic potential. The synergy between the insights garnered from mice and zebrafish models can propel us toward a future where we can harness the potential of seaweed-derived high-value compounds for human well-being.

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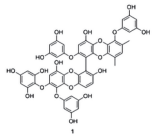

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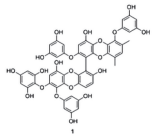

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