The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 4, Issue 4, 2026

UNDERSTANDING THE EFFECT OF MONOSODIUM GLUTAMATE ON GROWTH, OBESITY AND SUGAR LEVEL IN ZEBRAFISH MODEL

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DOI: https://doi.org/10.5281/zenodo.19638946

Keywords

Article History
Received: 22 February 2026
Accepted: 01 April 2026
Published: 18 April 2026

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Abstract

Monosodium glutamate (MSG), a popular flavor enhancer found in many processed foods, has raised growing concerns due to its possible role in driving metabolic disorders. This study aimed to investigate both the immediate and long term effects of MSG on growth patterns, fat build up, blood sugar, cholesterol and lipid level, and gene expression in zebrafish (Danio rerio) model. For the acute exposure phase, zebrafish embryos (24 hours post fertilization) were exposed to varying MSG concentrations (0 to 400 mg/L) for 72 hours. Along with other physiological data (hatching rate, mortality and developmental deformities), the expression of IL-1β and P53 was analyzed in these embryos through semi-quantitative PCR. In the chronic exposure phase, zebrafish larvae were exposed to sub lethal concentrations (0, 6, 60 and 100 mg/L) for 3 months. Histological changes, total lipids and sugar levels were analyzed in fish from the exposed and control groups. Statistical analysis was performed through one way ANOVA and Tukey's post hoc tests. Results revealed a concentration dependent increase in morphological deformities during early developmental phases of embryos. These deformities included bent tail (95% at 350mg/L), yolk sac edema (90% at 150 and 180 mg/L), and heart edema (95% at 100, 140 and 200mg/L), with significant embryo lethality (100%) at concentrations above 250 mg/L. At the molecular level, increased expression of IL-1β (2 fold) and altered expression of P53 gene were observed in these embryos. These data suggest increased inflammation and oxidative stress responses triggered by MSG. Phenotypic assessment of the chronically exposed groups showed significant increase in body weight (20% at 60mg/L compared to the control), Body mass index (BMI) increases (45% at 60 mg/L as compared to control and condition factor (100% increase) at 60 mg/L as compared to control. These are indicative of excessive fat accumulation and accelerated physical development. Biochemical assays of homogenized whole body tissues showed elevated levels of triglycerides (11.12 ± 0.48 in the control vs 16.00 ± 0.57 mg/g in the MSG group) and cholesterol levels (from 2.9 ± 0.21 to 7.24 ± 0.38mg/g) in MSG treated groups, but there was no significant increase in sugar levels. Histological examination of preserved tissues samples stained with hematoxylin and eosin highlighted tissues vacuolization and structural disorganization, showed that MSG induced structural damage. Overall, the study confirms MSG as a potent obesogenic and metabolic disruptor, causing developmental toxicity and long term metabolic disturbances, and validates zebrafish as a potent model for assessing metabolic syndrome, gene expression and toxicological risks.

I.INTRODUCTION

The medical condition known as obesity is defined by an excessive build-up and storage of body fat., leading to a body mass index (BMI) of 30 or higher (Panuganti et al., 2024). Obesity is an unhealthy condition because Numerous health hazards and consequences are linked to it (Fruh, 2017). The harmful effect of excess weight has been known for a long time. Ancient doctors like Hippocrates, noted that overweight people are more likely to die suddenly, and they recognized that being extremely overweight can be a disease because it makes normal body functions harder and shortens life by causing numerous serious health problems (Marghoub et al., 2023). Obesity happens when more calories are consumed then they are use (National Heart and Institute, 2022).

Obesity raises the risk of certain metabolic disorders, such as Type II diabetes, cardiovascular disease, and some malignancies (Brown et al., 2023). It is also linked to a number of metabolic dysfunctions, high blood pressure and high cholesterol (Graham et al., 2015). Extra fat causes health problem in two ways: (i) Physical issues (osteoarthritis, sleep apnea, skin changes and the social stigma of being overweight), (ii) Metabolic changes cancer, excessive blood pressure, diabetes, gallbladder disease, and heart disease) (Al-Abed, 2021). Fat cells act like hormone producing cells and release substances that can lead to these diseases (Arner, 2005).

Overweight people develop osteoarthritis especially in weight bearing joints knees and ankle (Messier, 2008). Individuals may experience stretch marks on the skin, darkened skin in body folds (acanthosis nigricans) and excessive hair growth on body and face in women (hirsutism) (Hidalgo, 2002). Overweight individuals often face public disapproval (Puhl and Heuer, 2010). Excess weight especially around the abdomen can reduce lung capacity (Dixon and Peters, 2018). Obstructive sleep apnea is more common in overweight (Westbury et al., 2023). In addition to multiple myeloma, obesity is linked to 13 different cancer types, including pancreatic, renal, thyroid, meningeal, stomach, colon, liver, gallbladder, ovarian, uterine, and ovarian cancers(Jin et al., 2023). Obesity also increases the risk of stroke and high blood pressure due to excess fat causing increase strain on the heart and blood vessels (Singh et al., 2023).

One of the major and important health conditions associated with obesity is diabetes (Figure 1). Obesity is the major risk factor for type 2 diabetes mellitus (Abranches et al., 2015). In about one-third of obese people, type 2 diabetes (T2DM) occurs. T2DM is 20 times more likely to develop in those over 35 than in those with normal BMIs (18.5-24.9) (National Heart and Institute, 2022). According to a study, 80% of T2DM people are obese or overweight (Abranches et al., 2015). T2DM is primarily caused by obesity driven insulin resistance and insufficient insulin secretion by the pancreas (Galicia-Garcia et al., 2020). Insulin is the hormone that helps cells takes in and uses glucose (sugar) and prevents the breakdown of fat and protein (Rahman et al., 2021). Insulin resistance develop when cell have poor insulin sensitivity, which results in elevated blood sugar levels (Szablewski, 2024).

In addition to glucose level, obese individuals also have high level of free fatty acids in their blood, which can be deposited in non-fat tissues, causing lipotoxicity and insulin resistance (Wondmkun, 2020). Different obesity related factors may interfere with insulin resistance such as accumulation of lipid metabolites from free fatty acids, inflammation and oxidative stress, levels of hormones such as adipokines, adiponectin, and leptin released by fat cells (Guilherme et al., 2008). Other factors of T2DM also include dysfunction of insulin producing beta cells. Insulin resistance may also be brought on by pro-inflammatory cytokines such as interlukin-6 *(IL6)* and tumor necrosis factor-α *(TNF-α)* (Dludla et al., 2023).

Glucose and lipid metabolism closely interact in diabetes a phenomena called diabetic dyslipidemia, It includes numerous tiny, dense LDL (bad cholesterol) particles, low HDL (good cholesterol), and elevated triglycerides leading to issue with glucose metabolism (Parhofer, 2015). Most people with T2DM have some form of dyslipidemia. This dyslipidemia often reflects insulin resistance rather than just high blood sugar (Maki and Benes, 2021).Good blood sugar

controls helps improve dyslipidemia but does not completely fix it (Wang et al., 2022). Statins are usually used in combination with other drugs like ezetimibe to lower LDL cholesterol (Nodari et al., 2007). Managing triglycerides levels is important for controlling T2DM because of its direct impact on glucose metabolism (Guilherme et al., 2008). Other treatments include fat transplantation and leptin hormone replacement, which can also improve insulin sensitivity (Coppari and Bjørbæk, 2012). Small weight reductions through diet and exercise can significantly improve insulin sensitivity(Tam et al., 2011). Mitogen-Activated Protein Kinases *(MNKs)* are non-essential enzymes which are activated by MAP kinases (Moore et al., 2016). MNKs including MNK1 AND MNK2 could also be a promising strategy to prevent obesity related weight gain and metabolic issues (Jin et al., 2023).

Figure 1: Factors and consequences of obesity.

A multitude of genetic, environmental, behavioral, and metabolic variables that affect fat distribution and energy balance combine to cause obesity (Romieu et al., 2017).Various genes and variants have been identified in humans linked to obesity (Annex 1), including the *fat and obesity* (*FTO*) gene and the *low- fat* gene *Apo lipoprotein A5* (*APOA5*) (Fonseca et al., 2020). Variations in the adiponectin gene (SNP 276 G allele) leading to insulin resistance and metabolic syndrome has also been linked with obesity in humans (Fonseca et al., 2020). Exome sequencing has identified variants in the *SYPL2* gene associated with morbid obesity (Jiao et al., 2015).

Non-genetic factors of obesity include imbalanced energy intake and expenditure due to sedentary life style coupled with over nutrition. Triglycerides are the form in which extra nutrients are kept in adipose tissue (Romieu et al., 2017). Nutritionally, obesity is usually the consequence of high amounts of saturated fat in diet, and a refined carbohydrate diet. Increased dietary intake combined with decreased exercise can lead to negative health outcomes, including increased coagulation and decreased fibrinolysis, metabolic effects (dyslipidemia and carbohydrate intolerance), endocrine effects (hyperinsulinism, insulin resistance), renal effects, and sympathetic nervous system activation (Wali et al., 2020).

Monosodium glutamate (MSG) is one substance that is frequently used to enhance the flavor of food. MSG has been associated with numerous health concerns (Niaz et al., 2018). These include obesity, metabolic disorders, Chinese Restaurant Syndrome, neurotoxicity, and reproductive issues (Niaz et al., 2018). Chinese Restaurant Syndrome referrers to burning feeling behind the neck, blisters on the anterior thorax and both arms occasionally, overall weakness and exhaustion, and heart palpitations, which usually starts after 20 minutes of eating (Bawaskar et al., 2017).

Research on both humans and animals indicates that MSG can be harmful even at low dosages (Annex 2). The typical daily consumption of MSG is between 0.3 and 1.0 grams, which may cause neuronal disruption and behavioral changes (Husarova and Ostatnikova, 2013). MSG exposure during infancy has been connected in rodents to obesity and metabolic problems like insulin resistance and decreased glucose tolerance. This is thought to be because MSG can make food taste better and interfere with leptin transmission in the hypothalamus, which can result in obesity (He et al., 2008).

According to official data of the European Food Safety Authority (EFSA); The "Acceptable Daily Intake" (ADI) concentration of MSG is 30 mg/kg body weight (BW)/day, and the "No Observed Adverse Effect Level" (NOAEL) concentration is 3200 mg/kg BW/day. The average daily intake of MSG is around 0.3-1.0 g/day in European countries and USA, and 1.2-1.7 g/day in Asian countries. There is no complete agreement in the literature about the safety of MSG, even though the Food and Drug Administration (FDA) includes it on the GRAS list (Generally recognized as safe)(Çakmakci and Salik, 2022). MSG administration has been shown to induce hyperinsulinemia and obesity in animal models.

The autonomic nerve system (ANS) imbalance is best illustrated by the pre-diabetic obese rat model that was created by neonatal MSG injection (Russo et al., 2021). The autonomic nervous system regulates energy homeostasis, which is the balance between energy intake and energy expenditure and is typically the cause of obesity(Sharma et al., 2022). This is followed by increased fasting blood glucose levels and development of type 2 diabetes mellitus (T2DM) (Hernández Bautista et al., 2019).The toxic effects of MSG on the CNS in animal studies are not directly applicable to humans due to differences in age, administration route, and dosage (Hernández Bautista et al., 2019)

Animal models are invaluable tools for studying obesity, diabetes, and their related complications. Rodent models of mice and rats are genetically engineered models which include knockout or transgenic mice where specific genes related to diabetes or obesity are altered. E.g. ob/ob mice lack the leptin gene, leading to obesity and insulin resistance (Kottaisamy et al., 2021). High fat and high sugar diets and certain chemicals like streptozotocin or alloxan are used to induced obesity and T2DM in rodents mimicking human dietary habits (Kottaisamy et al., 2021).Non- human primates can develop diabetes and obesity naturally or through diet and genetic manipulation (Havel et al., 2017). Pigs size and physiology make them good models for human diseases and they can also develop diabetes and obesity with similar complications (Hou et al., 2022). Dogs and cats are less common but have been used for specific studies, especially for spontaneous diabetes cases (Pandey and Chmelir, 2023).

Animal models help researchers understand the pathophysiology of diabetes and obesity, including insulin signaling, glucose metabolism, beta cell function and adipose tissue dynamics (Kottaisamy et al., 2021). However, the use of animals especially mammals in research raises ethical issues, and there are strict regulations and guidelines to ensure human treatment. Maintaining animal colonies and conducting experiments can be expensive, especially with larger animals like non- human primates (Singh et al., 2024).

As an alternative to animals, zebrafish has been used as model to study diabetes and obesity (Annex 2) (Zang et al., 2018). In recent years, zebrafish have become model of choice in different studies due to its amenability to genetic manipulation, rapid development, and genetic similarities to mammals for studying complex metabolic pathways (Henke et al., 2023). The transparent embryos of zebrafish allowing for real time visualization for investigating metabolic processes which is advantageous for studying disease progression and therapeutic interventions (Seth et al., 2013).

In regards to obesity studies, zebrafish and humans have similar pancreatic structures, with both having exocrine and endocrine parts (Lavergne et al., 2020). Zebrafish have comparable blood glucose levels (50-75 mg/dl) to humans (70-120 mg/dl). Zebrafish express genes involved in carbohydrate metabolism similarly to mammals. By 4 days post fertilization zebrafish can transcribe genes related to gluconeogenesis and lipolysis, essential for glucose homeostasis (Benchoula et al., 2019a).

Various researchers have used zebrafish to explore diabetes by manipulating there glucose levels with compounds like metronidazole, glipizide, and alloxan (Benchoula et al., 2019a). Zebrafish share similar adipose tissues and signaling pathways (e.g. leptin, melanocortin) with humans. Overfeeding zebrafish with high fat diets inducing obesity mimics human conditions. Zebrafish has also been used to study natural anti-obesity compounds and role of environmental factors in obesity development (Faillaci et al., 2018).

Interleukin genes were deliberately chosen for the study because of their critical role in promoting inflammatory processes, and because saroglitazar was investigating its effects on obesity and inflammation in MSG-obese Wistar rats. Many metabolic dysfunctions, including insulin resistance, are greatly influenced by cytokines including *TNF-α*, *IL-6*, and *IL-1β*, which are frequently raised in obese people. The researchers looked at how saroglitazar interacted with important inflammatory mechanisms, such as the *NLRP3* inflammasome and *NF-κB* signaling pathways, and assessed its effect on obesity-linked inflammation by measuring interleukin levels. Monitoring the production of interleukin before and after therapy yielded useful information about the medication's ability to reduce inflammation, which may be used to evaluate how well it works to address the more general metabolic problems linked to obesity (Nabi and Bhandari, 2022).

The study focused on the *p53* gene because of its critical function in controlling apoptosis and the cell cycle, particularly in response to oxidative stress and cellular damage. In an attempt to assess the effects of MSG on cardiac muscle, researchers discovered that increased *p53* expression was linked to cardiac toxicity and apoptotic pathways. This includes harm like myocardial degeneration and fibrosis, suggesting that *p53* plays a crucial part in oxidative stress and cardiac damage brought on by MSG (Hazzaa et al., 2020).

This research will examine the impact of MSG concentrations causing toxicity and developing diabetes and obesity in zebrafish. Developing diabetic and obese zebrafish model will provide a platform to study the dynamics of obesity and diabetes and potential treatment strategies. To analyze how changes in diet composition and exposure to chemicals, can affect gene expression and metabolic processes.

OBJECTIVES

- To identify the potential toxic effect of MSG by evaluating mortality and developmental deformities in larval zebrafish.
- To evaluate the effect of MSG on immunity and oxidative stress by analyzing the expression of *IL-1β* and *P53* gene in larval zebrafish.
- To evaluate the development of obesity by measuring growth, histology, total lipids and sugar levels in adult zebrafish model.

II. REVIEW OF LITERATURE

Altintas et al. (2025) investigated the sex specific effects of monosodium glutamate MSG induced obesity on cognitive function, anxiety like behavior, oxidative stress and genotoxicity in neonatal Wistar albino rats, offering valuable insights into obesity related pathologies. They administered subcutaneous MSG to 32 neonatal Wistar albino rats, effectively inducing obesity. The subjects were then evaluated using a comprehensive set of tools: The Morris water maze foe memory and learning performance, behavioral assays to gauge anxiety like responses, hippocampal assays for oxidative stress markers and genotoxicity assessments to evaluate DNA integrity. Their findings revealed a marked increase in abdominal fat accumulation across all sexes but more pronounced in females. Both sexes experienced impaired cognitive functions, particularly in learning and memory. Surprisingly, obesity did not significantly altered anxiety behavior and oxidative stress markers. However, one striking discovery was that only obese female rats exhibited signs of DNA damage, pointing to a possible sex specific susceptibility to genotoxic effects of MSG induced obesity. This study highlights the importance of considering sex as a biological variable in obesity research. It also underscores the potential long term cognitive and genetic risks associated with MSG exposure during early development.

Kahe et al. (2025) investigated potential link between MSG consumption and obesity, focusing on both human and animal studies to

gather evidence. They perform a comprehensive analysis of existing literature to identify mechanisms that connect MSG intake to weight gain. The results indicated that while MSG has been recognized as safe for consumption, there are significant concerns regarding its chronic use and its possible role in promoting obesity. The review highlights various mechanisms such as alteration in appetite regulation and metabolic processes, that could explain how MSG contribute to weight gain. Furthermore it discusses the challenges in studying MSG due to its widespread use in food and often hidden nature of its presence in food labeling. The review concludes to emphasize the urgent need for more rigorous mechanistic studies and randomized controlled trails to clarify the health impacts of MSG, particularly regarding weight management, to address public concerns effectively.

Kim et al. (2025) explored the possible neurotoxic effects of MSG on neural stem cells (NSCs) and hippocampal neurogenesis using both in vitro and in vivo models. Over a two week period, young male mice were administered either MSG or NaCl, while separate NSC cultures were assessed in a controlled laboratory setting. They measured cytotoxicity, gene expression levels, cell proliferation and behavioral outcomes related to spatial memory. Despite MSG known toxicity in mature neurons, findings showed it did not negatively influence the viability, growth and differentiation of NSCs. Moreover, hippocampal neurogenesis and spatial learning abilities remain intact. These results suggested that under the examined conditions, NSCs and the brain capacity for neurogenesis demonstrate resilience to MSG exposure.

