

EVALUATING LIPID METABOLISM ALTERATIONS BY FOOD ADDITIVES IN ZEBRAFISH MODEL

Submitted in partial fulfillment of the requirements for the award of

Bachelor of technology degree in

Microbiology

By

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**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **KEERTHIKA.M (40300031)** who carried out the project entitled "**Evaluating lipid metabolism alterations by food additives in zebrafish model**" under my supervision from October 2022 to April 2023

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ABBREVIATION

Hpf	Hour Post – Fertilization
LC-MS	Liquid Chromotography mass Spectrometry
FAO	Food and Agriculture Organization
WHO	World Health Organization
JECFA	Joint FAO/ WHO Expert committee
FAD	Food and drug Administration
BHA	Butylated Hydroxy Anisole
BHT	Butylated hydroxytoluene
FAs	Fatty Acyls
GPLs	Glycerophospholipids
PK	Polyketides
GLs	Glycerolipids
SPS	Sphingolipids
SL	Saccharolipids

ABSTRACT

Background of study: Lipids serve essential in cells as signaling molecules, membrane components, and source of energy. Defects in lipid metabolism are implicated in a number of pandemic human diseases including diabetes, obesity and hypercholesterolemia. Approaches for disease prevention and treatment. Numerous studies have shown that the zebrafish is an excellent model for vertebrate lipid metabolism. In this project we propose to employ zebrafish food additives on lipid metabolism during various stage of zebrafish development.

Methodology: Zebrafish embryos were exposed to various concentrations of tartrazine, Aspartame, Sodium benzoate (5.5ppm, 20ppm, and 200ppm) for 96 hours. The toxicity effects were investigated by analyzing the survival rate, locomotor activity, and biochemical changes of the lipid metabolism.

Result: The results showed that exposure to certain food additives led to significant alterations in lipid metabolism in zebrafish.

Conclusion: In this study, zebrafish were exposed to different concentrations of food additives commonly used in processed foods, such as Aspartame, tartrazine, sodium benzoate

Key words: Zebrafish embryos, Tartrazine, Aspartame, Sodium Benzoate, Lipid extraction, LC-MS analysis

CHAPTER 1

1. INTRODUCTION

Lipid metabolism plays a crucial role in maintaining cellular homeostasis and overall health. However, alterations in lipid metabolism can lead to various metabolic disorders such as obesity, diabetes, and cardiovascular diseases. Food additives are commonly used in the food industry to enhance taste, texture, and appearance of food products. However, some food additives have been shown to affect lipid metabolism in humans and animal models.

1.1 FOOD ADDITIVES

Many substances are incorporated into foods for functional purposes, and in many cases, these ingredients can also be found occurring naturally in some food. However, when they are used in processed foods, these chemicals have beyond me known as “food additives.” From a regulatory standpoint, each of the food additives must provide some useful and acceptable function or attribute to justify its usage. Generally, improved keeping quality, enhanced nutritional value, functional property provision and improvement, processing facilitation, and enhanced consumer acceptance are considered acceptable functions for food additives. One of the technologies driving the so-called Industry 4.0 paradigm shift is an additive manufacturing (AM). Scholars continue to debate the actual and potential effects of this technology on manufacturing, logistics, and operations strategy as well as on perspectives on sustainability and the overall business strategy. 53.8% of food items contained at least one food additive, and 11.3% had at least five. Artificially sweetened beverages, ice cream, processed sandwiches, biscuits, and cakes were the food categories most likely to contain food additives (in more than 85% of food items).

Citric acid, lecithins, and modified starches were the most widely used food additives (>10,000 products each). Some food additives with suspected health effects also pertain to the top 50: sodium nitrite, potassium nitrate, carrageenan, monosodium glutamate, sulfite ammonia caramel, acesulfame K, sucralose, (di/tri/poly) phosphates, mono- and

triglycerides of fatty acids, potassium sorbet, cochineal, potassium metabisulphite, sodium alginate, and bixin (>800 food products each). Six groups of food additives that frequently co-occur in food products were identified by us. Food additives are frequently used in commercials. Substances that are of little or no nutritive value, but are used in the processing or storage of foods or animal feed, especially in developed countries; include antioxidants; food preservatives; food coloring agents; flavoring agents; anti-infective agents; vehicles; excipients and other similarly used substances. Many of the same substances are pharmaceutical aids when added to pharmaceuticals rather than to foods. Food additives are substances added to food to preserve flavor or enhance its taste and appearance. Some additives have been used for centuries; for example, preserving food by pickling with vinegar, salting, as with bacon, preserving sweets, or using sulfur dioxide as in some wines. With the advent of processed foods in the second half of the 20th century, many more additives have been introduced, of both natural and artificial origins. It is sometimes wrongly thought that food additives are a recent development.

The business models, strategies, and operations of companies are changing as a result of this emerging technology, which has enormous advantages for the sustainability of their social, economic, and environmental systems (Niaki and Nonino, 2018b). Food products may contain a variety of additives to achieve desired effects like flavor, preservation, and sweetness. The European Food Safety Authority, the Organization for Economic Cooperation and Development, the International Conferences on Harmonization, and the United States Food and Drug Administration all publish lists of approved food additives along with guidelines for safe use. By using a variety of techniques, the food additives are specifically screened for cytotoxicity, genotoxicity, mutagenicity, and hepatotoxicity. Cytogenetic evaluation is used to conduct food additive safety studies, which include gene mutation assays, long-term carcinogenicity tests, reproductive and developmental toxicology tests, and activity tests for hyperactivity, anxiety, and depression. Inappropriate in vivo follow-up assays and subject specialization are these studies' limitations.

1.2 IMPORTANCE AND DISADVANTAGES OF FOOD ADDITIVES

Numerous people regularly consume a variety of food additives, which have both benefits and drawbacks. Food additives play a significant role in today's plentiful and nutrient-dense food sources, enabling people to enjoy a variety of wholesome, delectable, and secure foods throughout the year. Different positive effects of food additives on foods exist. However, some metabolites found to be carcinogens, like monosodium glutamate and nitrous compounds, may be present in food additives. Whether a food component is toxic or beneficial depends on how much it interferes with digestion, elimination, or metabolism. The interaction between various substances further complicates the description of the appropriate safety limits for human ingestion. The likelihood that chemical compounds will be toxic means that all new compounds should be thought of as toxic in the context of nutrition.

Due to the possibility of chemical compounds being toxic, all novel compounds in nutrition should initially be thought of as toxic until their safety has been established. Food additives can occasionally destroy vitamins in food (adding caramel to food has been shown to result in a vitamin B6 deficiency), are used to make poor-quality food look good, and can cause allergies in many people, including diarrhea, skin irritation, stomach disorders, vomiting, or an increase in body temperature. Additionally, it might reduce food's nutritional value. A number of food colorings have been prohibited because of their propensity to cause tissue damage and cancer. People who consume tartrazine as a food additive have been shown to experience a wide range of negative side effects and allergic reactions. These include migraines, anxiety, and attacks of asthma. (Kamal A et al., 2007)

1.3 FOOD ADDITIVES TYPES

Most food additives are tested in isolation, rather than in combination with other additives. The long-term effects of consuming a combination of different additives are currently unknown.

1. Anti – caking agent
2. Emulsifiers
3. Artificial sweeteners
4. Colours
5. Flavours
6. Antioxidants
7. Food acids
8. Monosodium glutamate
9. Sodium benzoate
10. Aspartame
11. Tartrazine
12. Sodium nitrate
13. Preservatives
14. Stabilisers and firming agents
15. Raising agents
16. Mineral salts
17. Antimicrobials

1. Monosodium Glutamate

Starting, we have MSG, which is the most common artificial additives. In addition to this, it can be found in several processed foods, such as your frozen dinners, that include ready to eat noodles, soups, processed meat, etc. Most of your canned food, which you see on the shelves is using MSG to preserve the food present inside.

According to the Food drug Administration, MSG is generally safe to eat. On the other hand, many companies use Glutamate property, which is an amino acid to enhance the flavors of your canned food. MSG is present in the natural form within tomatoes and cheese too.

Food corporations are using the fermentation process to produce MSG for food successfully. This fermentation process is the same one that your mother uses at to make yogurt and Dahi.

