

EFFECTS OF FENUGREEK SUPPLEMENTATION ON HIGH-FAT DIET INDUCED HYPERLIPIDEMIA IN ZEBRAFISH

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Submitted in partial fulfilment of the requirements for the award of

Master of science

in

Medical Biotechnology and

Clinical Research

by

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DEPARTMENT OF MEDICAL BIOTECHNOLOGY AND CLINICAL RESEARCH

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **VARSHA.B (41101014)** and who carried out the project entitled "**EFFECTS OF FENUGREEK SUPPLEMENTATION ON HIGH-FAT DIET INDUCED HYPERLIPIDEMIA IN ZEBRAFISH**" under our supervision from December 2022 to May 2023.


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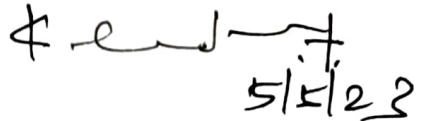

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DECLARATION

I **Ms. VARSHA.B (41101014)** hereby declare that the project report entitled **“EFFECTS OF FENUGREEK SUPPLEMENTATION ON HIGH-FAT DIET INDUCED HYPERLIPIDEMIA IN ZEBRAFISH”** done by me under the guidance of **Dr A.DAYANANDAN** at **Sathyabama institute of Science and Technology, Chennai** is submitted in partial fulfilment of the requirements for the award of Master of science degree in Medical Biotechnology and clinical research.

DATE: **05.05.2023**

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ABSTRACT

Humans are heavily affected by the causes of hyperlipidemia in high cholesterol diet. Hyperlipidemia in blood induce changes in lipid profiles, biochemical parameters and biochemical assay, the major cause of heart attack and stroke in humans. Extreme hyperlipidemia induced in mice and rabbits enables modeling many aspects of human lipid profiles, biochemical parameters and biochemical assay in blood. In the study we aimed to identify the effects of fenugreek on hyperlipidemia in high cholesterol diet zebrafish and report that feeding adult zebrafish (*Danio rerio*) with a high-cholesterol diet (HCD) resulted in hypercholesterolemia, remarkable lipoprotein oxidation, and fatty streak formation in the blood. Feeding an HCD and fenugreek treated fish supplemented in a live animal (adult zebrafish). The HCD feeding also increased lipid profiles, biochemical parameters and biochemical assay, and fenugreek treated diet feeding also decreased lipid profiles, biochemical parameters and biochemical assay. These results suggest that the novel zebrafish model is suitable for studying temporal characteristics of certain inflammatory processes of early hyperlipidemia.

Key Words: zebrafish, Hyperlipidemia, and Fenugreek.

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CHAPTER – 1

INTRODUCTION

1.1 HYPERLIPIDEMIA

Hyperlipidemia is a condition that incorporates various genetic and acquired disorders that describe elevated lipid levels within the human body. Hyperlipidemia is extremely common, especially in the Western hemisphere, but also throughout the world. Alternatively, a more objective definition describes hyperlipidemia as low-density lipoprotein (LDL), total cholesterol, triglyceride levels, or lipoprotein levels greater than the 90th percentile in comparison to the general population, or an HDL level less than the 10th percentile when compared to the general population.(Fredrickson DS.et.al,1971) Lipids typically include cholesterol levels, lipoproteins, chylomicrons, VLDL, LDL, apolipoproteins, and HDL.(Ballantyne CM.et.al,2000) Through a vast array of trials and studies, it has been consistently shown that elevated levels of LDL cholesterol increase a person's risk for the development of atherosclerotic plaques and subsequent vascular disease. In stark contrast, high-density lipoprotein (HDL) cholesterol assists in regulating cholesterol levels to prevent imbalances that would increase the risk of atherosclerotic vascular disease. Each patient's LDL cholesterol goal is conditional on their overall cardiovascular risk, and medical therapy should be independently tailored to the patient. Managing risk factors, such as hyperlipidemia, to diminish the risk for atherosclerotic cardiovascular disease is referred to as "primary prevention." The grounds for lowering LDL cholesterol derives from widespread epidemiologic data that reveals a positive, continuous correlation between LDL cholesterol levels, cardiovascular events, and patient mortality.(Fredrickson DS.et.al,1971) Treatment of hyperlipidemia continues to evolve as we better conceptualize the underlying pathophysiology, and we concurrently improve on preceding medical therapies. This article will overview the background, diagnosis, and most recent treatment guidelines for hyperlipidemia.