Taha et al. (2025) Investigated he structural and functional impact of MSG on rats testes and assess the potential protective role of resveratrol, a natural antioxidant. Their study involved four experimental groups of ten rats each, a control group, a resveratrol treated group 20 mg/kg, an MSG exposed group (60 mg/kg) and a combined MSG + resveratrol group, with treatments administered via oral gavage. Key hormonal markers such as testosterone, FSH and LH were measured, alongside assessment of oxidative stress (MDA, SOD, CAT), gene expression analysis (NLRP3, caspase-3, GSK-3β), histological evaluations and sperm quality analysis. Results revealed that MSG significantly impact testicular structure, reduced testosterone levels, disrupted the hypothalamic – pituitary gonadal axis, increased oxidative stress and expression of apoptotic and pyroptotic genes, and negatively affected sperm quality. However co-administration of resveratrol markedly mitigated these effects improving antioxidant enzyme activity, reducing lipid peroxidation, suppressing apoptotic and inflammatory responses, and restoring spermatogenic function, as evidenced by *Ki-67* expression. This study concludes that resveratrol exerts a significant protective effect against MSG induced testicular damage, highlighting its potential therapeutic role in maintaining male reproductive health under toxic stress.

Udom et al. (2025) investigated the adverse health impacts of MSG, a widely used flavor enhancer, with varying results depending upon dosage, frequency and duration of exposure. In a recent narrative synthesis conducted according to PRISMA guidelines, studies published between 2014 and 2024 were systematically retrieved from Scopus, Web of Science, PubMed, and Google Scholar. Quality assessment of selected animals and humans studies was carried out using the ARRIVE and Joanna Briggs checklists. The review highlighted that while MSG is generally recognized as safe at low concentrations, repeated or high concentration exposure is linked to multiple toxicological outcomes, including embryo toxicity, teratogenicity, obesity, hepatotoxicity, neurotoxicity, cardio toxicity, kidney toxicity and reproductive dysfunction. Furthermore significant alterations in lipid and glucose metabolism were reported , suggesting metabolic disruption. Their study concluded to emphasize the pathological implications of chronic MSG exposure and underscores the necessity for regulatory review and transparent labeling to guide consumer choices.

Gelen (2024) investigated the effects of monosodium glutamate (MSG) in both healthy and diabetic rats (induced with streptozotocin, STZ) on antioxidant activity, oxidative stress, glucose control, insulin secretion, and pancreatic health. The trial lasted 28 days and involved the

use of 65 mg/kg of STZ to induce diabetes. The findings showed that all treatment groups (STZ, MSG, STZ + MSG) had significantly higher levels of inflammatory markers (*TNF-α*, *IL-10*, *IL-6*, and *IL-1β*) than the controls ($p < 0.001$). The STZ + MSG group had the highest levels of malondialdehyde (MDA) ($p < 0.001$), indicating the most pronounced oxidative stress. Conversely, antioxidant defenses, such as glutathione (GSH) and superoxide dismutase (SOD), were significantly reduced ($p < 0.001$) in all treatment groups. Furthermore, insulin levels decreased ($p < 0.01$) in all groups, whereas glucose levels increased ($p < 0.01$) in the STZ and STZ + MSG groups. In the STZ + MSG group, substantial pancreatic injury was revealed by histopathological examinations. These results highlight the necessity for cautious MSG consumption and imply that MSG aggravates diabetes conditions.

Brant et al. (2023) utilized a water-avoidance stress (WAS) mice model of visceral pain to investigate the possibility that MSG produces visceral hypersensitivity. Four groups of mice were given therapy for six days: WAS + MSG gavage, WAS + saline gavage, sham-WAS + MSG gavage, and sham-WAS + saline gavage. Through ex vivo extracellular recordings, the acute effects of intraluminal injection of 10 μM MSG on jejunal extrinsic afferent nerve sensitivity to distension (0-60 mmHg) were investigated. Direct application of MSG was also done to untreated mice's jejunal afferents. After apical injection, the glutamate concentration in serum and the serosal compartment of the Using chambers were measured. he findings indicate that mice exposed to WAS + MSG had greater distension responses in their jejunal afferent neurons after receiving an acute intraluminal MSG injection. Wide dynamic range and high threshold units at both physiologic and noxious pressures (10-60 mmHg, $p < 0.05$) mediated this impact. MSG had no effect on the jejunal afferent nerves or on the other groups. Using WAS tissue, serosal glutamate rose ($p = 0.0433$), and mice treated to WAS + MSG had higher serum glutamate levels than those exposed to sham-WAS + saline. These results show that acute ex vivo treatment to MSG can sensitize jejunal afferent neurons in mice that have received repeated exposure. This could be a factor in the visceral hypersensitivity that IBS patients have been known to experience in reaction to MSG.

Kayode et al. (2023) analyzed the effects of MSG consumption on several metabolic conditions, particularly diabetes, hypertension, and obesity. Educate the public on the potential health problems linked to MSG usage. Encourage regulatory agencies to reconsider daily consumption thresholds in view of identified metabolic toxicities. MSG causes obesity through a number of processes, including the establishment of a hypothalamic lesion, hyperlipidemia, oxidative stress, leptin resistance, and increased expression of peroxisome proliferator-activated receptors (PPARs) Gamma and Alpha. Lower mass of pancreatic beta cells, increased oxidative stress and metabolic rates, reduced transport of glucose and insulin to skeletal muscles and adipose tissue, insulin insensitivity, reduced insulin receptors, and severe hyperinsulinemia were among the similar effects of MSG consumption on the induction of diabetes mellitus. The results indicate a nearly 4% yearly growth in the global market for MSG, with over 75% of the world's consumption—which is estimated to be over three million metric tons and valued at over $4.5 billion—occurring in Asia, especially in countries like China, Thailand, and Indonesia. The study indicates that because MSG has a substantial link with the initiation and progression of metabolic disorders, notably obesity, diabetes, and hypertension, it is important to use caution while taking MSG. In addition, it requires regulatory agencies to review and maybe revise the daily maximums for MSG intake in view of the metabolic toxicity observed at various concentrations.

Zazula et al. (2023) examined the early and long-term effects of obesity induced by MSG on systemic and muscle parameters in Wistar rats. The goal was to ascertain whether early MSG exposure led to adult-onset changes like decreased muscle mass, increased fibrosis, and peripheral insulin resistance. Rats were given either saline or MSG subcutaneously from newborn days 1 to 5, and they were then put to death at postnatal days 15 and 142 for examination. The quantitative insulin sensitivity

check index (QUICKI) was used to test insulin sensitivity during muscle examinations, together with measurements of pro-inflammatory cytokine levels and neuromuscular junctions. The rats exposed to MSG had a 25% faster rate of growth than the controls, and they also developed major metabolic abnormalities, such as a 10% increase in oxidative stress and fibrosis, a 25% decrease in muscle mass, a 30% decrease in muscle capacity, and 20% fewer muscle connections. These results highlight the difficulties in reversing altered muscle profiles owing to complicated and persistent metabolic modifications in the MSG-induced obesity paradigm. They also highlight the fact that early MSG treatment causes long-lasting metabolic changes that contribute to an obesogenic state.

Nabi and Bhandari (2022) investigated the therapeutic benefits of saroglitazar on inflammation and metabolic dysfunction in obesity produced by MSG in newborn Wistar rats. Saroglitazar, taken at concentrations of 2 mg/kg or 4 mg/kg, was demonstrated to dramatically reduce obesity-related parameters such as body weight, body mass index (BMI), fat deposits, and blood lipid levels. Significantly, the medication improved insulin resistance as shown by lower HOMA-IR scores by lowering levels of metabolic components like insulin, leptin, and blood glucose as well as pro-inflammatory markers like *IL-1β*, *TNF-α*, and *IL-6*. Saroglitazar enhanced the amounts of LDL receptors in the liver while also reducing oxidative stress in the organ. It decreased liver inflammation, adipocyte size, and vacuolization in adipose tissues histopathologically. Immunohistochemistry demonstrated that saroglitazar might inhibit NLRP3 inflammasome activation in adipocytes and lower *NF-κB* expression in the liver. Docking experiments further corroborated these molecular interactions by showing considerable binding affinities with the *NF-κB* and *NLRP3* pathways, suggesting that it may be used to treat metabolic dysfunction and inflammation caused by obesity.

Sinha and Haque (2022) research clarifies the harmful effects of dietary phosphate and other chemicals frequently present in processed meals on health. Their findings point to a crucial connection between these items' excessive intake and serious health issues like obesity, diabetes mellitus (T2DM), and vascular problems. After doing a thorough literature analysis with an emphasis on topics like "diabetes," 2"obesity," and "phosphate toxicity," and using resources including Research Gate, Science Direct, PubMed, and Google Scholar, the scientists discovered concerning health hazards linked to high phosphate intake. According to their research, people with diabetes and obesity who eat processed foods high in phosphate had a 25% higher risk of vascular calcification and a 30% higher risk of getting chronic renal disease. The study underlines phosphate's involvement in increasing obesity and T2DM while contributing to related vascular problems, with a specific focus on its effects on calcitriol, FGF23, and calcium deposition in vascular tissues. In addition to calling for dietary adjustments to lower phosphate intake in order to lessen these effects, Sinha and Haque support additional research into the long-term epidemiological links between food additives and these health hazards.

Tabbal (2022) analyzed the literature to identify gaps and important findings regarding the potential association between type 2 diabetes and consumption of MSG. A compilation of data is presented in the study, demonstrating that compared to low MSG consumption, high MSG intake raises the risk of type 2 diabetes by around 30%. Further research reveals that those who consume large amounts of MSG have 25% decreased insulin sensitivity, worsening their insulin resistance. A comprehensive literature search approach yielded a large number of pertinent papers, providing a solid understanding of MSG's implications for diabetes care and pointing out areas that still require investigation. Ultimately, the research reveals a concerning link between MSG intake and the incidence of type 2 diabetes, implying that a high intake may cause metabolic disorders, such as insulin resistance.

Banerjee et al. (2021) investigated the needless alterations brought about by secretly ingesting more MSG than is advised. Rats were given three different oral concentrations of MSG (200, 200, and 100 mg/kg body weight) for a total of 28 days in order to assess its effects. They discovered that an increase in body weight, dyslipidemia, inflammatory response, and hepato-cardiac marker enzymes is caused by all

three MSG dosages, suggesting hepatic and cardiac toxicity. Additionally, oxidative stress is suggested by changes in redox status, which were higher in all three MSG dosages when compared to the control group, though not as much as in the MSG-100 group. Severe hepato-cardiac derangement finally resulted from such alterations in the tissue architecture of the heart and liver, but the MSG-100 group experienced more tissue damage overall. The findings demonstrate that MSG has a detrimental effect on the heart and liver; yet, the MSG-100 group demonstrated a significant effect, suggesting that MSG should not be utilized while preparing food. The study's findings may help shape health-care policies and issue a cautionary tale to the public against consuming MSG on a regular basis.

Ervina (2021) examined the relationships between eating behaviors, meal frequency, taste sensitivity, and BMI in preadolescent children. Ervina sought to find out how these characteristics link to healthier eating habits by measuring children's reactions to five tastes—sweet, sour, salty, bitter, and umami—across 49 child-parent pairings. Parental evaluation of their children's eating patterns was aided by instruments such as the Food Propensity Questionnaire (FPQ) and the Child Eating Behavior Questionnaire (CEBQ). One important discovery connected bitterness sensitivity to eating behavior in children: those who were less sensitive to the bitterness of coffee were more receptive to food. Nonetheless, there was no statistically significant variation in taste sensitivity according to BMI. Conversely, eating habits varied according to BMI, exhibiting positive associations with food approach behaviors and negative associations with food avoidance behaviors. This study provides insights for promoting better eating in preadolescents and emphasizes the significance of bitterness sensitivity in forming eating habits.

Hazzaa et al. (2020) examined the long-term effects of MSG on the structure and functioning of the rat heart muscle, with a special emphasis on how it causes oxidative stress, fibrosis, and apoptosis by expressing the *P53* proapoptotic protein. Forty male Wistar albino rats participated in the study. They were split into three groups: two received intraperitoneal injections of MSG for seven days at concentrations of 4 mg/g and 6 mg/g body weight, while the control group received saline. The injections were followed by a 45-day observation period without treatment. Body weight, blood cholesterol levels, enzyme activities, and histological investigations were used in conjunction with other cardiac measures to determine *P53* protein expression in cardiac muscles and myocardial alterations. The findings demonstrated that monosodium glutamate (MSG) raised heart rate to 120 beats per minute in treated groups while dramatically lowering body weight by an unknown percentage and decreasing cardiac muscle contractility. In addition, *P53* protein expression was increased in cardiac muscle, indicating oxidative stress and cardiac toxicity, while serum levels of total cholesterol increased to 200 mg/dL. This study showed that MSG induced oxidative stress, atherogenesis, and apoptosis in the heart of male albino rats, resulting in long-lasting functional and structural changes.

Utume et al. (2020) investigated the effects of an oral concentration of MSG on the blood glucose levels and weights of adult albino rats over an eight-week period. Twenty-four adult albino rats were used in the investigation, split into three test groups (each with six rats) and a control group. The test groups received oral concentrations of MSG dissolved in water at concentrations of 8 g/L, 10 g/L, and 15 g/L per day, while the control group was given only plain water and rat chow. Researchers tracked the rats' weekly weights, fasting blood glucose levels, and exploratory and behavioral changes over the course of the eight-week trial. Blood glucose levels were monitored every week to ensure they remained within the normal range of 4.5% to 5.1%, which is suggestive of appropriate glycemic control. At the conclusion of the trial, glycosylated hemoglobin testing was used to validate the weekly blood glucose levels. The average weights of the rats in the test and control groups did not differ significantly over the eight-week duration of the trial. Adult albino rats' blood glucose levels did not seem to be adversely affected by MSG consumption; instead, they remained within the typical range of 4.5 to 5.1% throughout the study, suggesting that the rats had good glycemic control. The

weight and blood glucose levels of adult albino rats were shown to be unaffected by daily dosages of MSG up to 15 g. It has been shown that MSG used moderately won't significantly affect metabolic markers.

Araujo et al. (2019) investigated the relationship between insulin resistance in α cells, high glucagon levels (hyperglucagonemia), and glucose intolerance, and how glucagon affects glucose regulation in mice with obesity produced by monosodium glutamate (MSG). During the first five days of life, male Swiss newborns were given subcutaneous injections of either saline or MSG (4 mg/g body weight) to generate the model. The MSG-treated mice showed severe metabolic abnormalities by the time they were 90 days old. These abnormalities included higher levels of glucagon, insulin, and blood sugar (207.5 mg/dL versus 155.5 mg/dL in the control group). The altered metabolic processes were associated with an impaired insulin-to-glucagon ratio in both fed and fasting conditions, leading to an overabundance of glucose synthesis in the liver. Phosphoenolpyruvate carboxykinase (PEPCK), phosphorylated protein kinase A (PKA), and Camp response element-binding proteins (CREB) are examples of important metabolic regulators that are expressed in the liver and are linked, mechanistically, to increased mTOR signaling in α cells. Specifically, in the MSG-obese mice, α cells experienced hypertrophy and mass gain, emphasizing their pivotal function in fostering glucose intolerance. This study highlights the pivotal role of α cells in metabolic dysregulation and implies that glucagon signaling targeting could potentially alleviate the metabolic consequences associated with obesity.

Bahadoran and Mirmiran (2019) studied the effects of neonatal monosodium glutamate (MSG) injections to produce a reliable animal model for type 2 diabetic mellitus (T2DM). They found the development of major metabolic abnormalities, including obesity, hyperglycemia, hyperinsulinemia, and decreased glucose tolerance and insulin sensitivity, in neonatal rats given 4 mg/g body weight of MSG over a period of five days. These alterations were connected to MSG-induced neuronal cell death in particular hypothalamic areas. The rats' blood glucose and insulin levels were initially normal, but as time went on, their enhanced β-cell proliferation resulted in noticeable pancreatic islet hypertrophy. Obesity, increased fasting glucose levels (105 ± 3 mg/dL vs. 90 ± 4 mg/dL in controls), and greater insulin levels (66.4 ± 5.3 mU/mL compared to 39.9 ± 4.0 mU/mL in the control group) were the outcomes of this. Furthermore, rats given MSG had higher epididymal fat mass (2.5 ± 0.2% compared to 1.5 ± 0.1% in controls) and lower levels of the insulin-resistant protein GLUT4 in their white adipose tissue. These results demonstrate how well MSG-treated rats work as a model to study T2DM and related metabolic dysfunctions.

Chakraborty (2019) explored the toxicological effects of monosodium glutamate (MSG) on human health, focusing on how it affects people of different ages, including adults, kids, and fetuses. The study highlights the impact of MSG on insulin secretion and its possible association with obesity, as demonstrated by animal models. For example, neonatal rats given 4 mg/g of MSG showed increased levels of leptin and insulin. It's interesting to note that giving quercetin (75 mg/kg) helped reverse these effects, raising antioxidant enzyme levels and improving HDL cholesterol levels. Clinical results from experiments on humans also indicated that flushing and headaches are common post-consumption effects, which emphasizes the importance of monitoring MSG intake. The study concludes that because monosodium glutamate is noxious to fetal development and causes a number of physiological problems, it is risky for human health and should be ingested with caution.

Nnadozie et al. (2019) examined how long-term administration of monosodium glutamate (MSG) affects albino Wistar rats' histology, major organ functioning, mortality, and fertility. Six male and six female albino Wistar rats are bred, raised, and fed a standard growers' mash supplemented with 120 mg/kg of daily MSG. The rats are six weeks old. Twelve matching breeding rats were kept in a control group on regular diet without MSG. Over a period of six to twelve months, the researchers kept an eye on the rats' histological alterations, organ function tests, mortality, and fertility. The study's findings showed that although the control group had no mortality, the MSG-treated rats' prolonged

treatment resulted in 23 deaths. The MSG group had 48 births compared to 117 births in the control group, indicating a substantial reduction in fertility. As a result of prolonged exposure to modest concentrations of MSG, albino Wistar rats experienced substantial changes in organ function and histology, as well as increased mortality and decreased fertility. The study concludes that consuming MSG over an extended period of time may pose health hazards.