2. Sodium Nitrate

This chemical helps in preserving your meat products from harmful bacteria. In addition to this, it adds a salty flavor to your meat, while giving your meat a pinkish color. Furthermore, Sodium Nitrate blocks the growth of botulism and prevents your meat from spoilage. You can also find a higher amount of sodium nitrate in green veggies such as spinach, which can easily contain 500 to 1900 parts per million of sodium nitrate. Only 5% of your daily intake of sodium comes from meat, and the rest of it comes from the vegetables. When the sodium nitrate, which is present in the veggies, comes in contact with your saliva, which is not known to be harmful for human consumption During our research, we have found out that sodium nitrate, when it comes in contact with high heat, turns into amino acids and nitrates. That can turn in to nitrosamine, which not suitable for you to consume. It is best if you keep the levels of sodium nitrate to low in your diet.

3. Artificial sweeteners

When talking about losing weight and getting a toned body, most people will tell you its more to do with your daily diet than the exercise you do in the gym. Many cola and beverages companies use artificial sweeteners to enhance the flavor of their soft drinks to make them more pleasing to young kids and teenagers are aspartame, sucralose, and potassium. While it is true that in moderation, artificial sweeteners can help you reduce your weight, and people who are diagnosed with diabetes may use it on occasion to maintain their blood sugar levels, the fact remains that rampant use of artificial sweeteners is not good for the health in the long run.

4. High-fructose corn syrup

Another sweetener that is widely used in sodas is corn syrup. It is used in many of your fruit drinks, that say they are made from 100 natural fruits. Well, they are not lying to you as corn syrup does occur naturally in corn. HFCS has been linked to a variety of health

issue over the years and is considered really bad. It is counted as a major reason for growing obesity in the world. It is said to be one of the most versatile food additives that are present in the market as it is used across a variety of food.

5. Sodium Benzoate

Acidic foods including salad dressings, pickles, fruit juices, and condiments are frequently preserved using sodium benzoate. The FDA has determined that it is generally regarded as safe, although various studies have revealed potential negative effects that need to be taken into account. For instance, a study indicated that giving 3-year-old toddlers artificial food colouring and sodium benzoate enhanced their hyperactivity. Another study found that among 475 college students, consuming more drinks with sodium benzoate was linked to increased ADHD symptoms. Sodium benzoate can also be transformed into benzene when mixed with vitamin C, a substance that has been linked to the development of cancer.

The amount of benzene in carbonated beverages is the largest, and diet or sugar-free beverages are much more likely to generate it. One study that looked at the amount of benzene in various foods discovered that samples of cole slaw and coke had levels of benzene exceeding 100 ppb, which is more than 20 times the EPA's maximum contamination level for drinking water. Examine the food labels thoroughly to reduce your intake of sodium benzoate. A source of vitamin C, such as citric acid or ascorbic acid, should not be coupled with foods containing benzoic acid, benzene, or benzoate.

6. Aspartame

Aspartame is an artificial sugar agent. It is a white odourless powder, approximately 200 times sweeter than sugar. In Europe, it is authorized to be used as a food additive in foodstuffs such as drinks, sweets, dairy, chewing gum, energy-reducing and weight control products and as table-top sweetener. It is a methyl ester of the aspartic acid/phenylalanine dipeptide with the trade names NutraSweet, Equal and Canderel. Aspartame may act as a chemical stressor by altering liver function homeostasis and increasing protein oxidative damage. This might play a significant role in promoting apoptotic cell death leading to development of hepatotoxicity.

7. Tartrazine

Artificial azo dyes are playing a very important role in the appearance and enhancement of the quality of foods. Azo dyes are making products more attractive to consumers, particularly children (Hofer and Jenewein, 1997). Azo dyes have been in use for a long time in the food industry worldwide, but their uses are controversial in light of its adverse effects on health (Eman et al., 2000). Azo dyes contain at least one group in the molecule with two to three aromatic rings. The European Union has permitted some azo dyes to be used in food products such as tartrazine (Tz) (E102); Quinoline Yellow (E104) and Sunset Yellow (E110). TTZ is an orange-colored, water-soluble dye with inclusive applications in food industries, cosmetics, textiles, drugs, and pharmaceuticals (Merinas-Amo et al., 2019). TTZ is an anionic molecule having a benzene ring also shown in Fig. 1A. Food Additives (JECFA) reported the data of more than 300 studies on laboratory animals and clinical trials on human beings on toxicological concerns in 1964. From the report, it was found that TTZ has no cancer-causing potential while having little adverse effect on reproduction and development (Collins et al., 1990, Tanaka et al., 2008). Thus, intake of low concentrations of TTZ doesn't cause any genotoxicity in humans (Poul et al., 2009). It will be interesting to check the effects of Tartrazine on proteins found in milk.

Tartrazine is a synthetic lemon-yellow azo dye primarily used as a colorant in food products and cosmetics. Tartrazine is a monoazo pyrazolone dye used as a colorant in bakery products, flavoring cheese, carbonate toothpaste, drugs, and cosmetics. In addition, this dye is also used as a substitute for saffron in tropical cuisine. Although the acceptable daily intake of tartrazine for humans is 7.5 mg/kg body weight, prolonged use of this dye at higher concentrations is reported to increase the number of lymphocytes and eosinophils in gastric mucosal cells. Produce abnormal reproductive cells. In recent years, the zebrafish has emerged as a versatile vertebrate model for studying. Tartrazine is the best-known and one of the most commonly used food additives. Food colorants are also used in many medications as well as foods. There is no evidence that tartrazine makes asthma worse or that avoiding it makes asthma patients any better.

The use of tartrazine yellow additives in human food products is permitted within limits. However, studies have described this as a toxic component. The dye, which is intensively used in industry and commerce, enters the aquatic environment through releases of non-treated or inadequately treated effluents. However, further ecotoxicological research is needed. Tartrazine, e-number 102 Tartrazine usage ranged from 12.5 to 1092 mg/kg³. Another study from Uttar Pradesh reported that 59% of food items contained more than the maximum limit, with average quantities crossing the threshold of 100 mg/kg in most food commodities.

Tartrazine has been found by numerous researchers who have been examining the quality and safety of food products. We highlight various tartrazine detection and extraction techniques in this review paper. High-performance liquid chromatography, electrochemical sensors, thin-layer chromatography, spectrophotometry, capillary electrophoresis, and liquid chromatography-tandem mass spectrometry are a few of the analytical techniques that are available. Additionally, we go over the extraction procedures known as liquid-liquid extraction, solid-phase extraction, membrane filtration, cloud point extraction, and others. Additionally, a succinct overview is provided explaining tartrazine's synthesis, metabolism, and the highest levels allowed in various nations.

Tartrazine excretion and metabolism have been investigated in rats, rabbits, and humans. The route of administration affects the type of excretory products. The same color is excreted after intraperitoneal injection. Sulphanilic acid that is partially conjugated is excreted in equivalent amounts as a result of oral dosage.

The spectroscopic, microscopic and computational results support that the electrostatic, as well as hydrophobic interactions, played a very important role in Tz-induced BLG fibrillation.

8. Emulsifiers

Emulsifiers are used to keep one liquid evenly dispersed in another, such as oil in water. A long-chain fatty acid typically serves as the hydrophobic portion of an emulsifying agent's basic structure. The hydrophilic portion, which can be charged or uncharged, can be either charged or uncharged as well. In order to create a dispersion of tiny oil droplets,

the hydrophobic and hydrophilic portions of the emulsifier dissolve in the oil phase and the aqueous phase, respectively. Thus, emulsifiers help to create and maintain oil-in-water emulsions (such as mayonnaise), evenly distribute oil-soluble flavourings throughout a product, stop the production of big ice crystals in frozen foods (such as ice cream), and enhance the volume, uniformity, and fineness of baked goods.

9. Antioxidants

The different chemical compounds found in food are subject to oxidation through the addition of oxygen atoms or the removal of hydrogen atoms. Unsaturated fatty acids that undergo autoxidation (i.e., those that have one or more double bonds between the carbon atoms of the hydrocarbon chain) and enzyme-catalyzed oxidation are the two main types of oxidation that cause food to deteriorate.

Unsaturated fatty acids undergo autoxidation when molecular oxygen (O_2) and carbon-carbon double bonds come into contact. Free radicals, which are the by-products of autoxidation and are very reactive, are what give oxidative rancidity its off-flavours and off-odors. The rate of autoxidation can be slowed down by antioxidants that interact with free radicals (also known as free radical scavengers). These antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ), as well as the naturally occurring tocopherols (vitamin E derivatives).