1.2 HISTORY AND PHYSICAL

Regularly, patients presenting with underlying hyperlipidemia remain asymptomatic, therefore obtaining a precise and thorough history is essential. Upon taking a patients history, it is crucial to obtain a profound understanding of each patient's family history of cardiovascular disease, hyperlipidemia, and/or familial hypercholesterolemia; their diet and exercise habits; tobacco, alcohol, or drug use; the presence of coronary artery disease; risk factors or history of CAD; and/or symptoms of peripheral arterial disease or angina(Vodnala.D et.al,2012)In addition to obtaining a detailed history, a focused physical exam is also very important. Obtaining accurate blood pressure measurements, observing the patients skin for xanthomas, listening for carotid and femoral bruits for evidence of stenosis, listening for an S4 heart sound, and palpating for intact peripheral pulses in all four extremities are fast and simple physical exam findings that can assist in your diagnosis of hyperlipidemia.(Fredrickson DS.et.al,1971)

1.3 COMPLICATIONS

Complications from undertreated or untreated hyperlipidemia include all types of vascular disease, which may prove fatal down the road. These include, but are not limited to, coronary artery disease, peripheral artery disease, cerebrovascular accidents, aneurysms, type II diabetes, high blood pressure, and even death.(Ford I.et.al,2016)Statin medication complications include myopathy, renal injury, arthralgia, extremity pains, nausea, myalgia, elevated liver enzymes/hepatotoxicity, diarrhea, and rhabdomyolysis. Reportedly up to 5 to 20% of patients taking a statin medication experience a muscle-related intolerance. Nisse SE.et.al,2016) A lower dose of the statin should be attempted and/or attempting to transition to another lipid-lowering medication such as ezetimibe or evolocumab.

1.4 POSTOPERATIVE AND REHABILITATION

Aerobic exercise has shown to increase the levels of the HDL-cholesterol protein, with an anti-atherogenic action: the HDL that transports cholesterol from the walls of the arteries and peripheral tissues to the liver and the same HDL has an antioxidant and anti-inflammatory capacity that induces the release of nitric oxide (vasodilator compound). Estimates are that adding 10 minutes of physical activity to daily exercise increases the concentration of HDL by 1.4 mg/dl, and it has been calculated that, on average, an adequate training program can increase cholesterol HDL of 4.6%.(Franklin BA.et.al,2020)A combination of aerobic and anaerobic activity is equally capable of positively influencing blood lipid levels. Álvarez C. et.al,2019) One hour per week of resistance training (anaerobic activity) can improve the lipid profile and corresponds to a correct indication in order not to exceed in physical effort and maintain optimal health.(Bakker EA. et.al,2018)

1.5 DETERRENCE AND PATIENT EDUCATION

All patients diagnosed with hyperlipidemia must understand how the disease affects their organs and the health risks that it may pose. A proper diet, smoking cessation, and lifestyle modifications should always be discussed. If pharmacological therapy is necessary, discussion of the risks and benefits of each medication should be explored before initiation. The primary care physician and pharmacist alike have a duty to educate the patient on medication compliance, side effects, interactions, and the overall risks versus benefits of the medications prescribed. The patient must also understand the potential risks related to not taking the medication and what alternatives they may have as treatment options. (Fredrickson DS.et.al,1971)