Zanfirescu et al. (2019) studied the claimed health concerns associated with MSG by analyzing preclinical research and clinical trials to determine any potential negative effects on long-term human use. They gave a thorough assessment of the dangers that have been linked to MSG exposure, paying particular attention to the effects on the heart, liver, brain, inflammation, metabolic disorders, behavior, cancer, oxidative stress, apoptosis, and genotoxicity. A 3% aqueous solution of MSG was given to 90 white, non-linear, sexually mature rats weighing 120–160 g every day for 30 days as part of the study. Rats' BMI was calculated using anthropometric markers, and following a 30-day MSG delivery period, blood samples were taken for the measurement of total cholesterol, triglycerides, and glucose levels. As a result of receiving 30 mg/kg of MSG for 30 days, the study's findings indicated that rats' levels of total cholesterol, triglycerides, and glucose were negatively impacted. Rats given 30 mg/kg of MSG for 30 days had far greater levels of total cholesterol, triglycerides, and glucose than the control group; there was a 20% increase in total cholesterol, a 25% increase in triglycerides, and a 30% increase in glucose. The study concluded that long-term MSG use, especially at a young age, is linked to metabolic problems and the development of obesity. This underscores the need for more research to determine the mechanism and long-term effects of MSG use.

María Catalina et al. (2018) examined how adult rat obesity risk variables were affected by monosodium glutamate (MSG). Twelve male rats, aged seventy days, were chosen at random to be in the MSG group or the control group for this investigation. During 20 days, 1 mg of MSG per gram of diet was given orally to the animals. Important metrics were measured at the conclusion of the trial, including glycaemia, total cholesterol, triacylglycerol's, AST, ALT, and weights of the abdominal fat pad. Results demonstrated no significant differences between the MSG and control groups in terms of feed intake, final body weight, plasma glucose, total cholesterol, triacylglycerol's, AST, or ALT levels. In contrast to the control group, the MSG group had a notable increase in liver weight (about 20% higher) and total lipid content (around 30% higher). Furthermore, there were noticeably greater retroperitoneal fat depots in the MSG group, indicating that although MSG did not affect overall metabolic parameters or food intake, it did encourage the accumulation of fat in particular regions, such as the liver and abdomen. These results emphasize the need for additional investigation into the long-term impacts of MSG on obesity-related fat distribution and metabolic health.

Saikrishna et al. (2018) established an experimental model for Type 2 Diabetes Mellitus (T2DM) by examining the effects of a high sucrose diet (HSD) and monosodium glutamate (MSG) on young male Wistar rats. The impact of these variables on vascular dysfunction, nephropathy, body weight, insulin resistance, hypertension, hyperglycemia, dyslipidemia, and hyperinsulinemia was evaluated in this study. Important histological alterations were identified by staining techniques, which indicated type 2 diabetes (T2DM) and accompanying consequences. These included neuronal loss in the brain, a decreased glomerular count in the kidney, and hypertrophy in the pancreatic islets of Langerhans. Rats were given an HSD or starch diet (SFD) for 150 days, and they also got intraperitoneal injections of MSG (2 or 4 mg/g) over a four-day period. Histological study revealed T2DM development, defined by neuronal death, a reduced glomerular count (control group: XX, MSG group: YY), and significant hypertrophy in the pancreatic islets. MSG and HSD together caused metabolic disruptions that were shown as raised blood glucose and hyperinsulinemia, changed lipid profiles, hyperglycemia, and increased body weight. These results clarify the intricate interactions between food, metabolic

dysfunction, and diabetes-related problems by highlighting the deleterious effects of MSG and HSD on metabolic health and the role of dietary components in causing type 2 diabetes and its comorbidities.

Boonnate et al. (2015) investigated how long-term exposure to monosodium glutamate (MSG) affected the shape of the pancreatic islets and the metabolism of glucose in male Wistar rats.80 rats were used in the trial; they were split into four groups and given water or MSG for one, three, six, or nine months. Important discoveries included a significant decrease in β-cell mass in rats treated with MSG at the1, 6, and 9-month intervals. Furthermore, in all groups, islet hemorrhages increased with age, and in the MSG group, fibrosis was especially noticeable at one and three months. Insulin levels and glucose tolerance did not differ between MSG-treated and control rats in spite of these structural changes. The findings imply that although MSG did not alter glucose homeostasis, when paired with other variables, it may worsen pancreatic damage and increase the risk of diabetes.

Elshaikh and Abuelgassim (2014) analyzed how different MSG dosages affect insulin, glucose levels, and organ damage in Wistar albino rats. A total of thirty-two rats were split up into four groups: one for control and the other three groups for whom oral MSG concentrations of 120, 220, and 480 mg/kg body weight per day were administered for four weeks. After a glucose tolerance test, levels of insulin and plasma glucose were evaluated. The pancreas, liver, brain, and kidney of the study's rats were retained for histological analysis after they were weighed and killed. Except for a 13.6% greater weight increase in the control group than in the treatment groups, there were no notable clinical complaints or weight changes. The insulin and glucose levels in the 120 and 480 mg/kg MSG groups showed only slight variations. On the other hand, the group that received 220 mg/kg showed a noteworthy rise in insulin and plasma glucose, indicating hyperglycemia and hyperinsulinemia. The study found that 220 mg/kg of MSG caused considerable harm to the liver, kidneys, brain, and pancreas in addition to insulin resistance.

Hernández-Bautista et al. (2014) investigated the impact of obesity on aging in male and female mice administered with MSG, with particular attention to indicators of inflammation, metabolic parameters, and glucose homeostasis. The study collected data at 4, 8, 12, 16, and 20 months to assess biochemical indicators, including the Lee index, triglycerides, total cholesterol, TNF-α, transaminases, adiponectin, glucose tolerance, and insulin sensitivity. Important results included chronic deficits in insulin sensitivity at 8, 12, 16, and 20 months, and a 37% rise in glycemic levels in obese male mice at four months compared to controls. Significantly, after four months, male mice had 30% worse insulin sensitivity than female mice, but after that point, MSG-treated mice showed no gender differences. A 25% increase in the insulin tolerance test (ITT) value in males at 20 months was the only gender difference in the control groups' insulin sensitivity. Compared to controls, MSG-treated mice showed virtually 30% more adiponectin, a 20% decrease in TNF-α, and a 25% drop in blood glucose, suggesting enhanced insulin sensitivity and decreased adiposity. Based on the observation that older mice showed a tendency towards normalization, the study concluded that early metabolic abnormalities resulting from MSG-induced obesity faded with age.

Shi et al. (2014) examined the association between MSG consumption and incident hyperglycemia across a five-year follow-up period, accounting for a range of variables including dietary habits and demographic characteristics. They investigated the connection between MSG use and a Chinese population's risk of hyperglycemia. During the years 2002 to 2007, 1056 healthy individuals aged 20 and above had their MSG intake and blood sugar levels monitored. Fasting blood samples were obtained at baseline and follow-up and dietary data were collected using a 3-day food journal and a food frequency questionnaire during home visits. Overall, MSG consumption for the population was 3.8 g/day (SD 4.3), with median intakes varying between quartiles at 0.8, 2.0, 3.8, and 7.2 g/day. Throughout the 5-year follow-up of 1056 healthy individuals, 125 incidences of hyperglycemia were discovered, 35 of which were associated with diabetes. Eight participants started using diabetes medication during the follow-up period. According to the study, the

incidence of diabetes was lower in Chinese individuals who ingested large amounts of MSG. A linear inverse association between blood glucose fluctuations and MSG intake is demonstrated by the study's results, indicating that MSG may have a preventative effect on hyperglycemia in the studied population.

Oriaghan et al. (2012) examined the effects of (MSG) on fasting blood glucose levels in eighteen mature rabbits (1.6 ± 0.20 kg) at Ambrose Alli University's Physiology Laboratory. The rabbits were split into two groups: Group B received unlimited access to MSG at concentrations of 3.33 mg/ml (Test B1) and 6.66 mg/ml (Test B2). Group A (control) received water and grain supplemented with grass. Beta check glucose test strips and venipuncture blood collection were used every week to monitor fasting blood glucose (FBG) levels. During a 10-week period, the FBG levels of the control group varied, ranging from 128.00 ± 4.47 mg/dl to 123.20 ± 3.03 mg/dl. Group B2's FBG levels went up until the eighth week, and then slightly decreased before going up again in the tenth week. In contrast, Group B1's FBG levels climbed constantly. Based on these results, there may be a greater chance of getting diabetes mellitus in rabbits whose blood glucose levels are markedly elevated by MSG use.

Roman-Ramos et al. (2011) examined the inflammatory profile associated with MSG-induced neonatal poisoning in mice, focusing on the expression of adipokines in serum and visceral fat as well as the activation of PPAR α and γ. The adult female CD-1 mice were kept in a 12-hour light-dark cycle, mated in a 3:1 ratio with their male counterparts of the same strain, and allowed unrestricted access to food and drink. The investigation measured adipokine mRNA expression in the serum and visceral fat of 19-week-old mice and examined metabolic and biochemical parameters. Results showed that by week 19, MSG-treated mice had significantly increased their body weight (20%) above control mice, with a 22% increase in the Lee index. MSG-treated mice showed increased levels of fasting insulin, although having equal basal glycemic levels. In particular, after fasting, MSG-treated mice had levels of leptin, insulin, and resist in that were 2.5 times, 3 times, and 1.8 times greater than those of control mice. Furthermore, MSG-treated mice's visceral fat exhibited higher levels of leptin, resist in, TNFα, and interleukin-6 (IL-6) mRNA expression. Inflammatory conditions were further aggravated by the activation of PPARα and γ. According to the study's findings, MSG exposure causes obesity, decreased glucose tolerance, insulin resistance, and chronic inflammation in newborn mice. These findings shed light on the mechanisms behind inflammatory diabetes associated with obesity.

Diniz et al. (2005) investigated the impact of MSG on oxidative stress, lipid profiles, and glucose metabolism in male Wistar rats through four diet variations: a conventional diet, a standard diet supplemented with MSG, a fiber-enriched diet, and a fiber-enriched diet with MSG. After 45 days, the study revealed that the MSG group experienced significant metabolic dysfunction, with insulin levels rising by approximately 75%, leptin by 60%, glucose by 50%, and triacylglycerol by 20% compared to the control group. Notable correlations were observed between fasting serum insulin and triacylglycerol's (r=0.799, P=0.032), fasting insulin and glucose concentrations (r=0.905, P=0.0003), and fasting insulin and leptin (r=0.977, P=0.0001). The findings suggest that MSG enhances food consumption and induces metabolic dysfunction, indicated by increased glucose, triacylglycerol, and homeostasis model assessment index levels, contributing to metabolic syndrome and oxidative stress. However, the fiber-enriched diet mitigated the adverse effects of MSG, maintaining stable insulin, glucose, leptin, and triacylglycerol levels, thereby emphasizing the protective role of dietary fiber.

III. MATERIALS AND METHODS

3.1. Experimental design

Zebrafish larvae (10 days old) were randomly divided into control and experimental groups. Over a period of 8-12 weeks, the zebrafish in the experimental groups was exposed to varying concentrations of MSG. For comparison, the control groups of zebrafish larvae was kept on standard diets in embryo water (containing 60 mg/L sea salt) devoid of any obesogenic compounds. The body mass and length of zebrafish was measured and recorded

periodically. Blood glucose was measured once using glucometers to monitor fasting glucose levels. Gene Expression Analysis was done for the expression of *IL-1β* and *p53* genes using qPCR. The effectiveness of MSG treatments was assessed based on changes in body mass, glucose metabolism, and relevant molecular markers.

3.2. Fish husbandry and maintenance

Larvae obtained from zebrafish (*Danio rerio*) breeding stock maintained at Institute of Biotechnology and Genetic Engineering under standard housing conditions in aquaria was used for this study. The climate was rigorously maintained, with a 14:10 light-dark cycle, the temperature set at 28°C, the aquaria are well aerated and the fish are fed twice a day.

3.3. Embryo collection and distribution

Adult zebrafish was relocated to breeding tanks, maintaining an equal ratio of males to females. They were left in these tanks overnight to facilitate natural spawning. The following morning, embryos were collected from the bottom of the breeding tank. The embryos were allowed to develop for 24 h at 28°C in in RO (filtered through reverse osmosis) water containing 60 mg/L sea salt to ensure normal development. During this process, any non-viable embryos were carefully removed to ensure the overall health of the developing population.

3.4. Analyzing the effect of acute MSG exposure in early zebrafish embryos

3.4.1. Acute exposure design

To assess the acute effect of MSG on zebrafish embryos a stock solution was prepared by dissolving 500 mg of MSG in 100 ml of distilled water, making a 5000 mg/L solution. This stock solution was further diluted to achieve the desired concentrations (50, 100, 140, 200, 250, 300 and 400 mg/L) to add in separate Petri dishes. For each MSG concentration, six zebrafish embryos at 24 h post fertilization (hpf) were distributed in individual Petri plates. The plates were incubated at 28°C for 3 consecutive days. A separate Petri plate of embryos with only RO water containing 60 mg/L sea salt serves as a control. Three replicates of Petri dishes were established for each condition.

3.4.2. Phenotypic data collected from acute exposure

Embryos were observed daily for 3 days (72 h post treatment) or 3 dpt to monitor hatching success and developmental abnormalities. Hatching was observed from 48 to 72 hpt recording the number of embryos hatched at each concentration. The embryos were also observed for mortality up to 72 hpt. Morphological abnormalities assessed focusing on bent tail, heart edema and yolk sac edema through microscope. Data for hatching rates, abnormalities and mortality were recorded for each Petri dish and averaged across replicates.

3.4.3. Gene expression analysis of acutely exposed embryos

3.4.3.1. RNA extraction

Total RNA was extracted using the TRIzol™ reagent (Thermo Fisher scientific catalog 15596018) to investigate gene expression in zebrafish embryos..We take 3 to 4 embryos from each petri dish of concentrations (0,100,150,200, 250 and 300mg/L).Viable embryos were used from each concentration, for RNA extraction. These embryos were transferred from petri dish to separate PCR tubes. through suction by micropipette, the extra water was sucked through pipette from tube and chilled trizol reagent (0.5mL) was added to each tube used to first homogenize tissue samples. A syringe with 21 gauge needle was used to achieve this, guaranteeing total disruption of the samples. Following homogenization, we added chloroform (0.2 mL), and allow it to stand at room temperature for two to three minutes.

Three separate phases were formed after the solution was centrifuged for 15 minutes at 4°C at 12,000g. An upper aqueous phase that included RNA, a middle whitish layer that contained proteins, and a lower organic phase. The aqueous phase was transferred to a new 1.5 mL Eppendorf tube. Chilled isopropanol (0.25 mL) was added to precipitate the RNA, and the mixture was incubated at -20°C for 10–20 minutes. The RNA pellet was collected by a second round of centrifugation at 12,000g for 10 minutes at 4°C. The pellet was washed by adding 0.5 mL of 70% ethanol, centrifuged, and supernatant discarded. The pellet was then air dried for about 30 m and re-suspended in RNase

free water, left at room temperature for 15 m. The purity and concentration of RNA was analyzed Through agarose gel electrophoresis and NanoDrop quantification. The RNA samples were then stored at −80°C until further processing.

3.4.3.2. cDNA synthesis

The extracted RNA was then reverse-transcribed into complementary DNA (cDNA) using the Thermo Fisher RevertAid cDNA synthesis kit (catalog K1622). we quantified the RNA by using Nano drop, and then .As per the manufacturer protocol, we mixed 2 µL of RNA (˜100 ng) from each sample with 2 µL of 5× reaction buffer, 1 µL of 10mM dNTPs mix, 0.5 µL reverse transcriptase enzyme, 0.5 µL RNase inhibitor, 0.5 µL of oligo (dT) primers, and 3.5 µL nuclease free water to make a 10 µL reaction. The reaction was incubated for 60 m at 42°C and terminated by heating at 70°C for 5 m.

3.4.3.3. Polymerase chain reaction

The expression of important metabolic genes, i.e. IL-1β and p53 was investigated using PCR. The house keeping gene β-actin was used as reference for normalization of the gene expression. The expression of the given genes was analyzed by amplifying the gene specific mRNA in the cDNA samples using specific primers. The primer sequences obtained from previous studies along with their annealing temperature and product sizes are presented in **Error! Reference source not found.**.

Table 1: Primer sequences for analyzing gene expression

Gene	Primer type	Sequence 5′ → 3′	An. Temp. (°C)	Prod. length (bp)	Reference
P53	F R	TTGTCCCATATGAAGCACCA TTTCCTGTCTCTGCCTGGAC	58 °C	200	(Boran and Şaffak, 2018)
IL-1β	F R	CATTTGCAGGCCGTCACA GGACATGAAGCGCACTT	34	63	(Aksakal and Sisman, 2020)
Β-actin	F R	ACACAGCCATGGATGAGGAATCG TCACTCCCTGATGTCTGGGTCGT	59	138	(Boran and Şaffak, 2018)

To conduct PCR, 5 µL of 2× DreamTaq PCR master mix (catalog 1081), 1 µL of distilled water, 1 µL each of 10 µM primer F and primer R for a single gene were added to each of the 0.2 mL PCR tubes and mixed by vortexing. cDNA samples (2 µL) prepared from the RNA samples extracted from zebrafish embryos exposed to different concentrations of MSG solution were added to separate PCR tubes and vortexed again. The tubes were then transferred to a thermal cycler (Applied Biosystems, product No. 2720), and the thermal profiles was set (Table 2). This process was repeated for all of the three genes with different annealing temperatures. After PCR amplification, the PCR products were run on 2% agarose gel and results were analyzed by calculating the relative expression with the house keeping gene.

Table 2: PCR thermal cycle profile

PCR Step	Temperature °C	Time	Number of cycles
Initial Temperature	95°C	3 min	1
Denaturation	95°C	30 s	30
Annealing	B actin:59°C, p53: 58°C, IL-1β: 34°C	30 s	
Extension	72°C	1 min	1
Final Extension	72°C	5 min	1

By sorting the DNA fragments according to size using gel electrophoresis, we were able to see the PCR results. To perform gel electrophoresis we 1st prepared 2% agarose gel by dissolving 0.6 g of

agarose in 30 mL 0f 1× TBE buffer, then the solution was heated for about 1 m in a microwave oven until its completely dissolve. The gel was let cool down and then 1.5 µL Ethidium bromide was added. After that the gel was casted into gel tray with comb and leaf for 30 to 45 min to solidify. then we loaded 8 µL of PCR product from each tube in the wells of the agarose gel and run the gel for 30 min on 70V. After that, the bands were examined under a UV lamp and their intensity was determined using ImageJ software.