Many dietary components may also be oxidised by particular enzymes. These oxidation reactions' byproducts may cause food quality alterations. For instance, when fruits and vegetables like apples, bananas, and potatoes are chopped or bruised, enzymes called phenolases catalyse the oxidation of certain compounds (such as the amino acid tyrosine, for example). Melanin, a dark pigment, is the end result of these oxidation processes, which are collectively referred to as enzymatic browning. Agents that bind free oxygen (i.e., reducing agents), such as ascorbic acid (vitamin C), and agents that inactivate the enzymes, such as citric acid and sulfites, are examples of antioxidants that block enzyme-catalyzed oxidation.

1.4 FOOD ADDITIVES LINK WITH HUMAN DISEASES

You have probably heard that most Americans eat too much sodium. Your body needs a small amount of sodium to work properly, but too much sodium can be bad for your health. Diets higher in sodium are associated with an increased risk of developing high blood pressure, which is a major cause of stroke and heart disease.

Sodium attracts water, and a high-sodium diet draws water into the blood stream, which can increase the volume of blood and subsequently your blood pressure. High blood pressure is a condition in which blood pressure remains elevated over time. Hypertension makes the heart work too hard, and the high force of the blood flow can harm arteries and organs (such as the heart, kidneys, brain, and eyes). Uncontrolled high blood pressure can raise the risk of heart attack, heart failure, stroke, kidney disease, and blindness. In addition, blood pressure generally rises as you get older, so limiting your sodium becomes even more important each year.

1. Liver disease

The worldwide incidence of many immune-mediated and metabolic diseases, including those of the intestines and liver, is steadily increasing. Gut microbiota plays a central role in the pathogenesis of these diseases as it mediates environmental changes to the intestinal immune system. Various environmental factors including diet, food additives and medication also trigger the composition and functional alterations of microbiota, that is, dysbiosis, and this dysbiosis is closely associated with many chronic inflammatory diseases. However, the causal relationship remains unclear for the majority of these diseases. In this review, we discuss essential epidemiological data, known pathogenetic factors including those of genetic and environmental nature, while mainly focusing on the role of gut microbiota in the development of selected intestinal and liver diseases. Using specific examples, we also briefly describe some of the most widely used animal models including gnotobiotic models and their contribution to the research of pathogenetic mechanisms of the host-microbiota relationship.

2. Brain disease

Aspartame is an artificial sweetener used in many sugar free products. Aspartame is made of phenylalanine methanol and aspartic acid. Phenylalanine can cross the blood - brain barrier and might disrupt the production of neurotransmitters. Additionally, aspartame is a chemical stressor and may increase the brains vulnerability to oxidative stress. One study looked at the effects of a high aspartame diet. Participants consumed about 11mg of aspartame for every pound of their body weight for eight days. By the end of the study, they were more irritable, had a higher rate of depression and performed worse on mental tests. Another study found people who consumed artificially sweetened soft drinks had an increase risk of stroke and dementia, though the exact type of sweetener was not specified. Some experimental research aspartame intake in mice found that it impaired memory and increased oxidative stress in the brain. Another found that long - term intake led to an imbalance antioxidant in the brain.

3. Cancer disease

Toxicological investigations are used to assess the safety of food additives. The use of additives in the food industry has been approved as being safe scientifically and legally. Although the legal limitations of these food additives in these foods are being observed, eating some processed foods that include them may increase the risk of cancer in people. For instance, according to recent findings, the risk of colon and pancreatic cancer is increased by processed beef that contains nitrite and nitrate preservatives. According to research by Belpoggi et al., a soft drink like coke may also enhance the risk of several cancer types. In this study, rats on a typical diet were used in a trial where half were given regular tap water and the other half were given coke as a source of drinking water. It was determined that the group getting coke had a greater incidence of pancreatic tumour mass in males and females than the group receiving regular tap water. Similar research indicated that consuming beverages with food additives may raise the risk of developing cancer. According to this study, 600 Singaporeans who drank two or more non-alcoholic drinks per week for 14 years doubled their risk of developing pancreatic cancer, but those who drank fruit juice showed no such increase.

These cases make it abundantly evident that even though there was no officially announced safety concern for these compounds, some processed foods containing safe additives may raise the risk of carcinogenicity. As a result, one can hypothesise the following causes for why these additives might be somewhat carcinogenic in food products even though no carcinogenic risks were shown in experimental studies where they were used per se: potential food structural changes, potential harmful synergistic interactions with other carcinogenic byproducts in commercial additives, potential exposure to lengthy and unsanitary storage conditions, and potential for going over the saline limit.

1.5 LIPID METABOLISM

Lipid metabolism is the synthesis and breakdown of lipids in cells. It involves the breakdown of storage of facts of energy as well as the synthesis of structural and functional lipids. For example, lipids necessary for the formation of cell membranes are produced by the liver from fats that are consumed. The process of creating these fats is known as lipogenesis. Triglycerides and cholesterol make up the majority of lipids ingested by humans and discovered in their bodies. Fatty acids and membrane lipids are two more lipids types that can be present in the body. There are two sources of fats that organisms can use to get energy. From eaten dietary fats and from stored fat. Lipid metabolism is sometimes thought of as the digestion and absorption process of the dietary fat, however this is incorrect. Both types of fat are used by vertebrate to generate energy for the operation of organs like the heart. Lipids must be solubilized before their metabolism can start because they are hydrophobic substance. Lipid metabolism frequently starts with hydrolysis, which is accomplished with the aid of different digestive enzymes. Lipid metabolism also takes place in plants, albeit the procedure are some what different from those in animals. The absorption of the fatty acids into the intestinal wall's epithelial cells come next following the hydrolysis. Fatty acids are packed and delivered to the rest of the body in epithelial cells. (R.Paul Sing.et. at.,2012)

1.6 ZEBRAFISH AS A MODEL



Figure 1.1 Zebrafish as a model organism

The zebrafish is a freshwater fish belonging to the minnow family of the order Cypriniformes, native to South Asia. It is a popular aquarium fish frequently sold under the trade name "zebra danio." It is also found in private ponds. The zebrafish has become known as an excellent model organism for studies of vertebrate biology.

Vertebrate genetics, embryonic development for use in genetic studies as an animal model, the zebrafish was initially introduced by Streisinger and colleagues in the early 1980s. Zebrafish compared to 80% of human genes Zebrafish for understanding human genetic diseases Zebrafish possess several advantages over rodent models in the study of vertebrate development and diseases. One such popular animal model is that of the Zebrafish (*Danio rerio*), a teleost found in freshwater systems, which has been at the forefront of several research fields since the 1980s. When zebrafish models started to be used in research, the primary focus was to contribute to the field of developmental biology, with thousands of zebrafish mutant models being identified with highly conserved disease genes among different species.

This finding made researchers aware of the importance of the zebrafish model in other research fields and subsequently led to gene cloning through rigorous expansion of gene mapping resources. Today, zebrafish are being seen as a powerful model of acquired and genetic human diseases with increasing utility in translational research. The freshwater teleost is a popular animal model choice because of its high embryo yield, and rapid reproduction rate. The necessity of model organisms to recapitulate humans has led to the exploitation of several animal species, among which rodents

have been widely employed. Host-pathogen interactions are complex but the zebrafish can also be used to investigate pathogenic mechanisms. Such approach is feasible due to the conservation of innate immunity mechanisms in the fish, including mucus secretion, synthesis of piscidins and defensin enzymes, and the inflammatory response triggered by bacterial components (LPS, peptidoglycan, flagellin) interacting with Toll-like receptors. Furthermore, the zebrafish also has an adaptive immune system sharing. Arguably one of the most exciting aspects of the gut microbiota is its role in brain development and function. In that context, one of the limitations of the rodent models has been the use of relatively invasive methods to monitor the live development and activity of the brain. The transparency of the zebrafish, either in larvae or adult stages, allows live brain imaging, without the need for implanting a probe or a cranial window to perform two-photon imaging or light-sheet microscopy. Animal models are used extensively in medical research to better understand the causes of both human and animal diseases as well as to facilitate the creation of novel treatments. Though rodents are still the most popular research model used globally, zebrafish (*Danio rerio*) models have rapidly gained popularity among scientists in recent years. This is due to the fact that a tiny tropical freshwater teleost fish shares significant physiological, anatomical, and genetic similarities with mammals. In order to better understand the genetic, toxicological, and behavioral aspects of various human diseases, zebrafish serve as an excellent experimental model. Testing novel therapeutics, such as a new vaccine's safety, is also beneficial.