1.6 ZEBRAFISH

TAXONOMY AND NOMENCLATURE

Scientific classification of *Danio rerio*

Taxonomic rank : Species

Common name(s) : Zebra danio

Kingdom : Animalia

Subkingdom : Bilateria

Infrakingdom : Deuterostomia

Phylum :Chordata
Superclass :Actinopterygii
Class :Actinopterygii
Subclass :Neopterygii
Order :Cypriniformes
Family :Cyprinidae
Subfamily :Danioninae
Genus :Danio
Species :*D.rerio*

Various animal species have important roles as experimental models to advance biomedical research. Animal models provide consistency and validity of research results from in vitro studies or studies with rodents. Zebrafish has become a popular animal model for biomedical research. One reason that zebrafish are an important biomedical model is because zebrafish embryos are transparent and they develop outside of the uterus. This unique developmental process allows scientists to study the details of development starting from fertilization and continuing throughout development. Innovation and development of molecular techniques model in areas mainly related to diet-induced diseases, metabolic disorders, liver diseases, and intestinal diseases in humans. https://doi.org/10.1093/af/vfz020_

1.7 GENERAL FEATURES OF ZEBRAFISH

Danio rerio the Latin name for zebrafish formerly called *Brachydanio rerio* is a small tropical freshwater fish originating in the Ganges River and its tributaries in northern India (Tavares and Santos Lopes, 2013). In the natural habitat, zebrafish are usually found near the bottom of the water to minimize attack by predators. Currently, zebrafish are considered as a suitable model to investigate development, genetics, immunity, behavior, physiology, and nutrition. According to its feeding habits, zebrafish are classified as omnivores and they eat a variety of foods (euryphagous). During experimental trials, scientists use different types and levels of dietary feeds. The same amounts of ingredients are used for adult and larvae zebrafish. Moreover, the feeds and feeding regimes implemented by some laboratories for rearing zebrafish are varied and, in some cases, are implemented without formal evaluation (Castranova et al., 2011; Gonzales and Law, 2013). In the laboratory, to get reasonable research results, zebrafish should receive the appropriate type and level of dietary nutrient. Most of the time researchers use different commercial diets for zebrafish, but several commercial diets have undefined nutritional composition and may have an effect on experimental results (Gonzales and Law, 2013). The criteria to select animal models for biomedical research are directly related to the final goal of the research. The use of zebrafish as a biomedical model was suggested by George Streisinger and colleagues at

the University of Oregon, who launched the modern era for zebrafish in the field of biomedical research (Clark and Ekker,2015).

1.8 FENUGREEK

Fenugreek (*Trigonella foenum graecum*) is an annual plant belongs to the family Leguminosae. It is the famous spices in human food. The seeds and green leaves of fenugreek are used in food as well as in medicinal application that is the old practice of human history. It has been used to increase the flavoring and color, and also modifies the texture of food materials. Seeds of fenugreek spice have medicinal properties such as hypocholesterolemic, lactation aid, antibacterial, gastric stimulant, for anorexia, antidiabetic agent, galactagogue, hepatoprotective effect and anticancer. These beneficial physiological effects including the antidiabetic and hypocholesterolemic effects of fenugreek are mainly attributable to the intrinsic dietary fiber constituent which have promising nutraceutical value (Srinivasan, 2006). It is well known for its fiber, gum, other chemical constituents and volatile contents. Dietary fiber of fenugreek seed is about 25% which changes the texture of food. These days it is used as food stabilizer, adhesive and emulsifying agent due to its high fiber, protein and gum content. The protein of fenugreek is found to be more soluble at alkaline pH (Meghwal and Goswami, 2012). Fenugreek is having beneficial influence on digestion and also has the ability to modify the food.