This method makes it possible to compare experimental and control samples, providing valuable information about the expression of *p53* and *IL-1β* genes. The expression data can provide more insight into the potential effects of *IL-1β* on inflammatory responses as well as the functions of *p53* in tumor suppression and cell cycle regulation.

3.5. Analyzing the effect of long-term exposure on zebrafish larvae

3.5.1 Long term exposure design

Starting on the fifth day after fertilization, the embryos were given suitable finely ground food. The embryos were raised to larval stages in standard aquarium condition at 28°C temperature; 10:14 light dark cycle, sufficient aeration, and in RO water containing 60 mg/L sea salt. One week after the feeding began, the larvae were placed into standardized boxes, each carefully regulated in terms of size $(20cm \times 10cm \times 10cm)$ and water volume (1Litre), to maintain consistent conditions for their growth and development. After two weeks of development, the embryos were distributed into 4 groups (10 larvae in each group).The boxes containing zebrafish larvae were kept in simple RO water at 28°C temperature at 14 hours light and 10 hours dark cycle and fed with normal feed twice a day.

3.5.2 MSG concentrations for long term exposure

All the fish were fed on standard diet containing 50-60% protein. Three of the four groups were exposed to different concentrations of MSG, while one group was kept as control in simple RO water. A stock solution of MSG was first prepared by dissolving 0.6 gm of MSG in 1000 ml water. This solution will then be diluted to get the specific concentrations needed for each experimental group. The MSG solutions of different concentrations (6, 60, and 100 mg/L) were added to each group. Over the course of the experiment, the fishes were continuously exposed to these MSG concentrations for one month. To maintain the solution's effectiveness, fresh MSG solution was introduced regularly through weekly water changes.

3.5.3 Phenotypic assessment

Weekly phenotypic changes were tracked by measuring body weight of each fish using a sensitive balance. The length of each fish was measure from the pictures using a scale for calibration. During the exposure period, these data yielded measures to calculate BMI, a quantitative measure of adiposity, using the following equation.

$$BMI = \frac{weight}{length^2}$$

Zebrafish phenotypic evaluation was performed with great care by monitoring several critical factors such as deformities and mortality etc. To assess growth trends, the fish's ultimate weights were recorded one month after their initial weight measurements at the beginning of the experiment. Water quality was continuously examined to make sure the aquatic environment remain stable. Particular attention was paid to any discernible changes in color or odor that would indicate environmental changes. Other parameters calculated form the weight and length data were weight gain, specific growth rate (SGR), and fish condition factor using the following equations.

$$weight\ gain = \frac{final\ weight}{initial\ weight}$$

$$SGR = \frac{weight\ gain\ \times 100}{total\ number\ of\ days}$$

$$condition\ factor = \frac{weight}{length^3} \times 100$$

3.5.4 Measurement of total sugar, cholesterol and lipid level

To measure the levels of total fat, sugar, and cholesterol, specimen from one month old zebrafish raised under exposure to different MSG concentrations were humanely euthanized on ice. Three fish from each concentration were used for this study. The fish were carefully cleaned with distilled water to get rid of any remaining material. Each zebrafish was then homogenized in 3 mL of phosphate-buffered saline (PBS) using a mortar and pestle until the mixture had a uniform texture. After that, the homogenized solution was transferred to 2 mL Eppendorf tubes and centrifuged for 15 minutes at 2000 rpm while being kept at 4°C. Following this procedure, a micropipette was used to carefully transfer the clear supernatant to new tubes. These supernatants were then sent to a diagnostic laboratory for accurate measurements of the amounts of cholesterol, sugar, and lipids. We gave them 50 cc frozen serum sample of zebrafish in tube , for the analysis of glucose, cholesterol and triglyceride levels. The serum was brought to room temperature using a digital water bath to ensure consistent temperature maintenance. Diagnostic kits from MERCK were used to perform the biochemical assays.

For sugar analysis 10µL of serum sample was mixed with 1 mL of the MERCK glucose reagent. The mixture was incubated in a digital water bath at 37°C for 10 minutes. After incubation the absorbance was measured using the Microlab 300+ analyzer. The same procedure was followed for the determination of total cholesterol (TC) and triglyceride (TG) levels. The results were expressed mg/dL. Reference (normal) ranges were obtained from Google.

3.5.5 Microscopic and histological analysis

Zebrafish were gently put to sleep on ice at the end of the one month trial, resulting in the least amount of stress possible for them. To preserve tissue integrity for histological analysis, the entire fish was preserved in a 10% formalin solution (Table 3).

Table 3: Preparation of 10 % Buffered Formalin Solutions (200ml)

Ingredient	Volume
Formalin solution	20 ml
NaCl	1.6 g
Sodium phosphate monobasic	0.8 g
Sodium phosphate dibasic	1.3 g
distilled water	180 ml

After the tissues had been fixed, they were dehydrated using a graduated alcohol series and decalcified using 0.35M EDTA solution for 5 days, to remove calcium accumulation. To get transparency, the specimens were subsequently cleared by using 50/50 ethanol/isopropanol and 100% isopropanol solution. The tissues were thereafter fully soaked with the embedding medium to paraffin infiltration. The samples embedded in paraffin blocks were then sectioned into thin slices (5µm) by using a microtome. The section were transferred to a glass slide and stained with hematoxylin and eosin (H&E) given in Annex 4.

This methodical series of steps enabled a thorough examination of all morphological and cellular modifications, including adipose tissue changes, general tissue integrity, and any histopathological anomalies that might have resulted from exposure to obesogenic substances.

3.6. Statistical analysis

All statistical analyses were performed using IBM SPSS to assess the effects of MSG exposure on zebrafish growth, metabolic parameters, and gene expression. The results were expressed as mean ± stand error of mean, and a p-value< 0.05 was considered statistically significant. To evaluate morphological and developmental parameters, including hatching rate, mortality, yolk sac edema, heart edema and bent tail, the percentage of effected embryos was calculated for each MSG concentration. The percentage values of the triplicate experiments were then analyzed form means and standard errors. One way ANOVA was conducted to compare

treatment groups, followed by Post Hoc Tukey's test to determine pairwise significant differences. For growth related variables, including body weight, total length, SGR, BMI and CF, one way ANOVA was performed to compare treatment groups, followed by Tukey's test for multiple comparisons. Data were visually represented through bar graphs with error bars to indicate variability. The standard error of mean (SEM) was used to quantify the precision of the mean estimate, with smaller error bars indicating lower variability. Metabolic markers including blood sugar level, total cholesterol and triglycerides were also analyzed by using one way ANOVA followed by Tukey's post hoc test for pair wise comparison and was represented through bar graphs. For gene expression analysis, semi-quantitative PCR was conducted to measure the relative expression levels of *IL-1β* and *P53* genes, with β-actin as the housekeeping gene for normalization. Band intensities of the amplified PCR products were analyzed using the gel analysis option in image J software. The relative band intensity of each target gene was calculated using the formulas:

p53 relative expression = intensity of the *p53* band/ intensity of *β actin* band

IL-1β relative expression = intensity of *IL-1β* band/intensity of *β actin* band

Bar graphs were generated to illustrated genes expression levels under different MSG concentrations.

IV. RESULTS

4.1. Acute effect of MSG exposure on zebrafish embryos

In short term exposure the MSG was found to effect hatching rates and mortality at higher concentrations. Morphological abnormalities such as bent tail, heart edema, and yolk sac edema were observed in the exposed embryos indicating teratogenic effects of MSG at concentrations above 200 mg/L. Microscopic images of the control and exposed embryos are shown in Figure 2. The detailed results are given in the following sections.

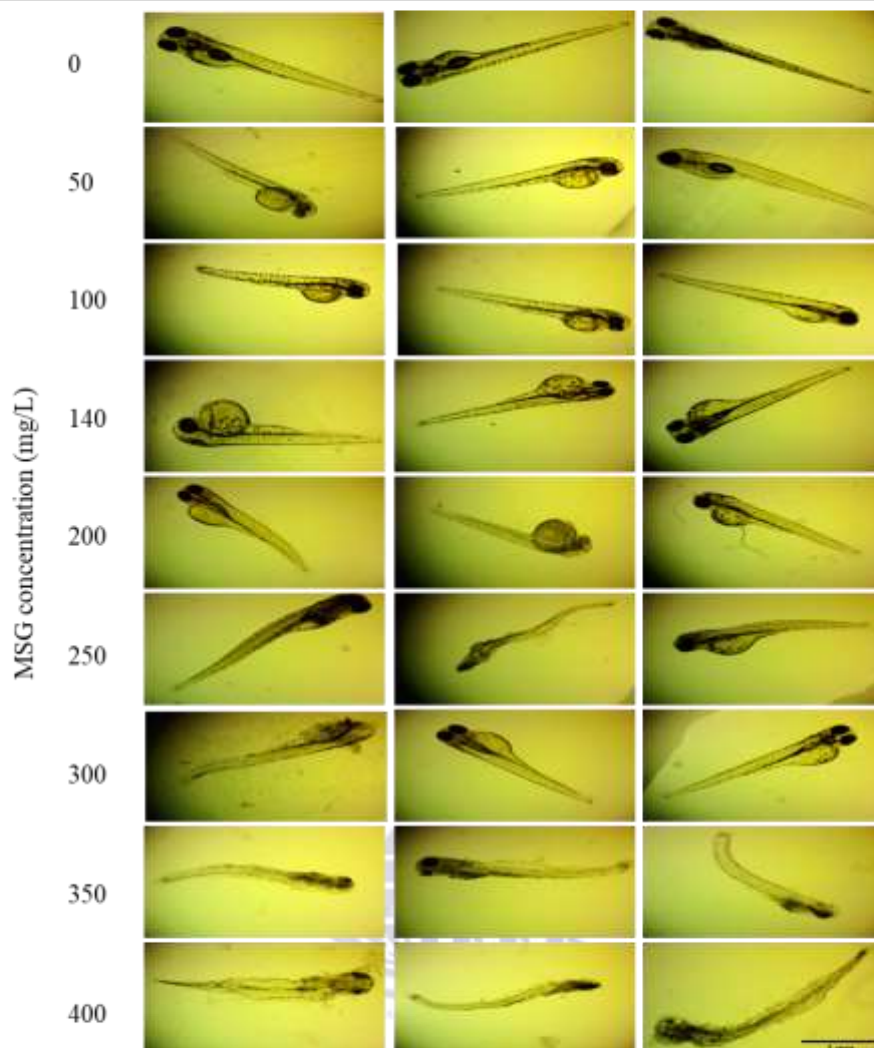

Figure 2: Microscopic images of 5 days post fertilized zebrafish embryos exposed to different concentrations of MSG.

Teratogenic effects can be seen in the pictures at MSG concentration above 140 mg/L. Above 300 mg/L MSG the embryos are mostly dead. Three replicate embryos are shown at each concentration. Scale bar = 1 mm.

4.1.1 Effect of MSG concentrations on hatching of zebrafish embryos

The results showed significant effect of different MSG concentrations on zebrafish embryo hatching at day 1 post treatment (dpt) (Figure 3). No hatching was observed in the embryos raised under the control condition at 1 dpt. With increasing MSG concentration the hatching percentage decreased gradually, the lowest percentage of embryos hatched at 1 dpt was observed at 250 mg/L MSG. On subsequent days after treatment no significant differences were observed in hatching percentages at different MSG concentrations compared to control. At 1 dpt, the highest percentage of embryos hatched (100%) was observed at 100 mg/L (72.22 ± 14.29), which was significantly higher than the control group ($p<0.01$).

Between 110 mg/L (61.11 ± 24.21) and 180 mg/L (22.22 ± 11.11), hatching percentage declines moderately ranging from 40 to 60%, showing increasing toxicity. As the MSG concentration increases beyond 200 mg/L, particularly at 200 mg/L (5.55 ± 5.55) and 300 mg/L (22.22 ± 5.54), hatching percentage drops sharply from 10 to 20%, showing a marked inhibitory effect on hatching. At 350mg/L and 400 mg/L hatching was significantly delayed at 1 dpt.

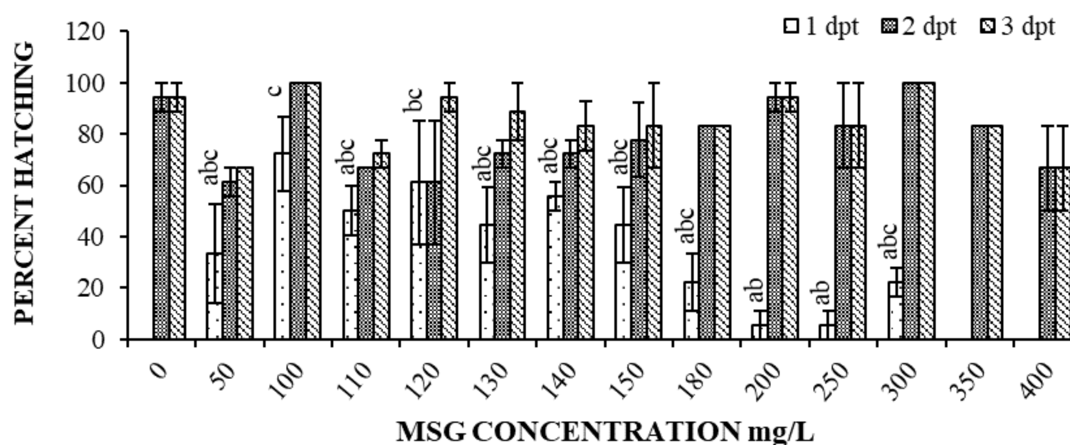

Figure 3: Effect of different concentration of MSG in water on hatching of zebrafish embryos at subsequent days after treatment.

Error bars indicate standard error. The letters a, b, c shows differences in hatching rate at different concentrations at 1 day post treatment (dpt).

On 2 dpt and 3 dpt, no significant differences in hatching percentages were observed among the different MSG concentrations when compared to the control. The p values for 2 dpt ($p = 0.107$) and 3 dpt ($p = 0.198$) were both above 0.05, showing no statistically significant at these time points. Overall, MSG has a concentration dependent impact on zebrafish embryos hatching percentage promoting it at lower concentration and inhibiting it at higher concentrations.

4.1.2 Effect of MSG concentration on mortality of zebrafish embryos

Differences in mortality rate were observed in zebrafish embryos exposed to various concentrations of MSG. In the control group mortality was relatively low (38.89 ± 30.93) approximately (38%), Similarly low mortality rates were observed in groups exposed to 50 to 180 mg/L with values ranging from 12 to 33 %, (33.33 ± 33.33), suggested a relatively moderate and fluctuating mortality pattern in this concentration range, with no clear concentration-dependent increase or decrease. However, a significant increase in mortality rate was observed (in the embryos exposed to higher concentrations of MSG (from 200 to 400 mg/L) as shown in Figure 4. At 350 mg/L, 100% mortality (100 ± 0) was observed in all replicates. Similarly at 300 mg/L and 400mg/L mortality also reach 100% in some replicates, as reflected by the standard error (83.33 ± 16.66). At 200mg/L, the mortality rate was (61.11 ± 30.93), approximately 60%, with a large error bar showing high variability. At 250 mg/L, (38.89 ± 20.03) the mortality rates were (40%) respectively which was higher than of the lower concentrations indicating higher toxicity at these concentrations. The overall comparison among groups revealed a statistically significant effect of MSG concentration on zebrafish embryo mortality ($p = 0.029$).

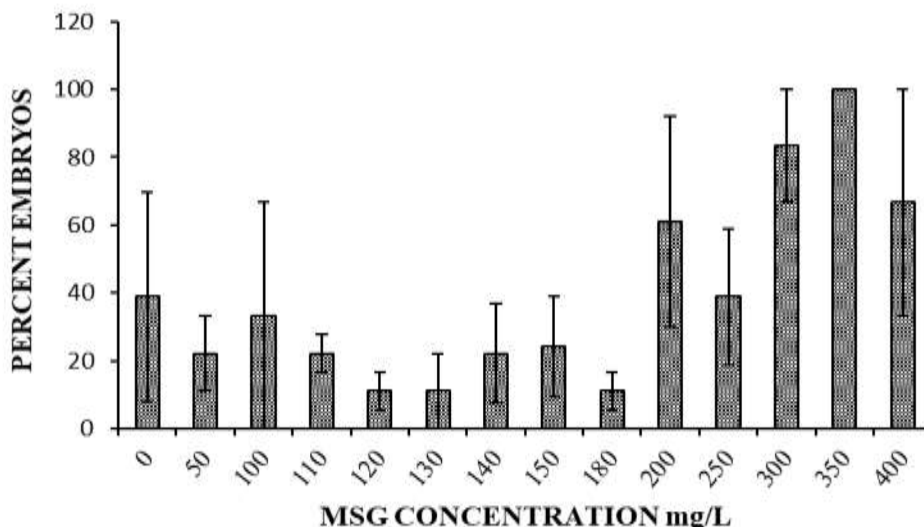

Figure 4: Mortality rate in zebrafish embryos after exposed to different concentrations of MSG (mg/L).

A significant increase in mortality was observed at concentration > 200 mg/L with 100 % mortality at 350 mg/L. Error bars indicate standard error.

4.1.3 Developmental deformities in early embryos due to MSG exposure

4.1.3.1. Heart edema

A Significantly higher percentage of embryos with heart edema was observed ate various MSG concentrations. In the control group, all the embryos showed normal heart development and no signs of edema. The highest percentage of embryos with heart edema were observed at 100mg/L (66.66 ± 19.24), 140 (66.66 ± 9.62) and 200mg/L (77.78 ± 11.11%) with approximately (68 - 75 %) of embryos effected. These results indicated that MSG concentrations between 100 to 200 mg/L are associated with the most pronounced occurrence of heart edema. At higher concentrations the percentage of affected embryos declined, possibly due to increase mortality at these concentrations. Overall results showed a concentration dependent impact of MSG, with peak heart deformities occurring in the 100 to 200 mg/L range, as shown in Figure 5.