Table 1.1 Taxonomy classification of zebrafish (*Danio rerio*)

Kingdom	Animalia
Phylum	Chordata
Class	Actinopterygii
Order	Cypriniformes
Family	Cyprinidae
Sub family	Danioniane
Genus	Danio
Species	Danio Rerio

1.6.1 HOMOLOGY OF ZEBRAFISH AND HUMAN

Zebrafish have been more useful in neuroscience over the past few decades because of their high degree of physiological and genetic similarity to humans, their ease of genetic manipulation, and their similar central nervous system (CNS) anatomy. It is being used as a model for many different brain illnesses because to its increased shelf life and quick development. The availability of zebrafish strains has made it possible to examine the effects of various strains on behavior, brain function, and medication responsiveness. They may therefore be the perfect species for medium- and high-throughput small molecule and genetic mutation screening.

Given their various advantages over alternative animal possibilities, their employment is both novel and logical. Zebrafish are an excellent research model due to their rapid growth, translucent embryos, and, most significantly, genetic make-up. Zebrafish may appear like an odd model for research involving humans because they are not mammals, lack arms and legs, have unique breathing mechanisms, live underwater, etc. However, as previously indicated, they are vertebrates, and humans and zebrafish share 70% of

the genes that code for proteins. Even more impressive is the fact that 84% of human illness genes have zebrafish analogs, demonstrating how similar the zebrafish and human genomes are.

Zebrafish as a genetic disease model. The qualities that distinguish the zebrafish as a model system include its capacity to effectively apply invertebrate-style genetics to challenges peculiar to vertebrates. Zebrafish are extremely genetically tractable due to external fertilization, high inventiveness, quick development, and large stocking densities. However, there are a lot of genomic tools available for zebrafish, such as a number of high-density genetic maps for mapping induced mutations and continuous zebrafish gene sequencing. Furthermore, genetics approaches, the main tool-of-trade for zebrafish biologists is a core group of mutants generated by random mutagenesis and selected on the basis of a common phenotype that can be used to dissect a particular process of interest.

CHAPTER 2

REVIEW OF LITERATURE

In this study on the use of liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography-tandem mass spectrometry (LCxLC-MS) for lipid profiling in zebrafish embryo samples. Jessica Legardi et.al., (2020) evaluated the performance of both techniques and compared their results. They found that both LC-MS and LCxLC-MS were effective in analyzing a wide range of lipid classes and molecular species, with LCxLC-MS providing more comprehensive coverage. They conclude that LCxLC-MS is a powerful tool for lipid profiling in zebrafish embryos and may have the potential for broader applications in lipidomics.

Mengmeng et.al., (2022) have discussed the use of comprehensive lipid profiling to investigate the effects of perfluorohexane sulfonate (PFHxS) exposure during various stages of early zebrafish development. The authors analyzed lipid profiles in zebrafish embryos and larvae exposed to PFHxS at different time points using liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography tandem mass spectrometry (LCxLC-MS). They found that PFHxS exposure led to significant changes in lipid profiles at different developmental stages, with alterations in several lipid classes and molecular species. The authors suggest that comprehensive lipid profiling can be a useful tool for studying the effects of environmental pollutants on lipid metabolism and early development in zebrafish.

Anderson et.al., (2011) discusses the role of lipid metabolism in zebrafish development and health. The authors describe how early lipid signaling pathways help to pattern the developing embryo, and then go on to discuss how zebrafish use various mechanisms to regulate lipid uptake, storage, and utilization in response to dietary changes. The article also delves into the molecular and genetic basis for these processes, and how they relate to human health and disease. Overall, the authors provide a comprehensive review of the current understanding of zebrafish lipid metabolism and its implications for research on nutrition and disease.

In Rosa L, Myares et.al., (2014) study, discusses the use of zebrafish as a model organism to study lipid transport and metabolism in vertebrates. The authors focus on the processing of lipids in the yolk of developing zebrafish embryos, which provides a convenient and well-characterized system for investigating the roles of specific genes and pathways in lipid metabolism. The article covers various aspects of yolk lipid processing, including lipid uptake, storage, mobilization, and utilization during embryonic development. The authors also discuss how the zebrafish model can be used to study lipid-related diseases and to test potential therapeutics for these conditions. Overall, the article highlights the usefulness of zebrafish as a tool for investigating the complex processes involved in vertebrate lipid transport and metabolism.

Arnold, et.al., (2012) discusses the potential link between artificial food colors and symptoms of attention-deficit/hyperactivity disorder (ADHD). The authors review studies examining the effects of artificial food colors on ADHD symptoms, including studies in which participants were given diets free of artificial food colors and studies in which participants were given supplements containing artificial food colors.

López-Serrano, A., et al., (2014) describes a study on the bioconcentration of ionic silver and silver nanoparticles in zebrafish embryos. The study exposed the embryos to either ionic silver or silver nanoparticles and measured the concentrations of silver in the embryos using inductively coupled plasma mass spectrometry. The results showed that both ionic silver and silver nanoparticles were readily taken up by the embryos, but that the bioconcentration of silver nanoparticles was significantly higher than that of ionic silver. The study also found that the silver nanoparticles caused developmental abnormalities in the embryos at concentrations that did not cause such effects with ionic silver. The study highlights the potential risks associated with exposure to silver nanoparticles and the importance of considering nanoparticle-specific effects when assessing the risks associated with nanomaterials.

Zebrafish have a high degree of genetic similarity with many commercially important fish species, making them an appropriate model for studying fish nutrition. Various research studies that have used zebrafish to investigate topics such as nutrient requirements, digestion, absorption, and metabolism. Zebrafish have great potential as a model organism for studying fish nutrition and can be used to improve the understanding of nutrient requirements and the development of more efficient and sustainable aquaculture practices (Ulloa, Pilar E., Juan F. et.al., (2014).

Dahm, Ralf, and Robert Geisler. (2006) discusses the use of zebrafish as a genetic model organism in the aquaculture industry. The article highlights the advantages of using zebrafish in genetic research, such as their small size, fast reproduction, and ease of genetic manipulation. The authors also discuss the potential benefits of using zebrafish research to improve the health and productivity of commercial aquaculture fish species. The article provides examples of successful genetic research using zebrafish as a model organism and suggests that further research in this area could lead to important advancements in aquaculture.

Myra L. Weiner (2016) studied the parameters and pitfalls that researchers should consider when conducting research on food additives, using carrageenan as a case study. The importance of rigor and transparency in research design, methodology, and data analysis, as well as the need to consider potential sources of bias and confounding factors that may impact study results. The author also emphasizes the importance of assessing the overall weight of evidence when evaluating the safety of food additives, including consideration of both positive and negative findings from multiple studies. Overall, this study provides a valuable overview of key considerations for researchers working in the field of food additive research.

Various advancements and achievements made using zebrafish as a model organism for developmental biology and biomedical research. The fundamental attributes that make zebrafish a valuable tool for studying human biology and disease, such as their transparency during early development, their rapid external development, and their relatively small size. The technological developments that have enabled increasingly complex and sophisticated zebrafish research, including CRISPR/Cas9 genome editing,

single-cell sequencing, and live imaging techniques. In addition, there are some of the challenges and limitations of zebrafish research, such as differences in physiology between zebrafish and humans. Overall, the success of zebrafish research over the past 25 years and looks to the future of this model organism as an important tool for understanding human development and disease (Mary C. Mullins, 2021).

The authors discuss the advantages of using zebrafish in translational research, including their genetic and physiological similarities to humans, their rapid development and easy maintenance, and their suitability for high-throughput drug screening. They provide examples of recent studies that have used zebrafish to investigate a range of diseases, including cancer, neurodegenerative disorders, and infectious diseases. The potential of zebrafish for personalized medicine, by enabling the study of individual genetic variations and their effects on disease susceptibility and drug response. Overall, zebrafish are a valuable tool for translational research, and that their use in this context may ultimately lead to new insights into disease mechanisms and the development of more effective treatments (E. Elizabeth Patton and David M. Tobin 2019).

Barbara Tavares and Susana Santos (Lopes 2013) discuss the various areas of research where zebrafish have been used, such as neuroscience, cardiovascular research, and cancer research, and provide examples of recent studies that have used zebrafish to gain insights into disease mechanisms and potential treatments. They also describe the various experimental techniques that can be used in zebrafish research, such as transgenesis, mutagenesis, and chemical screens, and highlight the advantages of these techniques in identifying new drug targets and potential therapeutics. Additionally, the authors discuss the ethical considerations of using zebrafish in research and highlight the importance of implementing humane practices to ensure the welfare of the animals. Overall, emphasizes the significant role that zebrafish can play in advancing biomedical research and the potential for this model organism to contribute to the development of new therapies and treatments.

L. rhamnosus administration to zebrafish results in a reduction in lipid content, which is associated with changes in the gut microbiota and alterations in the expression of genes involved in lipid metabolism in the host. *L. rhamnosus* may be a potential therapeutic

agent for managing lipid disorders in humans, and zebrafish can serve as a valuable model for studying the effects of probiotics on host physiology. (Falcinelli, Silvia, et al., 2015)

Fang, Longhou and Chao Liu et.al., (2014) discusses the use of zebrafish as a model organism for studying dyslipidemia, a condition characterized by abnormal levels of lipids in the blood that can lead to atherosclerosis and other cardiovascular diseases. The authors suggest that zebrafish models can provide valuable insights into the genetics and molecular pathways involved in dyslipidemia and serve as a useful tool for the development of new therapies for cardiovascular diseases.

Gultekin Fatih, et. al. (2020) explores the impact of food additives on the human microbiota. The authors review the different types of food additives, their prevalence in the food industry, and their potential effects on the gut microbiome. They discuss the mechanisms by which food additives may alter the gut microbiota, including disruption of the intestinal barrier, changes in gut motility, and promotion of bacterial growth. The authors also consider the potential health implications of alterations to the microbiota, including the risk of obesity, metabolic syndrome, and inflammatory bowel disease. Finally, a need for further research to understand the complex relationship between food additives and the microbiota, and to determine the best strategies for minimizing potential harms while ensuring the safety and quality of our food supply.

The study by Kamel et al. (2013) aimed to investigate the potential neurobehavioral effects of sodium benzoate exposure in male rats. Sodium benzoate is a common food preservative, and its safety has been a subject of debate in recent years. The rats exposed to sodium benzoate exhibited significantly reduced motor coordination and cognitive function compared to the control group. Additionally, the experimental group displayed increased anxiety levels compared to the control group. The authors concluded that exposure to sodium benzoate may lead to neurobehavioral alterations in male rats, and that further studies are needed to investigate the potential implications for human health.

Aspartame, a commonly used artificial sweetener, have showed an effect on oxidative stress and immune response in Wistar albino rats. Consumption of aspartame led to an

increase in oxidative stress and a decrease in the activity of antioxidant enzymes in the rats' blood cells. Additionally, aspartame was found to suppress the immune response in the rats. The authors suggest that the use of aspartame may have negative health implications and recommend further research in area (Arbind K. C et.al., 2014).

The effects of tartrazine, a commonly used food color additive, on neurochemical alterations in rat brain sub-regions. The researchers administered tartrazine orally to rats and examined the levels of various neurochemicals in different brain sub-regions. Tartrazine led to significant alterations in the levels of neurotransmitters such as dopamine, norepinephrine, and serotonin in the hippocampus and striatum regions of the rat brain. Tartrazine consumption may have negative effects on brain function (Bhatt, Diksha et al., 2018).

In Tural Emon et al. (2015) study shows the effects of the popular food additive sodium benzoate on neural tube development in chicken embryos. By administering different doses of sodium benzoate to the embryos they have evaluated their effects on neural tube closure and other morphological abnormalities. Sodium benzoate showed a dose-dependent increase in neural tube defects, with higher doses resulting in more severe abnormalities. The study suggests that sodium benzoate may have adverse effects on neural tube development and highlights the potential risks associated with the use of food additives in processed foods.

The four food dyes (tartrazine, sunset yellow FCF, erythrosine, and brilliant blue FCF) had significant negative effects on the development and growth of the cucumber, brine shrimp, and zebrafish. Tartrazine and sunset yellow FCF were found to be the most toxic, with the highest mortality rates and developmental abnormalities. Motta et al., (2019) study suggests that the use of these food dyes in food products may have adverse effects on aquatic ecosystems and highlights the need for further research into the potential environmental risks associated with the use of food additives.

Bearth et.al., 2014 discusses the impact of consumer perception on their acceptance of artificial food additives. The authors conducted a survey among a representative sample of Swiss consumers to investigate the factors that influence their perception of risk and

benefit associated with artificial food additives. The study found that consumers' risk perception was influenced by their level of knowledge, trust in authorities, and the type of food product. In contrast, their perception of benefits was mostly influenced by the product's taste, cost, and nutritional value. The authors also found that consumers who were more concerned about their health were more likely to perceive risk associated with artificial food additives. The study highlights the importance of understanding consumers' perceptions of food additives and suggests that transparent communication about the benefits and risks of artificial food additives can improve their acceptance.

CHAPTER 3

3. AIM AND SCOPE OF THE PRESENT INVESTIGATION

3.1 AIM

Aim of this study is to explore how food additives alter lipid metabolism using zebrafish as a model system

3.2 SCOPE

- To analyze the neurodegenerative phenotypic changes, and survival rate during embryonic and larval stages of zebrafish treated with selective food additives.
- To study the behavioral activities by analyzing embryonic and larval locomotor activity of zebrafish treated with selective food additives

CHAPTER 4

4. MATERIALS AND METHODS

4.1 MATERIALS

- a. Fish tank
- b. Sterile petri plates
- c. Sterile micropipettes
- d. Eppendorf centrifuge tubes
- e. Sterile tips
- f. Measuring cylinders
- g. Culture flasks

4.1.2 Chemicals and reagents

The chemicals used in our study are as follows

1. Sodium Chloride (NaCl)
2. Potassium Chloride (KCl)
3. Calcium Chloride Dihydrate ($\text{CaCl}_2\text{H}_4\text{O}_2$)
4. Magnesium Chloride Hexahydrate ($\text{Cl}_2\text{H}_{12}\text{MgO}_6$)
5. Sodium Hydroxide (NaOH)
6. Methylene Blue
7. Methanol
8. Chloroform
9. Acetone
10. Ammonium acetate
11. Acetonitrile
12. Aspartame
13. Tartrazine
14. Sodium benzoate
15. Ethanol
16. Distilled water

4.1.3 Instruments

- A. Autoclave
- B. Stereozoom Microscope
- C. Cooling centrifuge
- D. Vortex
- E. Lyophilization
- F. Sonication
- G. LC -MS
- H. Horizontal shaker

4.2 METHODS

4.2.1 Preparation of E3 Medium

A 60X Stock i.e., 34.8 g of Sodium Chloride (NaCl), 1.6g of Potassium Chloride (KCl), 5.8 g of Calcium Chloride Dihydrate ($\text{CaCl}_2\text{H}_2\text{O}_2$), and 9.78 g of Magnesium Chloride Hexahydrate ($\text{Cl}_2\text{H}_{12}\text{MgO}_6$) was prepared by dissolved in water, to a final volume of two liters. The pH was adjusted to 7.2 with Sodium Hydroxide (NaOH) and autoclaved. For the preparation of 1X medium, 16.5 ml of the 60X stock was diluted to 1 liter. 100 μL of 1% methylene blue (Sigma-Aldrich) was added to the 1X medium (Cold Spring harbor protocols).

4.2.2 Collection of Zebrafish Embryos

Wild-type adult (<8 months old) Zebrafish were procured from a specialized supplier. The Adult wild Zebrafish were reared and kept at aquatic research laboratory at Sathyabama Institute of science and technology under standard laboratory conditions of temperature $28 \pm 0.5^\circ\text{C}$, pH 7.2 ± 0.2 On a 14:10 Dark/light photoperiod according to the standard breeding protocols (Westerfield 1995). Zebrafish embryos were obtained from spawning adults within 0.5h of spawning and rinsed in embryo medium. Fertilized embryos were staged under stereo microscope (ZEISS) according to the description of (Kimel et al., 1995). Zebrafish embryos were collected at the end of spawning of adults and then placed in a sterile culture flask which contains E3 medium. The zebrafish embryos were kept in

their normal temperature 28.7°C. Embryonic developmental stage was analyzed in the range of 24hpf, 48hpf, 72hpf, 96hpf (hpf: Hours post fertilization). Medium was changed regularly for every 24-hour interval with fresh E3 medium. Dead embryos were removed.