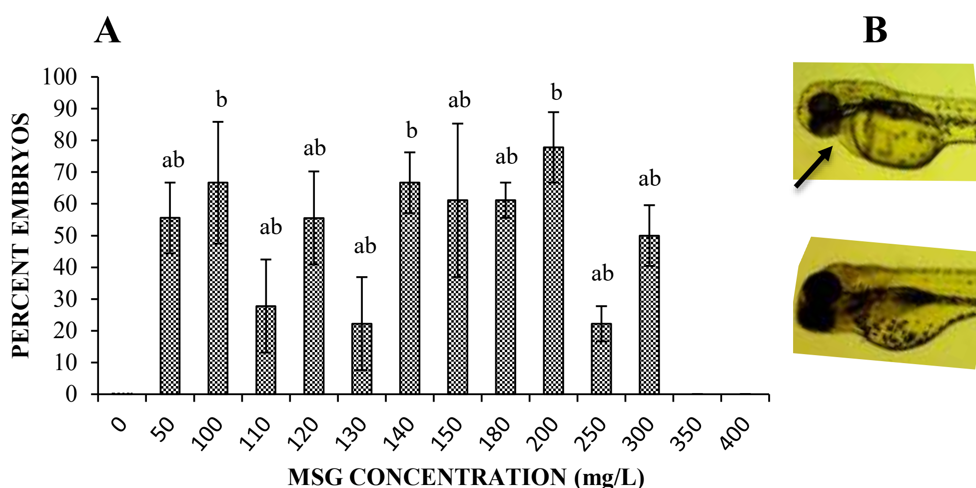

Figure 5: Heart edema observed in zebrafish embryos after exposure to different MSG concentrations. Peak deformities occurred at 100, 140 and 200 mg/L, indicated concentration dependent cardio toxic effect of MSG. (A) Graph showing percentage of embryos exhibiting heart edema at

different MSG concentrations. **(B)** Microscopic pictures of embryos showing heart edema (above, arrow), and normal embryo (below). Error bars indicate standard error.The letters a, b, c shows differences among different concentrations.

4.1.3.2. Bent tail

Highly significant effect of MSG was observed causing bent tail deformities in embryos ($p<0.001$). At lower MSG concentrations 50 to 150 mg/L the percentage of embryos with bent tail deformity were remains relatively low with some fluctuations. A marked increased in bent tail deformity was observed at higher concentrations from (250 to 350 mg/L) with a highest percentage of embryos with bent tail observed at 350 mg/L (77.77 ± 14.69%). The percentage declines slightly at 400 mg/L, possibly due to increase embryo mortality at extreme conditions as shown in Figure 6.The increasing deformity rate at higher concentration suggesting a threshold beyond which MSG exerts pronounced teratogenic effects, potentially disrupted normal tail development and skeletal structure.

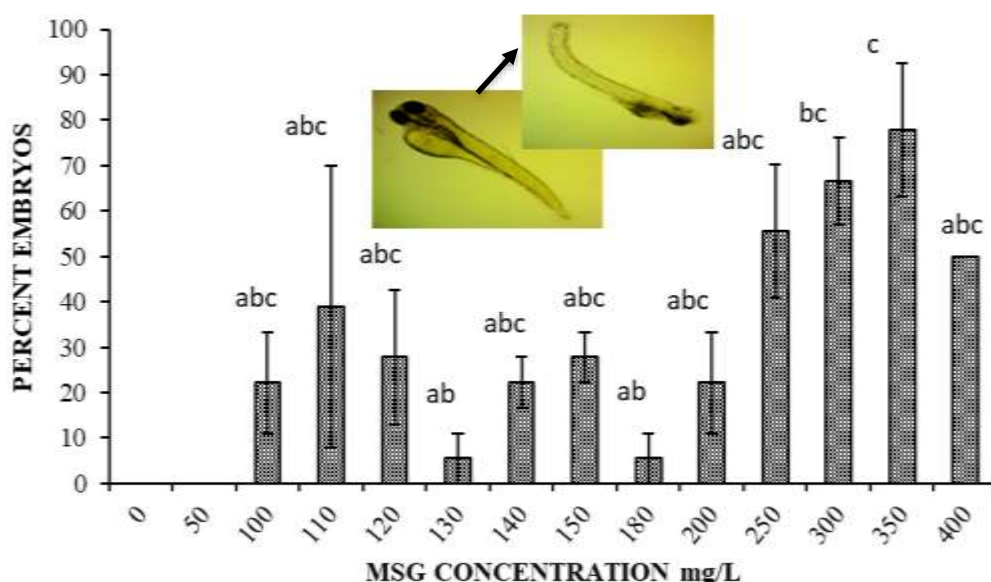

Figure 6: Deformed bent tail observed in zebrafish embryos after exposure to varying concentrations of MSG (mg/L).

A significant increase ($p<0.001$) was observed at higher concentrations, peaking at 350 mg/L (77.77 ± 14.69% embryos), decline at 400 mg/L was due to increased embryo mortality. Error bars indicate standard error, different alphabets on the bars shows that groups are significantly different. The microscopic image shows the normal embryo below the arrow and bent tailed embryo above the arrow.

4.1.3.3. Yolk sac edema

Significantly increase in yolk sac edema was observed with MSG treatment ($p<0.001$). The results indicate a concentration dependent effect, with a fluctuating trend in deformity prevalence. At lower concentrations 50 to 110 mg/L the percentage of effected embryos remains relatively high with peaks observed at 150 mg/L (66.66 ± 19.24%) and 180 mg/L (66.66 ± 9.62%) shows that MSG disrupted normal osmoregulation and vascular development. At 120 mg/L a notable decline occurred, suggesting possible embryonic adaptation or differential embryonic sensitivity at this concentration. At higher concentrations 200 mg/L (33.33 ± 19.24% embryos) to 250 mg/L (27.77 ± 5.55% embryos), the prevalence of yolk sac edema declined progressively, specifically, at 200 mg/L due to increase embryonic lethality at extreme toxicity levels as shown in Figure 7.These findings suggests that MSG exerts terotogenic effects including yolk sac edema being a prominent developmental abnormality at specific concentrations.

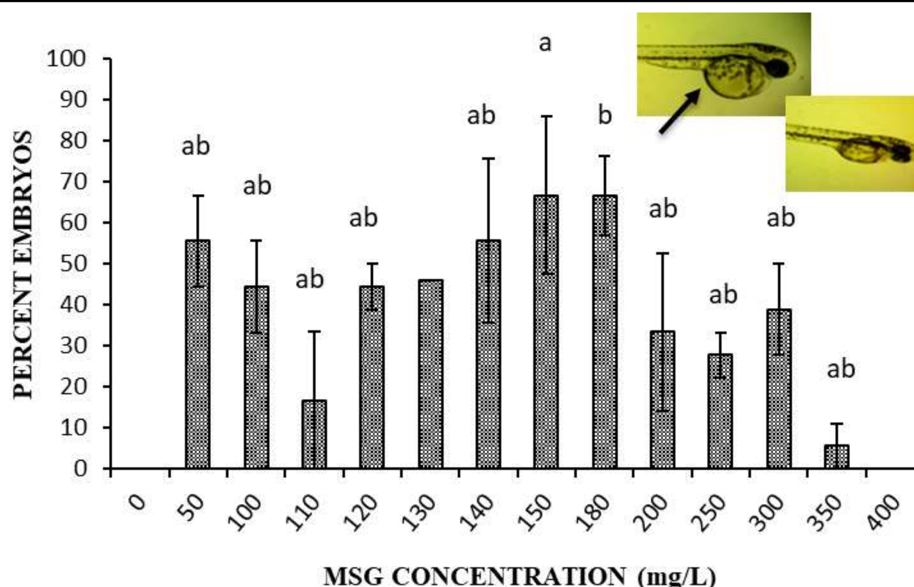

Figure 7: Occurrence of yolk sac edema in zebrafish embryos at different concentrations of MSG mg/L.

A concentration dependent but fluctuating trend is observed with significant peak at 150 mg/L and 180 mg/L indicated disrupted osmoregulation and vascular development. Error bars indicate standard error letters a, b and c shows that groups are significantly different.The microscopic images show normal embryo below and yolk sac edema (arrow) exhibited embryo above.

4.2. Effect of of MSG on gene expression of zebrafish embryos

4.2.1. Effect of MSG exposure on relative expression of *P53* gene

The gel electrophoresis pictures demonstrated how the expression of *p53* gene fluctuated in response to varying concentrations of MSG. *β-actin* was used as housekeeping gene for this analysis. The relative expression of *p53* gene was calculated by dividing its band intensity with *β-actin* band intensity. A closer look at the *p53* bands revealed a distinct pattern, at 100 mg/L indicated a reduction in expression. This trend is showed by the bar graph, which showed a corresponding dip. However, as MSG concentration increases to 120 mg/L and 200 mg/L, the *p53* bands become more intense, reflecting a rise in expression levels. The peak is observed at 250 mg/L, where the band is darkest and aligns with the highest value in the graph. At 300 mg/L MSG concentration, the band intensity slightly diminishes, mirroring a small drop in the quantified data as shown in Figure 8. These findings suggest a concentration dependent regulatory effect of MSG on *p53* expression. While lower concentrations appear to suppress *p53*, higher concentrations lead to an upregulation, possibly indicating a cellular stress response.

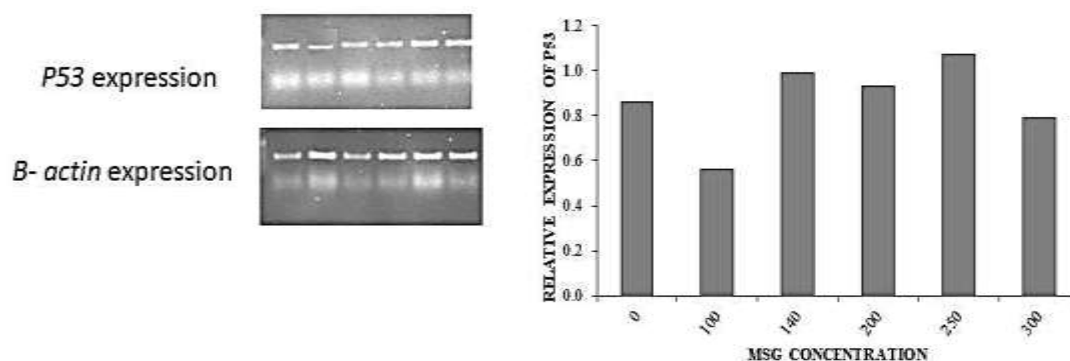

Figure 8: Gel electrophoresis (left) and bar graph (right) showing concentration dependent modulation of *p53* gene expression in zebrafish exposed to increasing MSG concentrations.

β-actin gene was used as housekeeping gene, a clear suppression at lower concentrations and peak expression at 250 mg/L suggest a stress induced regulatory response.

4.2.2. Effect of MSG exposure on relative expression of *IL -1B* gene

The gel electrophoresis and bar graph collectively demonstrated the effect of MSG on *IL-1β* expression in zebrafish. The electrophoresis bands showed a progressive increase in *IL-1β* expression with rising MSG concentrations, while *β- actin* validating its role as housekeeping gene. At 150 mg/L, *IL-1β* expression was slightly up regulated compared to the control, as indicating by a faint but visible band.

The intensity of *IL- 1β* bands further increased at 200 mg/L and 250 mg/L, corresponding to a rise in the bar graph, suggesting enhanced transcriptional activity. The highest expression was observed at 300 mg/L (Figure 9), where both the darkest *IL-1β* band and the peak in the bar graph align, indicating significant induction of the inflammatory marker. These finding suggest a concentration dependent effect of MSG, with increased *IL-1β* expression likely reflecting an inflammatory response in zebrafish.

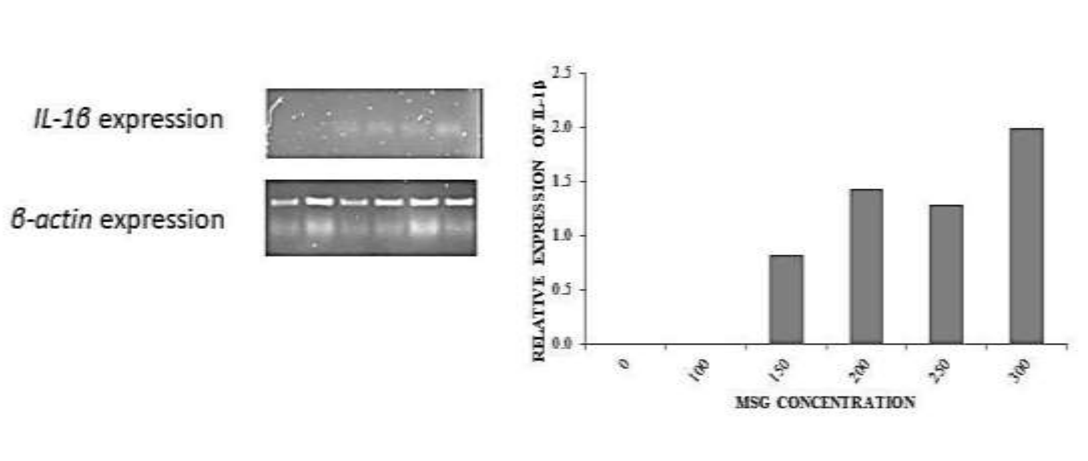

Figure 9: Concentration dependent up regulation of IL-1β gene expression in zebrafish, exposed to MSG (150-300 mg/L).

With peak expression at 300 mg/L, indicating a strong inflammatory response.

4.3. Long term effect of MSG on zebrafish growth

Figure 10 shows pictures of zebrafish in order to obtain data on changes in weight and length at subsequent days post treatment (20, 34 and 49 dpt). Visual observation reveals that zebrafish in the control group exhibited steady growth overtime, showing a progressive increase in body

size. Measuring the weight at different time points identified an increase in bodyweight at 60 mg/L MSG concentration.

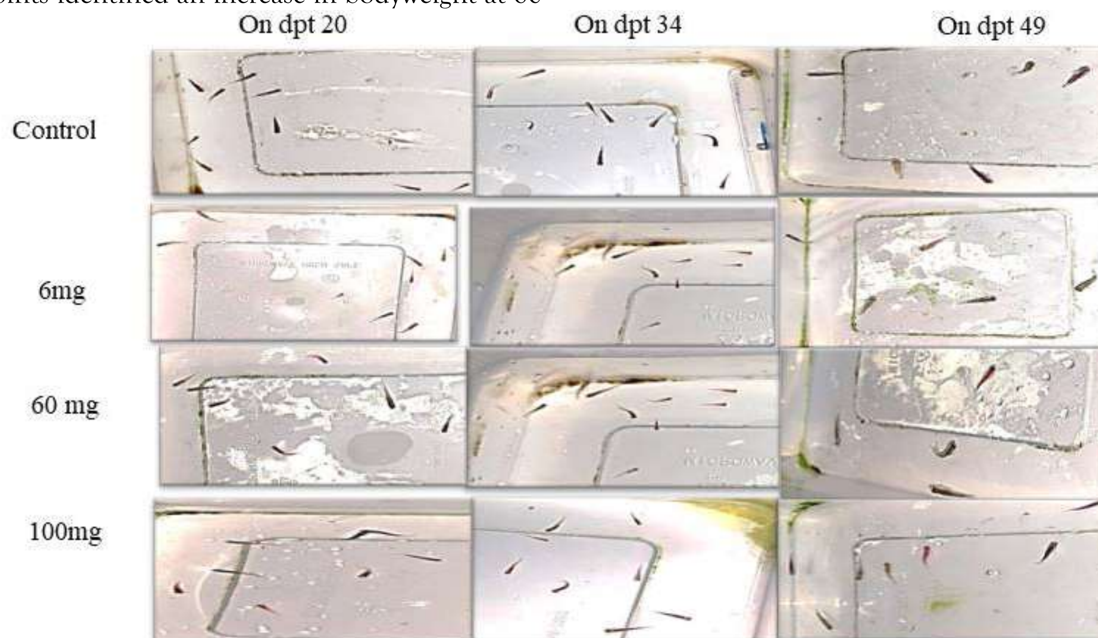

Figure 10: Pictures of zebrafish raised for Long term at varying MSG concentrations with data collected on 20, 34 and 49 dpt.

No significant differences were observed in the length of zebrafish exposed to different MSG concentrations. Despite this overall increase in size, qualitative observations suggest that excessive MSG exposure may lead to irregular growth patterns, particularly at the highest dosage. Detailed results of the effect of MSG on zebrafish growth are presented in the following sections.

4.3.2. Effect of MSG on zebrafish weight in long term exposure

No statistically significant difference between the groups for initial weight ($p>0.05$). For the weight at 34 dpt, significant difference ($p<0.05$) between the groups was observed (Figure 11). At 49 dpt the weight was also significantly different among groups ($p<0.05$). The graph data indicated a concentration dependent increased in weight, with higher MSG concentration leading to significant increase in zebrafish body weight particularly in later stages of development.

At 49 dpt, zebrafish exposed to 60mg/L, shows the most pronounced increase in weight (116.80 ± 7.94 mg) compared to control (81.2 ± 5.34 mg). The average weight of zebrafish grown at 100 mg/L was less than control (66.42 ± 3.45 mg) showing the toxic effect of MSG at extreme concentrations. MSG at 60 mg/L showed markedly higher weight at day 34 and 49 compared to the control groups. It shows that MSG treatment has strong significant effect on weight at subsequent days after treatment.

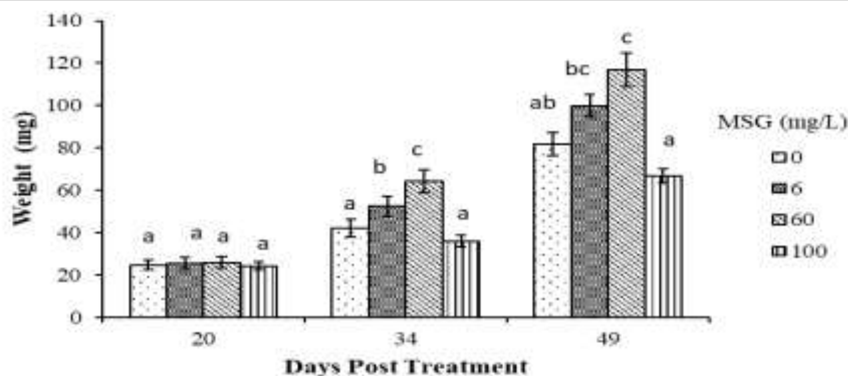

Figure 11: Effect of MSG on Zebrafish body Weight at dpt (20, 34, 49).