4.2.3 Experimental Treatments

The experimental groups used for the study comprise the following (i) control group (untreated zebrafish embryos) (ii) zebrafish embryos treated with food additives viz., Aspartame (20ppm), Tartrazine (5.5ppm), and Sodium benzoate (200ppm). The embryos were exposed with additives from 18hpf to 96hpf. Each flask contains 50 zebrafish embryos in 40ml of E3 medium.



Figure 4.1 Food additives expose Control, Aspartame, Sodium benzoate, Tartrazine

4.2.4 Evaluation of spontaneous tail coiling in embryos and locomotor activity in larvae of zebrafish

Spontaneous tail coiling were evaluated at 24hpf and the locomotor activity of the larvae i.e., 48hpf to 96hpf were evaluated as described previously by **Behra et al., 2002; Westerfield, 1993**. A petri plate was marked with graph lines of 1 cm distance. Embryos were allowed to swim in the petri plate for 1 minute and the total distance crossed was noted. The duration of the swimming movements was measured with a stopwatch from the recorded film. The results were documented and compared.

4.2.5 Lipid extraction

A comprehensive method for lipid extraction from zebrafish larvae, aimed at preparing samples for chromatographic analysis. The process begins by adding 1000µl of methanol to 96 hpf zebrafish larvae, followed by storing them at -20°C. Next, the supernatant is transferred to a new centrifuge tube, and 2000µl of chloroform is added to the remaining aqueous phase. The sample was homogenized for a duration of 2-5 minutes using a sonicator followed by vortexing and centrifugation at 14000rpm for 5 minutes. The supernatant is divided into two Eppendorf tubes and the above process is repeated with one part of the supernatant by adding 1000µl of chloroform to it and centrifuging at 14000rpm for 5 minutes. The organic phase is combined with the remaining supernatant from the previous step and dried using a lyophilizer.

The lipid extraction process is finalized by dissolving the dried lipid extracts in a mixture of acetone/acetonitrile/ammonium acetate (40:40:20, v/v/v), and stored at -80°C prior to analysis. This methodology enables researchers to isolate and analyze the different lipid compounds present in zebrafish larvae, providing valuable insights into their roles and functions in the organism's physiology (Mengmeng Xu et.al., 2022).

4.2.6 LC-MS analysis

4.2.6.1 Principle

LC-MS (Liquid Chromatography-Mass Spectrometry) analysis is a powerful analytical technique used for the identification and quantification of compounds in complex mixtures. It is commonly used in the fields of pharmaceuticals, environmental monitoring, and metabolomics. The technique involves separating the components of a mixture using liquid chromatography, which separates the components based on their physical and chemical properties. The separated components are then ionized and passed through a mass spectrometer, which detects and quantifies the ions based on their mass-to-charge ratio. LC-MS analysis provides high sensitivity, selectivity, and accuracy in identifying and quantifying compounds. It can detect a wide range of compounds, including small molecules, peptides, and proteins, and can provide information on their molecular weight, structure, and fragmentation patterns.

4.2.6.2 Instrumentation

Lipid analysis was performed by a 1290 infinity liquid chromatography system from Agilent (Palo Alto, CA) coupled to a high-resolution quadrupole time-of-flight MS (QTOF, Compact. Bruker Daltonik, Bremen, Germany) with an electrospray ionization (ESI) source in positive and negative modes. The separation of lipids was achieved by a Kinetex EVO C18 column (2.1 mm × 100 mm, 2.6 µm, Phenomenex, USA). The solvent A: water: acetonitrile (40:60, v/v), and solvent B: acetonitrile: 2-propanol (10:90, v/v), both containing 10 mM ammonium formate and 0.1% (vol) formic acid. The gradient was as followed: 0 min 15% B; 2 min 30% B; 3 min 48% B; 20 min 82% B, 21 min 99% B; 30 min 99% B; 30.1 min 15% B; 32 min 15% B, with flow rate of 0.3 mL/min, column temperature of 45 °C, and injection volume of 5 µL. The MS data were collected in auto MS/MS acquisition with 5 spectra/s. The MS parameter settings were as follows: capillary voltages of ±5000 V and ±4000 V in positive and negative modes, respectively; end set plates of ±500 V; nebulizer gas (N₂) pressure of 4 bar; drying gas flow rate of 6 L/min; and the drying gas temperature of 200°C and 250°C in positive and negative modes, respectively. The m/z range in MS detection was from 60 to 2000 in positive mode, and from 50 to 2000 in negative mode. The MS calibration solution (sodium formate) was analyzed before every injection, and the pooled samples were measured.

4.2.7 Software

LIPID MAPS online tool was used to perform searches with product ion peak lists on a variety of lipid classes, where structures are generated by computational methods or present in the LIPID MAPS structure database.

4.2.8 Statistical Analysis

The locomotor activity was analyzed by One Way ANOVA. SPSS 15ver software was used to perform One Way ANOVA in order to determine the significance of difference in the studies between the Control groups and Test groups. All data are reported as a mean ± standard error (SEM) and *p < 0.05 and **p < 0.01 were considered statistically significant.

CHAPTER 5

5. RESULTS AND DISCUSSION

5.1 MORPHOLOGICAL CHANGES

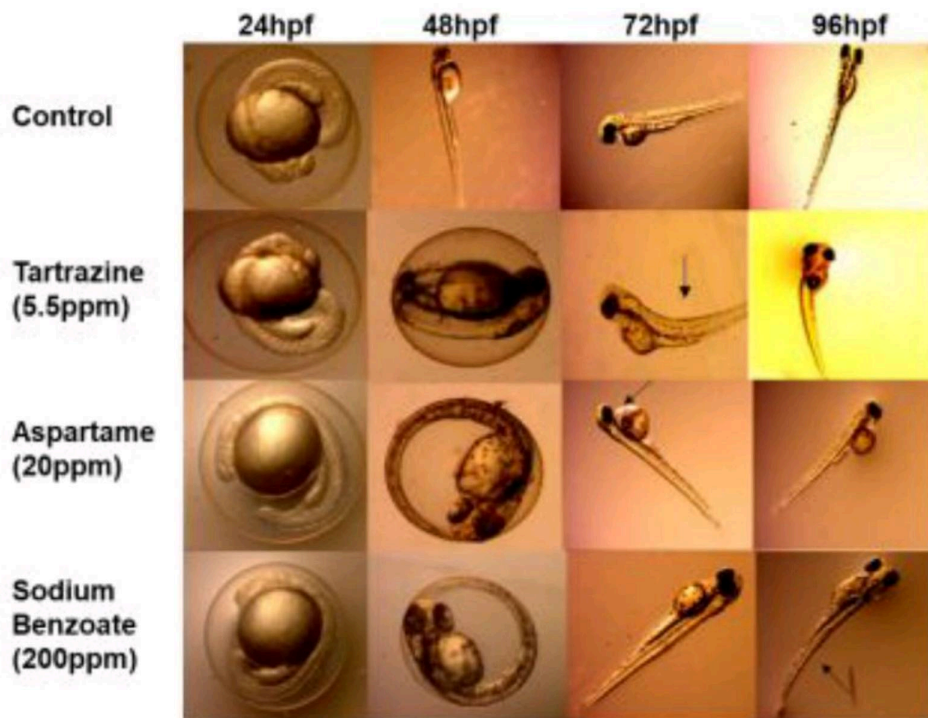


Figure 5.1 Display of phenotypic changes at different developmental stages of zebrafish exposed to Tartrazine, Aspartame, and Sodium benzoate at 18th hour of post-fertilization, photographed in lateral orientation through a stereomicroscope.

The development of the embryos was monitored from 24hpf to 91dpf after they were exposed to the food additives at the critical developmental period (18hpf). The exposed groups to tartrazine at 72 hpf and 96 hpf at 5.5 ppm concentration and to sodium benzoate at 96 hpf at 200 ppm showed significant morphological changes like bent tail and curved spine structure in the zebrafish larvae; cardiac edema was seen in aspartame-exposed groups even at low concentrations from 72 hpf and body pigments and accumulation of yolk sac were also observed in groups. The normal hatching period for zebrafish embryos is at 48hpf, when compared to the control, all exposed groups showed a delay

in hatching (Fig. 5.1). We can observe morphological changes in the treated groups with concentration less than the recommended ADI value of the food additives.