A significant, concentration dependent increase in weight was observed at 34 and 49 dpt in groups exposed 6 and 60 mg/L, indicating an obesogenic effect. No significant difference was observed at 20 dpt. Error bars indicates standard error, the letters a, b, c shows difference among different concentrations.

4.3.3. Effect of MSG on zebrafish length in long term exposure

For length at 20 dpt, significant differences were observed between groups ($p<0.05$). However, at 34 and 49 dpt there was no statistically significant difference between groups ($p = 0.166$ and 0.643, respectively).Suggesting that MSG exposure has no meaningful impact on these measurements. Results on 20 dpt showed that at 6mg/L zebrafish exhibit slightly smaller length (10.64 ± 0.77 mm) compared to control group (11.69 ± 0.49 mm), but this reduction is not highly pronounced. At 60 mg/L zebrafish length is notably smaller (9.09 ± 0.36 mm) compared to control (Figure 12). At 100mg/L the reduction in length was more prominent (9.82 ± 0.67 mm). The graph shows a clear difference in mean length at 100 mg/L across all time points, with zebrafish in this group displayed the slowest growth rate.

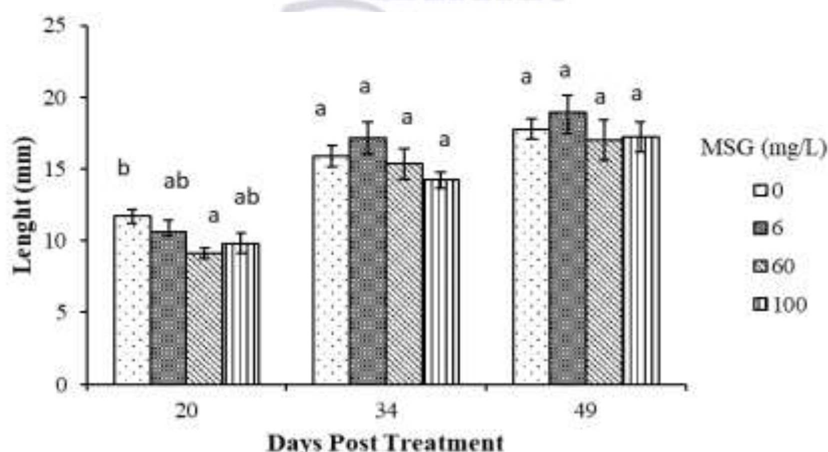

Figure 12 : Zebrafish length on dpt (20, 34, 49), after exposed to varying MSG concentrations.

A significant reduction in length was observed at 20 dpt ($p=0.029$), particularly at higher MSG concentrations, while no significant differences were observed at later stages, error bars indicates standard error, the letters a, b, c shows difference among different groups.

4.3.4. Effect of MSG on zebrafish weight gain in long term exposure

The results found that on 20 dpt zebrafish exposed to 6mg/L MSG showed a slight but noticeable increase in weight gain (25.40 ± 2.99 mg) as compared to control group (24.72 ± 2.31 mg), suggests that even low concentration of MSG can influence metabolic process leaded to

weight gain (p = 0.961) but there is no significant difference between groups (Figure 13). Similarly, zebrafish exposed to 60 mg/L, exhibited a marked increase in weight gain (26.05 ± 2.57 mg), and indicated a clear concentration dependent effect. P value less than 0.05 for weight gain indicated that MSG exposure has significant effect on zebrafish weight gain. These findings showed that lower concentration of MSG may cause steady long-term effects, without triggered the physiological thresholds observed at higher concentrations.

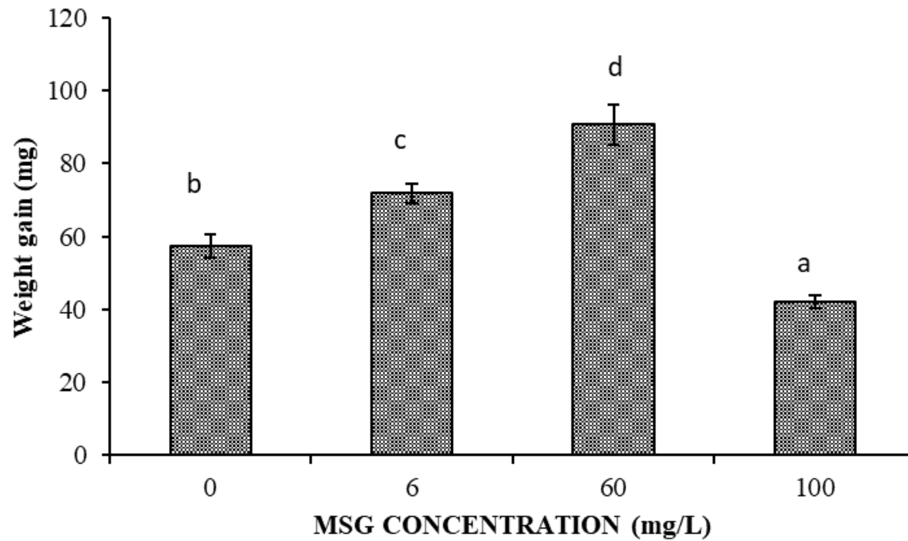

Figure 13: Weight gain in zebrafish at different intervals after exposure to different concentrations of MSG,

A significant weight gain was observed at 60 mg/L (98-100mg), weight loss was observed at 100 mg/L, showed a threshold level beyond which MSG led to weight gain, error bars represent standard error, the letters a, b, c, d shows difference among different groups.

4.3.5. Effect of MSG on specific growth rate of zebrafish in long term exposure

Slight non-significant differences in specific growth rate (SGR) among different MSG treated zebrafish groups were observed (Figure 14). At 0 mg/L the baseline SGR was recorded at approximately (177.75 ± 22.01%). Zebrafish exposed to 6 mg/L MSG exhibited slightly higher SGR (198.37 ± 33.84%) compared to control group. The most pronounced effect was observed at 60 mg/L MSG where SGR peaked (243.21 ± 48.18%), indicated that MSG at this concentration optimally stimulate growth process.

The larger error bar at this concentration suggested variability in individual responses possibly due to differential metabolic adaptation. Exposure to 100 mg/L MSG results a noticeable decline in SGR (129.31 ± 16.97%) dropping below the control group levels (Figure 14). This reduction suggested a threshold effect, where excessive MSG concentrations may induce metabolic stress, toxicity or growth inhibition. The smaller error bar at this concentration suggested a more consistent inhibitory effect across the population.

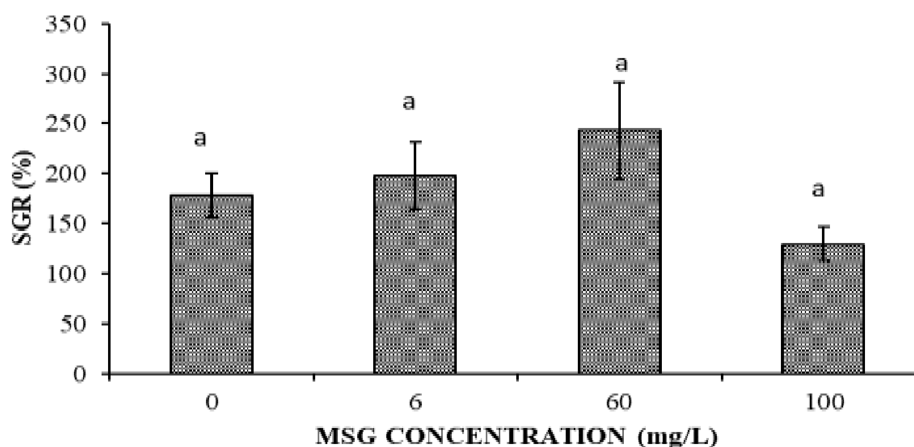

Figure 14: Specific growth rate observed in zebrafish on different MSG concentrations.

A higher SGR was recorded on 60 mg/L (250%), significantly different from control, SGR at 100 mg/L was reduced, showing the terotogenic effect of MSG at higher concentration in Zebrafish. Error bars represent standard error, the shared letter a shows that there is no significant difference among different groups.

4.3.6. Effect of MSG on BMI of zebrafish

MSG significantly impact BMI in zebrafish at all measured time points among treatment and control groups. Significant changes for BMI ($p<0.05$) at 20dpt, means that MSG exposure had strong impact on BMI of zebrafish at early stages of exposure (Figure 15). For BMI at 49dpt the p value was also less than 0.05, the effect of MSG on BMI is weaker as compared to first two time-points. The strongest impact was seen on 34 dpt, suggesting that MSG exposure causes peak changes in BMI at that stage.

On 20dpt, zebrafish exposed to 6 mg/L MSG showed a slightly higher BMI (0.22 ± 0.008) compared to control (0.176 ± 0.004), indicating mild growth promotion, while 60 mg/L (0.30 ± 0.012) caused a markedly higher BMI, suggesting strong growth stimulation, and 100 mg/L (0.258 ± 0.016) led to a slightly lower BMI, implying potential metabolic stress. By 34dpt, the 6 mg/L group exhibited BMI similar to the control group (0.14 ± 0.016), reflecting sustained growth. The 60 mg/L group (0.21 ± 0.04) reached the highest BMI at 34 dpt, indicating peak growth and individual response differences, and the 100 mg/L group (0.15 ± 0.019) showed a lower BMI compared to the 60 mg/L group. On 49dpt, the 60 mg/L group (0.33 ± 0.07) sustained the highest BMI (Figure 15), and the 100 mg/L group (0.20 ± 0.030) remained with a BMI even lower than the control group, reinforcing growth inhibition or metabolic toxicity.

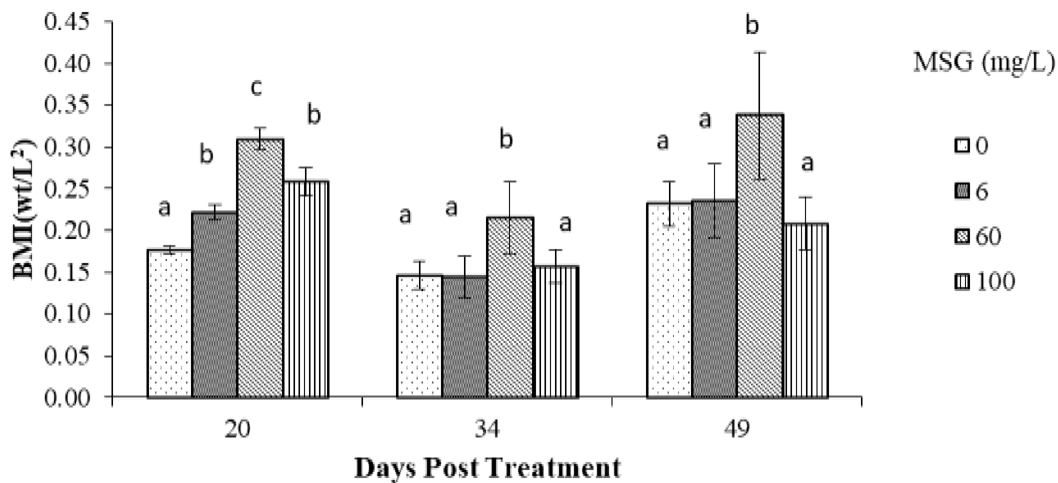

Figure 15: BMI calculated of zebrafish on 20, 34, and 49 dpt at varying MSG concentrations.

The highest BMI was observed at 49 dpt on 60 mg/L, significantly different from low concentration groups, BMI on 100 mg/L was lower due to toxic effect of MSG beyond threshold level. Error bars represent standard error, letters a, b, c shows difference among different groups.

4.3.7. Effect of MSG on zebrafish condition factor

The condition factor (CF) of zebrafish showed variation depending upon MSG concentration and exposure duration (Figure 16). At 20dpt, the CF remain stable in the control group (1.5 ± 0.070), exhibited a slight increase at 6 mg/L, peaked at 60 mg/L (3.44 ± 0.19), and then declined slightly at 100 mg/L (2.84 ± 0.40), indicating initial weight gain and possible metabolic effects, these differences was statistically highly significant ($p = 0.000 < 0.05$). By 34dpt the CF remain stable in the control group, moderately increased at 6 mg/L, declined from its earlier peak at 60 mg/L (1.92 ± 0.28), and dropped further at 100 mg/L, suggesting metabolic stress at higher MSG concentrations , with effect still statistically significant ($p = 0.0002 < 0.05$).

At 49dpt, the control group CF remained relatively unchanged (1.47 ± 0.90), showed minimal variation at 6 mg/L, slightly decreased at 60 mg/L, and reached its lowest value at 100 mg/L (1.44 ± 0.21), as shown in Figure 16, the effect of MSG on CF at this point remained moderately significant ($p<0.05$). overall this pattern suggests that prolonged exposure to increasing concentrations of MSG compromises growth efficiency and disrupt metabolic homeostasis, with the lowest CF observed at 100 mg/L on day 49, indicating severely affected overall health in zebrafish.

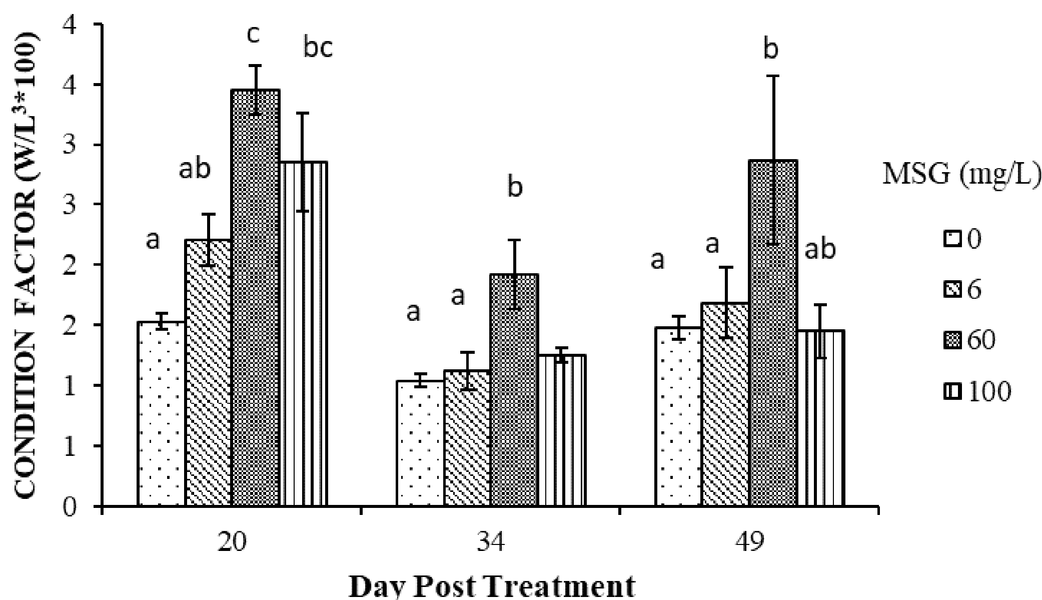

Figure 16: Condition factor calculated of zebrafish on 20, 34, and 49 dpt, upon exposure to different concentrations of MSG.

The highest CF was recorded on 20 dpt at 60 mg/L, the CF is decreases as the duration and concentration of MSG increases, the lowest CF recorded on 49 dpt at 100mg/L. Error bars represent standard error, letters a, b, c shows difference among different MSG concentrations.

4.4. Effect of MSG on triglyceride, cholesterol and sugar level of zebrafish

4.4.1. Effect of MSG on triglyceride level in zebrafish

Triglyceride levels of zebrafish showed a concentration dependent response to MSG exposure, suggesting that different concentrations of MSG significantly influenced lipid metabolism and led to variation in triglyceride concentrations. The results showed a clear rise in triglyceride levels as MSG concentrations increases with error bar reflecting variability in each group (Figure 17).

At 0 mg/L, triglyceride levels were at their lowest (11.12 ± 0.48 mg/g), serving as a reference point. A slight increase appeared at 6 mg/L MSG, though the overlapping error bars indicate this change was not statistically significant. However at 60 mg/L MSG, triglyceride levels (13.95 ± 0.40 mg/g), showed a notable jump, with reduced error bar overlap compared to the control suggesting a significant difference ($p<0.05$). The most dramatic increase is observed at 100 mg/L MSG (16.00 ± 0.57) as shown in Figure 17, where triglyceride level peaked, and the error bar remained distinctly separate from lower concentrations, confirming a statistically significant effect of MSG on triglyceride level ($p<0.05$). These results suggested that MSG exposure significantly impact lipid metabolism in zebrafish, with stronger effect at higher concentrations.

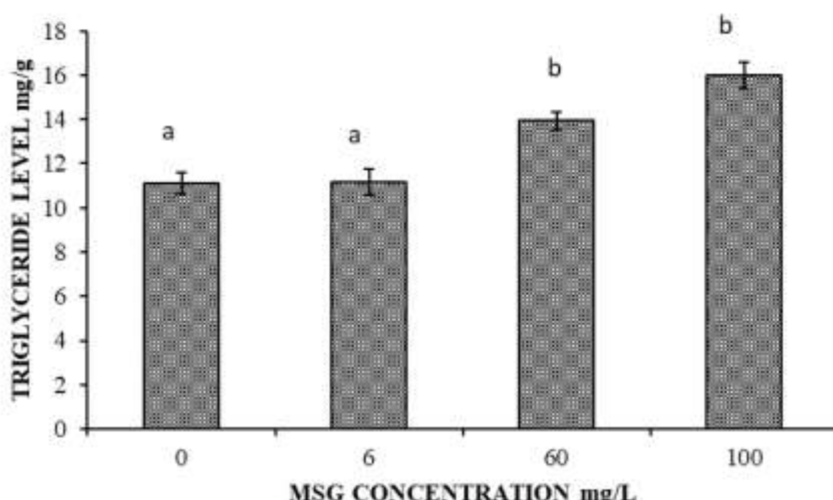

Figure 17: Effect of varying concentrations of MSG on Triglyceride level of zebrafish.

Upon treated with different MSG concentrations, the triglyceride level increases as MSG concentration increases, 100mg/L showed the highest triglyceride level ($p<0.05$). Error bars represent standard error letters a, b shows difference among different groups.