5.2 SURVIVAL RATE

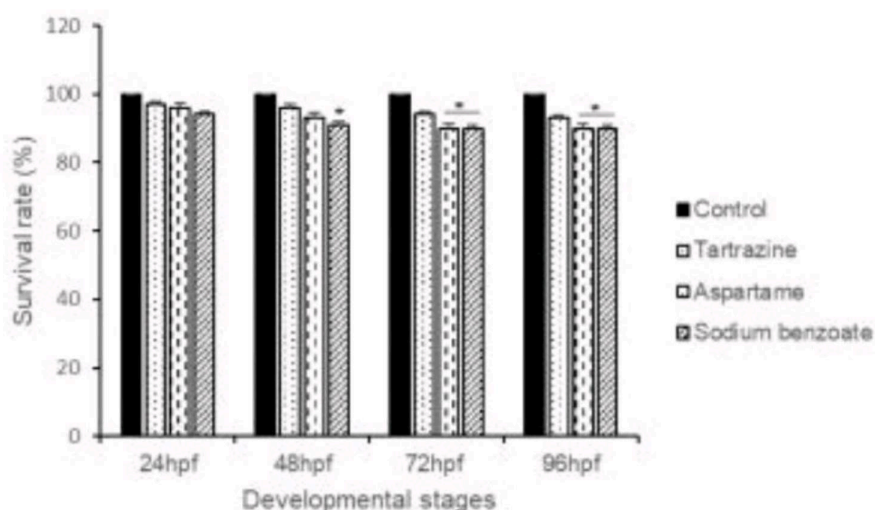


Figure 5.2 The survival rate of embryos following exposure to various food additives A) Tartrazine, C) Aspartame D) Sodium benzoate from 18 hpf to 96 hpf. Data are presented as Mean $\pm$ SEM (ANOVA, n = 50. *p $\leq$ 0.05)

The survival rate of embryos exposed from 24hpf to 96hpf showed a significant decrease in the aspartame and sodium benzoate exposed group at 72 hpf and 96 hpf (Fig. 5.2). At 48hpf a significant decrease was observed in the sodium benzoate group. There is no significant changes in the tartrazine group when compared to the control.

5.3 EFFECTS ON LOCOMOTOR ACTIVITY

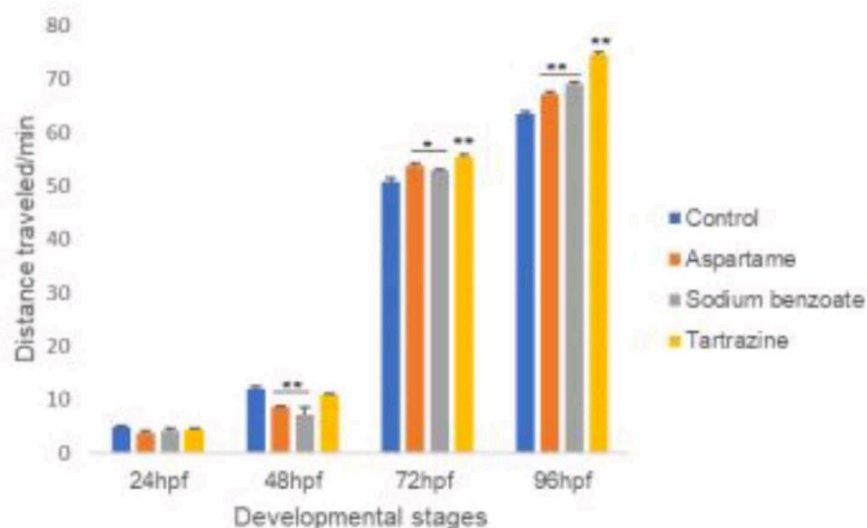


Figure 5.3 Locomotor activity of zebrafish embryos after exposure to Aspartame (20ppm), Sodium benzoate (200ppm) and Tartrazine (5.5ppm) from 18-96 hpf. Locomotor activity measured by distance traveled per min, at 24hpf locomotor activity calculated as a spontaneous tail coiling. Data are presented as Mean $\pm$ SEM (ANOVA, * $p \leq 0.05$, ** $p \leq 0.001$ relative to control) $n=5$

In comparison to control, locomotor activity was significantly reduced over the course of aspartame and sodium benzoate at 48hpf. No significant changes were observed at 24hpf. In contrast, an increase in locomotor activity was observed at 72hpf and 96hpf in all food additives exposed groups. The activity was increased for a long time exposure to additives which shows hyperactivity compared to the control group. This finding shows the food additive effects on zebrafish behavior and that nerve growth specific for locomotor activity is affected.

5.4 LC MS analysis for control result

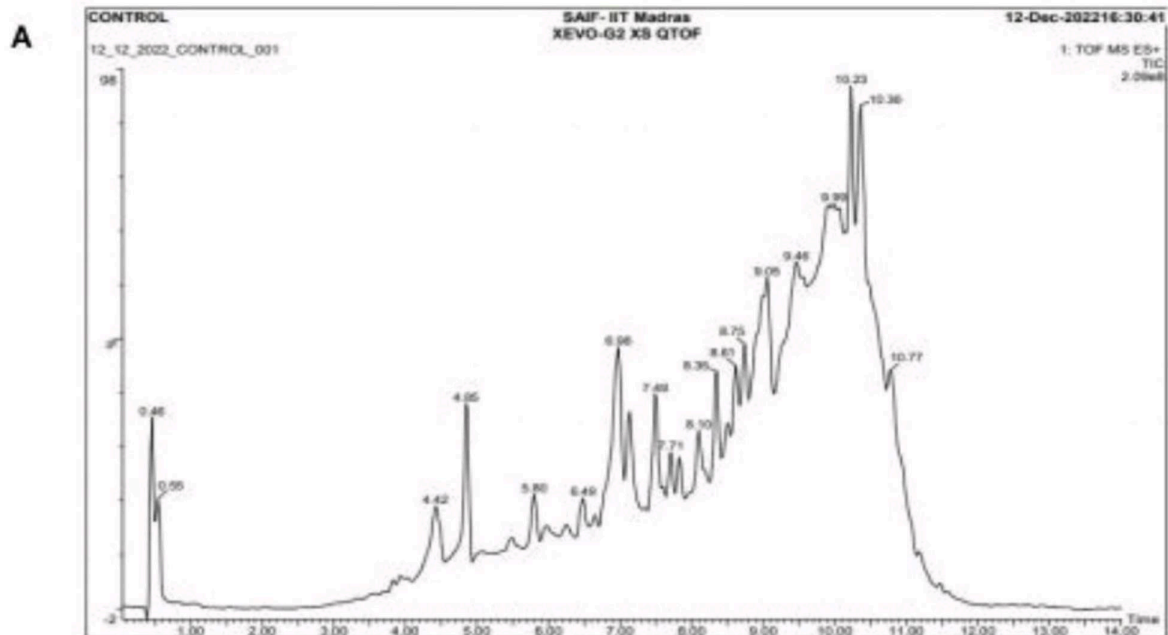


Figure 5.4 Lipid profile of zebrafish larvae at 96hpf after exposure to food additives control from 18-96hpf.

Table: 5.1 Identified on the lipids in control result

Mass value	Percentage	Lipids Name
430. 9140	74. 07	Arachidic acid(d3), O-decanoyl-R-carnitine, Dodeca-2E,4E-dienoic acid 4-hydroxy-2-phenylethylamide, Isononadecasphinganine
202. 1813	100. 00	4,8 dimethylnonanoyl carnitine, 6-Keto-decanoylcarnitine, Penaresidin A.
475. 3250	100. 00	1-(2-methoxy-tricosanyl)-sn-glycero-3-phosphoethanolamine, Formononetin 7-O-rutinoside
701. 4973	67. 05	Arachidic acid, (7Z)-14-hydroxy-10,13-doxoheptadec-7-enoic acid, 10-oxo-nonadecanoic acid
417. 3381	96. 21	5,7,9,11,13-tetradecapentaenoic acid, 6-hydroxytrideca-7,8-dien-10,12-diynoic acid, 9,9-dimethoxy-nonanoic acid
288. 2994	51. 07	Nudic Acid B, 9-amino-nonanoic acid, Heneicosylic acid, Zooxanthellactone
297. 9197	53. 79	11-dehydro-2,3-dinor-TXB2, Chlorosphaerolactylate A, (+)-5-Deoxykievitone
316. 3232	100. 00	Tricosylic acid, delta2-THA, 10-oxo-docosanoic acid, Ceriporic acid B
568. 3405	55. 51	Fujikinetin 7-O-laminaribioside, Acacetin 7-glucuronosyl-(1->2)-glucuronide
401. 2890	59. 57	Arachidic acid, Dihydroxychaulmoogric acid, Heneicosa-4E,10Z,12Z,20-tetraen-1,14-diyn-3,19-diol, thuwalenyne C, Cabreuvin

5.5 LC - MS analysis for Aspartame result

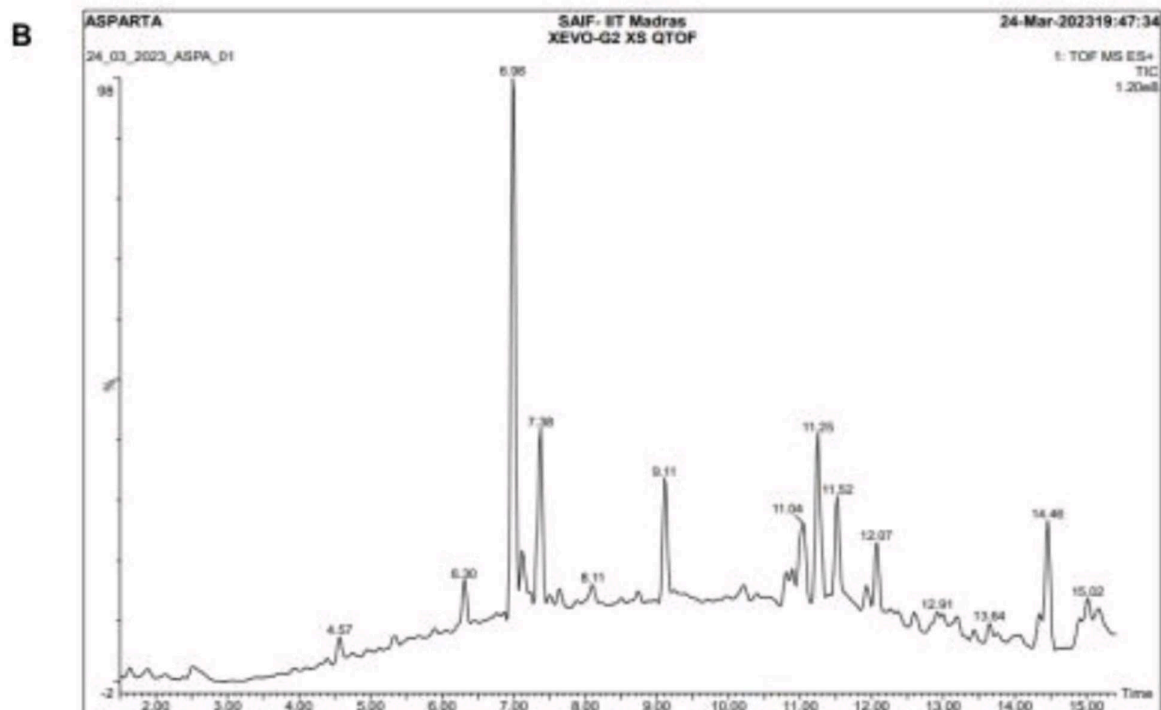


Figure 5.5 Lipid profile of zebrafish larvae at 96hpf after exposure to food additives aspartame from 18-96hpf.

Table: 5.2 Identified on the lipids in Aspartame result

Mass value	Percentage	Lipids Name
227. 6674	0.46	Triangulyne E, Lindenyl myristate, Myristyl lindenate, N - Lignoceroyl taurine
359. 7343	4.54	6 – prenylcatechin, Robustigenin , Belamcandin, Daipalitin, Sermundone
928. 6650	51.00	Liamocin B1, Thermozeaxanthin – 13, Undecaprenyl trihydrogen, diphosphate
213. 0210	100. 00	Tramutin, traumatin, patulolide C, Pinosylvin
410. 1656	18. 72	Shibic acid, Fulvinervin, Lcaritin, Melitemin, Tinderflame B

302. 3072	9. 95	Sphinganine, Enigmol, Nonanoylcarnitine, 2,6 dimethylheptanoyl
244. 6559	78. 80	2-ethylacryloylcarnitine, Tiglylcarnitine, BRN 2646116, N-(α -heptanoyl), homoserine thiolactone
313. 2362	11. 47	Nudic acid B, 9-amino-arachidic acid, arachidic acid, Isoarachidic acid
440. 4108	31. 20	9-N-palmitoyl theonine, bacillamidin B, Polycerasoidol, Hyrtial, Canrenoic acid, prednisone

5.6 Figure 5.4 and 5.5 Lipid profile of zebrafish larvae at 96hpf after exposure to food additives A) Control, and B) Aspartame from 18-96hpf.

In our study, various classes of lipids could be annotated in zebrafish embryos, including fatty acyls (FAs), Glycerophospholipids (GPLs), Polyketides(PK), Prenol lipids, Saccharolipids, Sterol lipids, Glycerolipids(GLs), Sphingolipids(SPs). In total 736 lipids were annotated with MS/MS spectra and the highest numbers were found for Fatty acyls(FAs) and Polyketides, and Glycerophospholipids(GPLs). Lipid profiling of zebrafish larvae at 96hpf after exposure to aspartame and non-exposed group (control) is shown in Fig. 5.3. The common lipids observed from control and aspartame exposed group were Bacillamidin B, Linolenyl myristate, Ascr#30, Undecanoylcarnitine, 11-amino-undecanoic acid (Fatty acyls), Prosopinine (Sphingolipids), Cholic acid (Sterol lipids), Salvurnin (prenol lipids), Kuwanol, Licoagroside F, Irisone A, Milldurone, Aflatoxin B1, Tricin, Cerasin, Chrysin, Wairol, Leridal (Polyketides). Fatty acyls are common to all living organisms. Fatty esters serve many roles from storage compounds and signaling to the oxidative metabolism of fatty acids. Octadecanoids, eicosanoids, docosanoids, and further lipid groups are collectively termed oxylipins. They are most known for their role in pro- inflammatory processes as well as resolution of inflammation, oxidative stress, and

the immune system. Arachidonic acid can act as a substrate for a number of other lipid modifying enzymes (e.g., cyclooxygenase) that are critical for cell movements required to pattern the early zebrafish embryo. Glycerophospholipids (GPLs) showed more complicated changes in levels in developing zebrafish. GPLs are important components of cellular membranes and precursors for bioactive mediators. Polyketides can play important roles in the development of zebrafish embryos.

CHAPTER 6

6. SUMMARY AND CONCLUSION

6.1 Summary

Our findings suggest that certain food additives commonly found in processed foods may have negative effects on lipid metabolism and contribute to the development of metabolic disorders. Further research is needed to determine the mechanisms underlying these effects and to evaluate the safety of these food additives in humans.

6.2 Conclusion

Food additives are substances supplemented during the processing of food either to enhance the nutritive value or quality or to enhance taste, flavor, freshness, and appearance. In this present study, exposure of selective food additives (tartrazine, aspartame and sodium benzoate) on early developmental stage of zebrafish, significant changes in physiological, behavioral and lipid profile were observed. Physiological changes are identified from the neurodegenerative phenotypic data and those abnormalities are certainly caused by the exposure of food additives. Further the locomotor activity has been increased in embryos exposed to certain food additives indicating hyperactivity, which is a symptom of ADHD. Changes in lipid composition were identified at 96hpf using LC-MS analysis. A decrease in polyketides, fatty acyls such as Bacillamidin B, Linolenyl myristate, Ascr#30, sphingolipids concentration and an increase in sterol, prenol lipids and fatty acyl (Undecanonylcarnitine, 11-amino-undercanoic acid) were observed during embryogenesis when compare to the control. Findings from the lipid analysis may prove the effects of aspartame on lipid profile in zebrafish larvae. In conclusion, zebrafish as a model organism for evaluating lipid metabolism alterations by food additives provides a powerful tool for understanding the potential health risks associated with the consumption of certain food additives. This research has important implications for the food industry and public health, and could ultimately lead to the development of safer food additives and the implementation these use of potentially harmful food additives.

CHAPTER 7

7. REFERENCES

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