4.4.2. Effect of MSG on cholesterol level in zebrafish

The results showing the impact of different concentrations of MSG on cholesterol level (mg/g) are presented in Figure 18. The finding revealed a clear concentration dependent increase, where higher MSG concentration corresponds to elevated cholesterol levels.

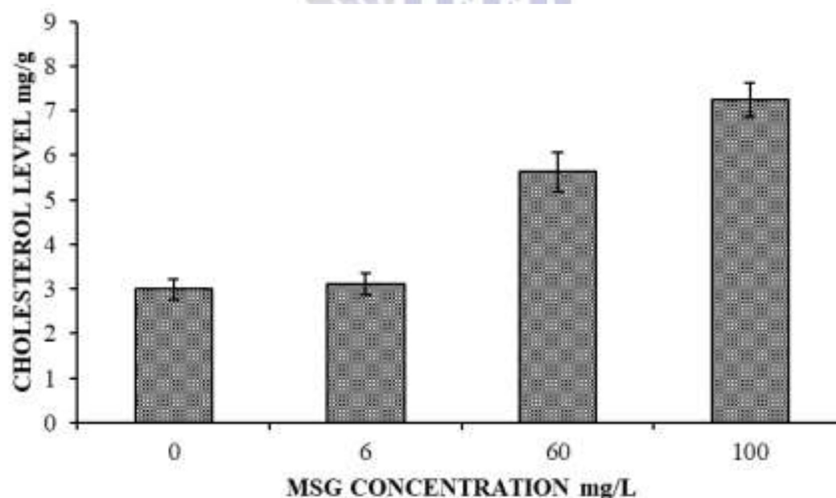

Figure 18: Effect of different concentrations of MSG on cholesterol level of zebrafish.

A clear concentration dependent effect of MSG on cholesterol level, peak observed at 100 mg/L significantly different from low concentrations groups. Error bars represent standard errors.

At the lowest concentrations 0 mg/L and 6 mg/L cholesterol level remain (2.9 ± 0.21 mg/g), relatively stable and comparable. However, a notable rise occured at 60 mg/L (5.6 ± 0.45 mg/g), nearly doubling the cholesterol levels observed in the control group. The most significant increase was seen at 100 mg/L (7.24 ± 0.38 mg/g), indicating a strong link between MSG exposure and hypercholesterolimia, although some varaibility exists, as shown by the error bars in Figure 18 the overall trend remains consistent, reinforcing the positive correlation

between MSG concentration and cholesterol accumulation. A highly significant of different concentrations of MSG on cholesterol levels was confirmed ($p<0.05$), suggesting that changes in MSG intake result in measurable alterations in cholesterol levels.

4.4.3. Effect of MSG on total sugar level of zebrafish

Figure 19 illustrates the impact of different MSG concentrations on zebrafish sugar levels (mg/g). Notably, the control group (0 mg/L) exhibited the highest sugar levels (7.32 ± 0.46 mg/g), while those exposed to 6 mg/L, 60mg/L and 100 mg/L of MSG (6.51 ± 0.42 mg/g), showed a slight decline. However the variations in sugar level among the MSG treated groups were relatively minor, implying that MSG exposure did not cause any significant fluctuations in sugar levels. The error bars highlights some degree of variability in the data, yet no clear pattern of hyperglycemia or hypoglycemia emerge as MSG concentration increases.

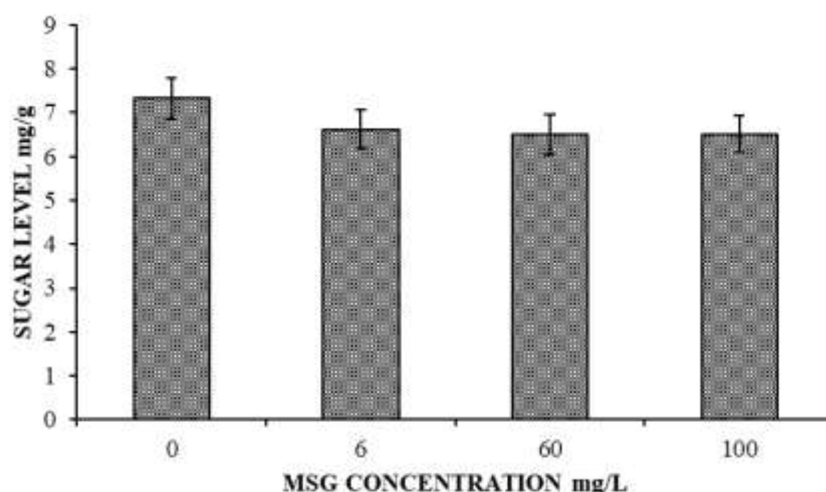

Figure 19: Effect of different concentrations of MSG on sugar level of zebrafish.

There was no significant effect of increasing MSG concentrations on sugar level (0.344>0.05), showed that these concentrations of MSG and within this duration, sugar level are not disrupted. Error bars represent standard errors.

4.5. Toxic effect of MSG on zebrafish histology

4.5.1. Histopathological analysis of gills

A histopathological analysis of zebrafish gills after exposure to MSG reveals a concentration dependent structural damage highlighted its toxic effects (Figure 20). In the control group gills architecture remains intact with clearly defined lamellae. However at a concentration of 60 mg noticeable changes emerged, including epithelial lifting, slight thickening of lamellae and early signs of hyperplasia, indicated initial tissue stress. At a significantly higher concentration of 100 mg/L the damage become severe, characterized by disorganized lamellae, epithelial degeneration and fusion of secondary lamellae as shown in Figure 20. These alterations suggest that oxidative stress, apoptosis and inflammation was contributed to the observed tissue deterioration. These finding showed that, MSG exposure appeared to compromised gill structure and impair respiratory function in zebrafish.

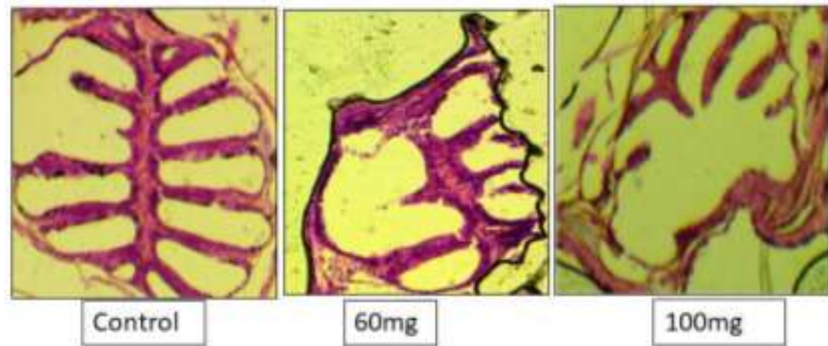

Figure 20: Toxic Effect of varying MSG concentrations on Gills of zebrafish.

Severe structure distortion occurs at 100 mg/L, showing epithelial degeneration and lamellar fusion.

4.5.2. Histopathological analysis of eye region

A detailed histopathological analysis of zebrafish eyes followed exposure to MSG revealed a progressive decline in structural integrity, directly correlated with increasing dosage (Figure 21). In the control group the retinal structure intact, with well -defined layers, clear cornea and an unaltered lens. At a concentration of 6 mg/L slight disruptions in retinal organization and minor thinning was observed Exposure to 60 mg/L led to more pronounce thinning, noticeable disarray within retinal layers, and the emergence of vacuolization, accompanied by mild lens changes.

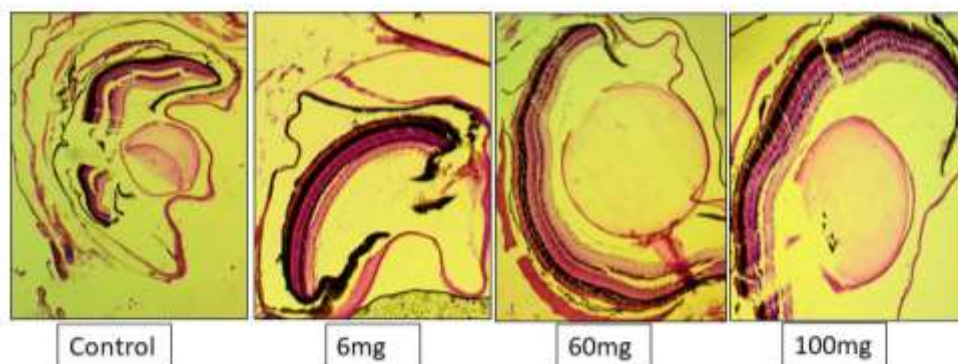

Figure 21: Concentration dependent Ocular damage observed in Zebrafish exposed to MSG.

Showing progressive retinal degeneration, lens deformities and signs of neurotoxicity at 60 and 100 mg/L.

The most extreme exposure 100 mg caused severe degeneration of retina, significant structural breakdown and substantial lens deformities, suggested irreversible ocular damage. These findings suggested that MSG has the potential to induce neurotoxic effects and oxidative stress, which may compromised vision and overall eye function in zebrafish (Figure 21).

4.5.3. Histopathological analysis of intestine

Histopathological examination after exposure to MSG revealed a clear concentration dependent decline in intestine health (Figure 22). In a control group the intestinal architecture remained largely intact showing only minor vacuolization and slight shortening of the villi.

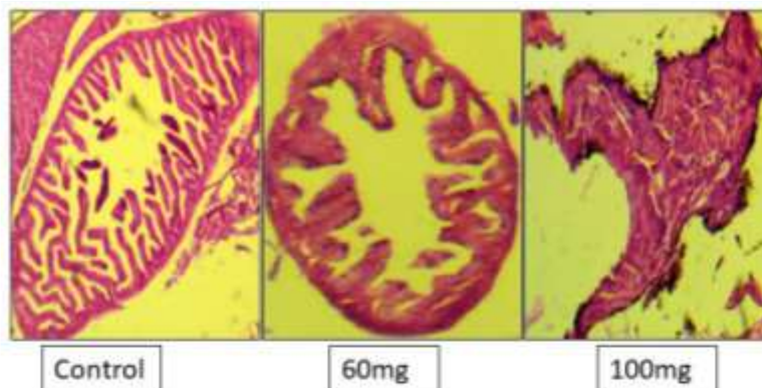

Figure 22: Toxic concentration dependent effect of MSG exposure on zebrafish Intestine.

Showing villous atrophy, epithelial disruption and mucosal damage at 60 and 100 mg/L.
However, at 60 mg/L noticeable damage emerged including moderate villous atrophy, widening of the intestinal lumen and epithelial breakdown, changes that suggest impaired absorption and possible inflammation. The most severe effects appeared at the highest concentration of 100 mg/L as shown in Figure 22, where extensive villous degeneration, loss of epithelial integrity and significant mucosal damage become evident, pointed to cytotoxic effect on gut tissue. These findings highlight the progressive deterioration of intestinal structure with increasing MSG exposure, which may ultimately disrupt nutrient absorption and digestive efficiency.

4.5.4. Histopathological analysis of female and male reproductive tracts

The ovarian histology of zebrafish exposed to control and 60 mg/L MSG revealed concentration dependent alteration in follicular development. In control group ovarian follicles appears structurally intact with a well-defined oocyte architecture and normal follicular arrangement. At 60 mg/L ovarian tissue exhibited signs of degenerative changes, included disrupted follicular organization, cytoplasmic vacuolization, and reduced oocyte integrity. The presence of arthritic follicles suggested impaired oogenesis and potential reproductive dysfunction (Figure 23). The results showed that MSG had deleterious effect on female reproductive health by disrupting ovarian structure and function.

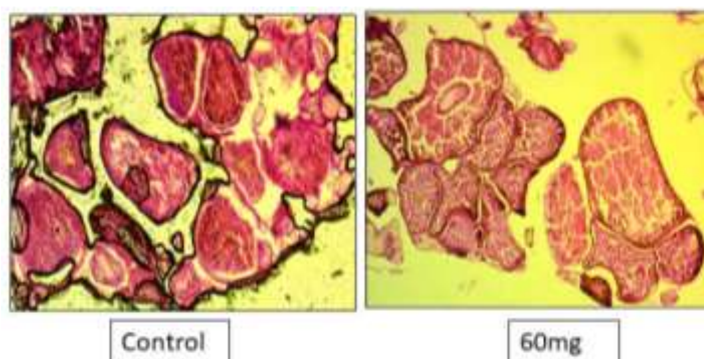

Figure 23: Concentration dependent ovarian damage in zebrafish expose to MSG.

Showing disrupted follicular structure, cytoplasmic vacuolization and signs of impaired oogenesis at 100 mg/L.
The histological analysis of zebrafish testes between the control group and 100 mg/L MSG exposed group's revealed significant structural alterations indicative of testicular toxicity. In the control group seminiferous tubules appeared well organized with a dense population of spermatogenic cells as shown in Figure 24. In contrast the testes of MSG exposed groups exhibited disrupted seminiferous tubules

architecture, reduced germ cell density and signs of vacuolization. The presence of degenerative changes including loss of germ cells suggests that high-concentration MSG exposure may impair spermatogenesis and compromise male reproductive function (Figure 24). These findings highlighted the potential endocrine-disrupting effects of MSG on male fertility.

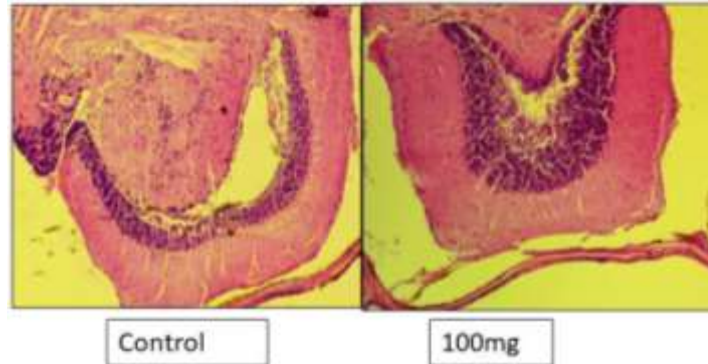

Figure 24: Histological changes in zebrafish testes after 100 MSG exposure, showing disrupted seminiferous tubules, reduced germ cell density and vacuolization indicating impaired spermatogenesis and testicular toxicity.

4.5.5. Histopathological analysis of blood capillaries

The histological sections of zebrafish exposed to increasing sections of MSG revealed a concentration dependent alteration in vascular morphology (Figure 25). In the control group the capillaries appeared well organized with intact endothelial lining and normal branching pattern. At 60 mg/L exposure mild structural disorganization is observed, with slight dilation of capillaries and irregular endothelial cell arrangement. At 100 mg/L MSG the capillary network showed significant alteration, including increased vessel dilation, endothelial disintegration and potential hemorrhagic regions, indicated vascular fragility and compromised blood flow (Figure 25). These results suggested that MSG may induce vascular damage potentially affected circulation and tissue perfusion.

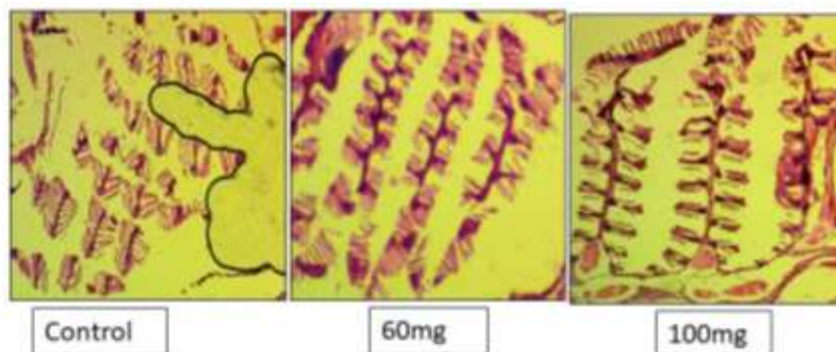

Figure 25: MSG induced vascular damage in zebrafish. With increasing concentrations 60mg/L and 100 mg/L, causing capillary dilation, endothelial disruption, and hemorrhagic signs, indicate impaired circulation and tissue perfusion.

4.5.6. Histopathological analysis of heart

The histological comparison of zebrafish cardiac tissue between the control and 60 mg MSG exposed groups showed a noticeable structural difference indicated potential cardio toxic effects (Figure 26). In the control group the myocardial fibers appeared relatively intact with well-defined muscle striations and a compact arrangement of cardiomyocytes. In 60 mg/L MSG exposed group, the heart tissue exhibited signs of structural disorganization, increased cellular density and possible hypertrophic changes as

show in Figure. The presence of myocardial disarray, congestion or increased interstitial space suggested potential inflammatory responses or signs of fibrosis. These alterations indicated that higher concentrations of MSG may induce cardiac remodeling, possibly linked to oxidative stress and metabolic disturbances effected cardiovascular health.

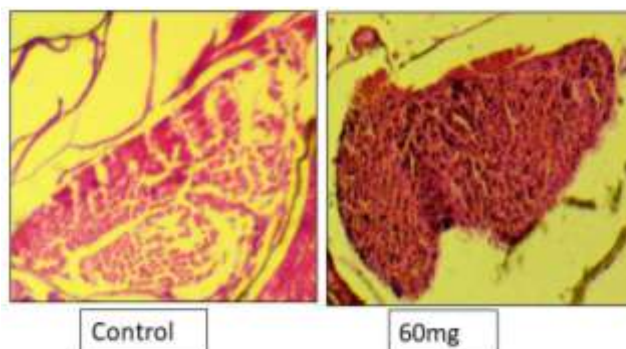

Figure 26: Histological changes in zebrafish heart after 60 mg/L MSG exposure. Showing myocardial disarray, increased cellular density, and signs of hypertrophy.

4.5.7. Histopathological analysis of liver

Histopathological examination of zebrafish liver following exposure to different concentrations of MSG, revealed a clear concentration dependent impact of MSG on liver structure. In control group, the liver appears largely intact with well-defined hepatocytes and minimal alterations. However as the dosage increased, noticeable disruption appears including hepatocellular vacuolization, tissue disorganization, and heightened vascular congestion early indicator of liver distress. At the extreme concentration of 100 mg/L severe pathological condition become evident, such as fibrosis, extensive necrosis and breakdown of cellular integrity (Figure 27), all pointing to substantial hepatotoxicity. These findings underscore the progressive liver damage, induced by higher concentration of MSG, reinforced concerned about its toxicological effect.

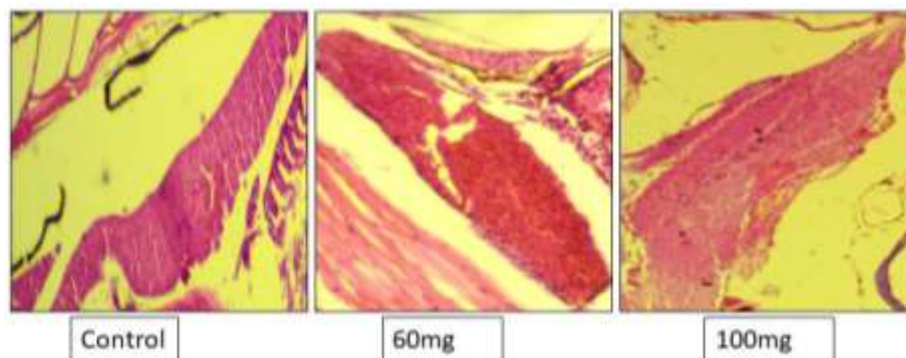

Figure 27: Harmful effect of MSG exposure on Zebrafish Liver. Showing progressive hepatocellular vacuolization, necrosis and fibrosis, indicating severe hepatoxicity.

V.DISCUSSION

The present study identified new insights regarding the influences of MSG on growth, obesity, sugar levels and key molecular markers associated with metabolic dysfunction in a zebrafish model. Using a well-rounded approach that combines phenotypic observation with biochemical, histopathological and molecular analysis, our research adds to the ongoing debate surrounding MSG induced metabolic disturbances. It also reinforces the value of zebrafish as a reliable model for studying metabolic disorders.

High MSG concentration significantly impaired hatching rates and mortality in zebrafish embryos, in the current study, possibly due to osmotic and metabolic stress. Early hatching observed in zebrafish exposed to MSG in

previous studies may be due to stress induced acceleration of embryonic development. According to Rajendran et al. (2024), that environmental stressors, including chemical exposures like MSG, can induce oxidative stress and metabolic disturbances that disrupt normal embryogenesis. In response embryos may initiate premature hatching to escape the toxic environment. This early emergence is thought to be regulated by increase enzymatic activity, particularly hatching enzymes and changes in chorion structure that occur under stress condition.

Exposure to toxins like MSG may activate the hypothalamic pituitary interregnal (HPI) axis in zebrafish embryos, leading to increased cortisol levels. Elevated cortisol a primary stress hormone, has been linked to accelerated development and premature hatching in zebrafish (Alsop and Vijayan, 2008). MSG can cause osmotic imbalance and oxidative stress due to accumulation of glutamate and sodium ions. This imbalance can affect cellular homeostasis and induce early escape behavior, including premature hatching (Mohammadbakir, 2016). According to Jennifer et al. (2017) zebrafish hatching involves the enzymatic activity of chorionase, which break down the chorion (egg membrane). MSG induce stress may trigger premature activation or overexpression of this enzyme.

In the current study, zebrafish embryos showed increased mortality with rising MSG concentrations, with 60% mortality at 200 mg/L and 100 % mortality observed at concentrations of 350 mg/L and above. When compared to previous studies in other animal models and zebrafish, these concentrations appear relatively low. Nnadozie et al. (2019) reported mortality in Wister rats administered 120 mg/kg day of MSG over a period of 6 to 12 months, but the deaths were linked to chronic organ damage rather than acute toxicity. Similarly, Elshaikh and Abuelgassim (2014) observed toxic effects such as liver and pancreatic damage in rats at 220 mg/kg, yet without reporting acute mortality. Banerjee et al. (2021) also found that oral administration of 100-200 mg/kg MSG in rats over 28 days led to liver and cardiac toxicity, with no mention of 100 % mortality.

These studies suggest that mammalian model require much higher cumulative concentrations, typically administered orally over extended periods, to elicit toxicity or lethality. In contrast zebrafish embryos, in the current study, responded with significant mortality within 72 hours of direct aquatic exposure to relatively lower MSG concentrations. While LC50 data for MSG in zebrafish are limited, other reports suggested lethality generally occurs between 300-500 mg/L, which aligns with the findings of the current study. This indicates that zebrafish embryos are more sensitive to MSG exposure than adult mammals, due to differences in developmental stage, exposure route, and species-specific physiological responses.

MSG exposure induce concentration dependent teratogenic effects in zebrafish embryos, in the current study, and showed various deformities including bent tails, heart edema, and yolk sac edema especially at higher concentrations. These results align with Hernández Bautista et al. (2019), who reported similar embryonic abnormalities in rodents exposed to MSG. Likewise, Utume et al. (2020) observed heightened mortality rates and developmental defects in rats subjected to higher concentrations of MSG, indicating that the compounds toxicity extends across species. Shi et al. (2014) further emphasized that higher MSG intake is associated with lower risk of developing hyperglycemia which aligns with our findings. It was a Prospective cohort study involved 1056 healthy Chinese adults aged 20 years and older, followed from 2002 to 2007.

At the molecular level gene expression analysis showed that MSG exposure led to a significant increase in *IL- 1β* and *p53* expression. *IL-1β* is well known pro inflammatory cytokine involved in metabolic inflammation and insulin resistance, while *p53* play a key role in regulating cell death and apoptosis Hazzaa et al. (2020). These results suggest that oxidative stress and inflammation may be major contributors to MSG induced metabolic disturbances, a concept previously observed in rodent models (Hazzaa et al., 2020). On a molecular level, our study revealed up regulation of *IL-1β* and *P53* genes, corroborating previous reports by Nabi and Bhandari (2022) that linked MSG exposure to inflammation and oxidative stress markers in

animal models like Wistar rats. Elevated *IL-1β* expression points to inflammatory activation, aligning with Roman-Ramos et al. (2011), who found increased pro inflammatory cytokines in MSG treated mice. Likewise, our findings on *p53* overexpression is similar to Hazzaa et al. (2020) research ,who associated increased *p53* levels with cardiac toxicity and apoptosis in MSG exposed rats. This supports the hypothesis that MSG induced metabolic dysfunction stems from oxidative stress and inflammatory responses and inline with the other observations in the current study.

Our findings reveals a clear, concentration dependent increase in body weight and body mass index (BMI) in zebrafish subjected to MSG, particularly at higher concentrations (60mg/L and 100mg/L). These results are in line with previous rodent studies, which suggest that MSG disrupts metabolic balance, triggering abiogenesis and fat accumulation. Zazula et al. (2023) observed heightened body fat, metabolic dysfunctions and diminished muscle mass in Wistar rats exposed to MSG during early life. Similarly, Bahadoran and Mirmiran (2019) documented MSG induced hyperphagia and excessive adipose tissue accumulation in neonatal rats, suggesting that MSG may contribute to obesity by impairing leptin signaling. Zanfirescu et al. (2019) also noted significant weight gain and fat accumulation in rats exposed to MSG, closely resembling the findings of the current study. However, unlike rodent models, zebrafish in our study did not exhibit pronounced glucose imbalances, hitting at potential specie specific metabolic responses. However the consistency in weight gain patterns across species underscores MSG role in obesity development.

Zebrafish exposed to 60 mg/L of MSG experienced a more pronounce increase in size by 49 dpt, indicating that higher MSG concentrations may accelerate weight gain. While at 100 mg/L MSG concentration the weight of zebrafish is significantly decline suggesting threshold level or toxic effect of MSG on zebrafish developmental process. Overall, these findings highlight a concentration-dependent relationship between MSG exposure and zebrafish growth. While low concentrations (6 mg) appear to modestly promote length increase, while higher concentrations (60 mg/L and 100 mg/L), do not increase the length, suggested that zebrafish length may be regulated differently. This study underscores the relevance of zebrafish as a model for investigating MSG-induced obesity and metabolic disorders.

The impact of MSG at higher concentration suggested that prolonged exposure disturbed normal physiological growth mechanisms, due to metabolic stress, oxidative damage or interference with growth regulating pathways. These results suggest that prolonged exposure to MSG contributes to increase body mass in zebrafish, supporting its obesogenic effect. The results of SGR, in the current study, showed that even low concentration of MSG concentrations can enhance growth, due to metabolic stimulation or improved nutrient assimilation. This support the hypothesis that MSG exposure induces metabolic changes, potentially leading to increased fat accumulation.

A striking concentration dependent increase in triglyceride and cholesterol levels was observed in MSG treated zebrafish, while blood sugar levels remained unchanged. These findings point towards an increase in fat synthesis (lipogenesis), and a suppression of fat breakdown, leading to dyslipidemia. These results are parallel to the findings reported by Diniz et al. (2005), who observed heightened lipid profiles following MSG exposure in rats, raising concerns about its role in dyslipidemia. Similarly,Kayode et al. (2023) emphasized that MSG alter lipid metabolism, contributing to obesity and insulin resistance. Our results also aligns with previous studies linking MSG consumption to a higher risk of metabolic syndrome and cardiovascular diseases (Niaz et al., 2018).

While mammalian models have frequently exhibited MSG induced hyperglycemia and insulin resistance, in previous studies Araujo et al. (2019), emphasized the role of insulin resistance and glucagon dysregulation in MSG-induced obesity in rodents, raising questions about whether zebrafish experience comparable metabolic shifts. Although our study identified increased lipid accumulation, further investigations are necessary to determine whether zebrafish undergo similar disruptions in

glucoregulatory mechanisms. Unlike in some rodent studies where MSG exposure resulted in insulin resistance and elevated blood glucose level (Kayode et al., 2023), our zebrafish model did not exhibit significant glucose alterations. However, similar to our results, another study has observed a rise in cholesterol levels post MSG exposure without marked glucose metabolism disruptions (María Catalina et al., 2018). This suggests that MSG may primarily affect lipid metabolism rather than directly impair glucose regulation. Variations in the effect of MSG on sugar levels could stem from specie specific metabolic differences or indicate that a longer exposure period may be needed to trigger noticeable changes in glucose regulation.

Histopathological analysis, in the current study, further confirmed the toxic effects of MSG. Zebrafish exposed to MSG exhibited severe tissue deterioration in the gills, intestines and eyes including abnormal cell growth (hyperplasia), lamellar fusion (gills), villous atrophy (intestines), and retinal disorganization (eyes). These structural changes are strong indicators of oxidative stress, metabolic toxicity, inflammation and overall physiological dysfunction, reinforcing the idea that MSG exposure has systemic toxic effects beyond metabolic disruption. Similarly, hepatic changes have previously been noted by Chakraborty (2019), who observed hepatic fibrosis and lipid accumulation in rodents exposed to MSG. Nabi and Bhandari (2022) further identified hepatic steatosis and oxidative damage in Wister rats following MSG exposure, findings that strongly parallel our zebrafish observations. These data reinforce that MSG induced stress impacts multiple organ systems at the cellular level.

Our findings support the argument that zebrafish serves as a useful model for studying MSG induced metabolic and physiological disorders as it replicate pattern observed in mammalian models. In previous research Benchoula et al. (2019a) and Zang et al. (2018) have validated zebrafish as reliable system for metabolic studies due to their genetic resemblance to mammals and their ability to develop obesity like phenotypes when subjected to high fat diets. In another study, Bahadoran and Mirmiran (2019) demonstrated that MSG exposure in rodents and non-human primates leads to obesity, insulin resistance and metabolic syndrome. By revealing similar metabolic disruptions in zebrafish, our study strengthens the case of using non mammalian models in metabolic research. The results of our study reinforce this perspective, emphasizing zebrafish as a practical platform for identifying metabolic disruptors and testing potential therapeutic interventions.

From a human health perspective, these findings are particularly relevant given the widespread use of MSG as a food additive. While agencies like the FDA and EFSA classify MSG as a safe, growing evidence suggests that excessive intake may contribute to metabolic imbalances, as noted in the results of the current study and the discussion of previous studies. These studies highlights are potential risks associated with chronic MSG consumption, underscoring the need for further research.

VI.SUMMARY

This research was conducted to understand the effects of MSG a widely used flavor enhancer, on growth, obesity and sugar metabolism using zebrafish as a model organism. MSG is commonly present in processed foods and has been long associated with metabolic issues such as obesity and T2DM. despite its popularity and the fact that its generally considered safe in moderate amounts, recent reports suggests that MSG may have serious long term health effects , especially when consume regularly in high concentrations. The aim of the study was to evaluate both the short term (acute) and long term (chronic) effects of MSG exposure on zebrafish in terms of physical growth, fat storage, biochemical markers like sugar, cholesterol and lipid, and the expression of key metabolic and inflammatory genes.

The study was conducted in two major phases. In the first phase called acute and short term exposure zebrafish embryos just 24 hours after fertilization were exposed to increasing concentrations of MSG ranging from (0 to 400 mg/L) for 3 days. Observations were made for mortality, hatching, and developmental deformities (bent tails, heart edema, yolk sac edema) and gene expression. In the second phase called chronic exposure, zebrafish larvae were continuously exposed to lower sub lethal

concentrations of MSG (0, 6, 60 and 100 mg/L) for three months. During this period the physical and metabolic health of fish was carefully monitored. Measurements included body weight, length, BMI, SGR and condition factor , which reflect fat accumulation and growth. Histological sections of the exposed fish were studied for histopathological effects on tissues and organs. Furthermore, total lipids and sugar level of these fish were analyzed to evaluate the effect of MSG on lipid accumulation and obesity.

The results showed that exposure to MSG at concentrations above 200 mg/L caused significant increase in embryo mortality and developmental deformities, indicating that MSG has toxic effects even at early stages of development. The highest mortality rates were seen at 300 and 400 mg/L, where all most all embryos failed to survive. Furthermore, MSG delayed hatching at higher concentrations, suggesting interference in embryonic development. Developmental deformities like heart edema, yolk sac edema and bent tails were observed in zebrafish embryos exposed to 200-250 mg/L of MSG.

Gene expression analysis revealed increased levels of *IL-1β*, an inflammatory gene, and altered expression of *P53*, a gene involved in regulating cell stress and apoptosis. These changes reflect that MSG induces inflammation and oxidative stress, even during early development. Furthermore, gene expression analysis using PCR confirmed that *IL-1β* was overexpressed, indicating that MSG trigger inflammation. Meanwhile, *p53,* a gene responsible for managing cell cycle and apoptosis, also showed irregular expression. This suggests that MSG exposure leads to oxidative stress, cell damage, and possibly long term effect on organ function and metabolism.

In chronic exposure group, the fish exposed to 6 and 60 mg/L showed a clear increase in weight and BMI compared to the control group. This demonstrates that MSG causes excessive fat buildup and rapid physical development, supporting the idea that it act as an obesogenic agent. Interestingly total lipid and cholesterol levels were significantly higher in the exposed groups, although the blood sugar levels did not increase, indicating that MSG primary impact was on fat metabolism rather than sugar metabolism.

To further understand the effect at cellular level, histological (tissue) analysis of zebrafish organs was conducted. The results showed tissue damage, vacuolization, and structural disorganization, especially at higher concentration of MSG. These are strong signs of metabolic stress and confirmed that MSG affects not only external growth parameters but also internal organ health. The microscopic examination of these tissues confirmed the toxic effect of MSG on organs integrity.

Biochemical assays were performed to measure total lipid, cholesterol and sugar content in the homogenized tissues of the zebrafish. The most significant findings were related to cholesterol and lipid accumulation, which were much higher at higher concentrations of MSG. These changes are consistent with obesity and fat metabolism disorders. The absence of significant elevation in blood sugar however shows that MSG might contribute more to fat related complications rather than directly causing high blood sugar or diabetes, although long term exposure might lead to it over time.

Statistical analysis using one way ANOVA and Tukey's post hoc test confirmed that the difference observed between the control and MSG treated groups were statistically significant ($p<0.05$). This supports the reliability of the findings. Graphical presentations of the data further illustrated the concentration dependent effects of MSG on growth, biochemical markers, and tissue damage and gene expression changes. Notably the increase in inflammatory and stress gene expression aligns with the physical and histological evidence of obesity related inflammation and oxidative stress.

The selection of Zebrafish for this research was strategic and scientifically appropriate. Zebrafish are increasingly used in biomedical research due to their genetic similarity with humans, transparent embryos, short life cycles and easy maintenance. Their liver, pancreas and fat storage patterns are remarkably similar to those in humans. They also exhibit similar blood glucose levels and gene expression patterns, making them an excellent alternative to mammalian models like mice and rats for studying metabolic diseases. This study

strengthens the case for using zebrafish to model complex human metabolic conditions such as obesity and inflammation caused by environmental or dietary chemicals like MSG.

In conclusion, this study provides strong evidence that MSG is not just a harmless food additive, but may act as a potent metabolic disruptor. It causes developmental deformities during early life stages, promotes fat accumulation and weight gain, and disrupts gene expression patterns related to inflammation and oxidative stress. Although no major increase in blood sugar was observed in this study, the significant rise in lipid, cholesterol, and inflammation related genes indicates that MSG contribute to metabolic syndrome, particularly obesity and possibly early signs of insulin resistance. These findings highlight the potential health risks of excessive MSG consumption especially in children and individuals with a genetic predisposition to obesity or diabetes.

This research also emphasizes the need for regulatory authorities to reconsider the safe limits of MSG usage in processed foods, given it proven harmful effects at higher concentrations. Lastly the study proven zebrafish as a reliable and effective model for studying metabolic diseases, toxicological effects of food additives, and for testing future therapeutic strategies targeting obesity and related conditions.This study provides opportunity for additional research to examine the long term effect of MSG on human health and to create safer food consumption practices.

CONCLUSIONS

- MSG exposure in zebrafish leads to notable metabolic changes.
- Substantial growth abnormalities, including increased body weight and lipid accumulation was observed in the exposed zebrafish.
- Evidence of tissue toxicity and inflammatory responses that align with previous results observed in mammalian and rodents.
- Results strongly suggest that MSG act as an obesogenic and metabolic disruptor.
- Zebrafish prove to be a valuable model for further research into MSG related metabolic disorders and possible therapeutic interventions.

RECOMMENDATIONS

Although this study provides valuable insights, additional research is needed to:

- Investigate whether extended MSG exposure influences glucose metabolism and insulin resistance.
- Explore the molecular mechanisms underlying MSG-induced obesity and lipid imbalances.
- Assess intervention strategies such as dietary modification and antioxidant treatment, to mitigate MSG related metabolic toxicity.
- Conduct comparative studies across different species, particularly in mammalian models, to improve the translation of findings to human health.

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