1.9 PYTOCHEMISTRY

Fenugreek contains a number of chemical constituents including steroidal saponin. Diosgenin component has been found in the oily embryo of fenugreek. There are two furastanol glycosides, F-ring opened precursors of diosgenin that have been reported in fenugreek also as hederagin glycosides. Alkaloids such as trigocoumarin, nicotinic acid, trimethyl coumarin and trigonelline are present in stem. The mucilage is a standing out constituent of the seeds (Khare, 2004). There is about 28% mucilage; a volatile oil; 2 alkaloids such as trigonelline and Choline, 5% of a stronger-smelling, bitter fixed oil, 22% proteins and a yellow coloring substance are present in stem (Grieve, 1984). Fenugreek contains 23–26% protein, 6–7% fat and 58% carbohydrates of which about 25% is dietary fiber (US Department of Agriculture, 2012). Fenugreek is also a rich source of iron, containing 33 mg/100 g dry weight (US Department of Agriculture, 2001).

1.10 LEAVES AND SEEDS

The leaves contain seven saponins, known as graecunins. These compounds are glycosides of diosgenin. Leaves contain about 86.1% moisture, 4.4% protein, 0.9% fat, 1.5% minerals, 1.1% fiber, and 6% carbohydrates. The mineral and vitamins present in leaves include calcium, zinc, phosphorous, Iron, riboflavin, carotene, thiamine, niacin and vitamin C (Rao, 2003). Yadav and Sehgal (1997) found that fresh leaves of fenugreek contain ascorbic acid of about 220.97 mg per 100 g of leaves and β -carotene is present about 19 mg/100 g. On the other side, it was reported that 84.94% and 83.79% ascorbic acid were reduced in sun and oven-dried fenugreek leaves

respectively. Fresh leaves are used as vegetables in the diets. It was found that there was a better retention of nutrients in the leaves of fenugreek. The leaves of fenugreek should be stored in either in refrigeration conditions, or dried in oven, or blanched for sometime (about 5 min) and should be cooked in pressure cooker. Fenugreek is known for its pleasantly bitter, slightly sweet seeds. The seeds are available in any form whether whole or ground form is used to flavor many foods mostly curry powders, teas and spice blend. Fenugreek seed has a central hard and yellow embryo which is surrounded by a corneous and comparatively large layer of white and semi-transparent endosperm (Betty, 2008). List of chemical constituents is shown in table below. The chemical composition of fenugreek (such as seeds, husk and cotyledons) showed that endosperm had the highest (4.63 g/100 g) saponin and (43.8 g/100 g) protein content. As against this, husk contains higher total polyphenols. The extracts of endosperm husk, and fenugreek seed at about 200 µg concentration exhibited antioxidant activity 72%, 64%, and 56% respectively by free-radical scavenging method (Naidu et al., 2010). The seeds of fenugreek contain about 0.1–0.9% of diosgenin and are extracted commercially. The structure of diosgenin is shown in image. The plant tissue cultures from seeds of fenugreek when grown under optimal conditions have been found to produce as much as 2% diosgenin with smaller smaller amounts of trigogenin and gitongenin. Seeds also contain the saponin (fenugrinB) Fenugreek seeds have been found to contain several coumarin compounds as well as a number of alkaloids (e.g., trigonelline, gentianine, carpaine). The large amount of trigonelline is degraded to nicotinic acid and related pyridines during roasting (Acharya et al., 2006). The major bioactive compounds in fenugreek seeds are believed to be polyphenol compounds, such as rhaponticin and isovitexin (He et al., 2015). Small amount of volatile oils and fixed oil has been found in fenugreek seeds (Sowmya and Rajyalakshmi, 1999). Blank et al. (1997) have found the odor active compounds based on the fenugreek aroma detection with the help of Gas Chromatograph and these includes the olfactometry diacetyl, 1-Octene-3-one, sotolon, acetic acid; 3-Isobutyl-2-methoxypyrazine, butanoic acid, isovaleric acid, 3-isopropyl-2-methoxypyrazine, caproic acid, eugenol, 3-Amino-4,5-dimethyl-3, linalool, (Z)-1,5-Octadiene-3-one, 4-dihydro-2(5H)-Furanone with characteristic aroma of buttery like, roasty/earthy, metallic, pungent, paprika like, sweaty/rancid, flowery, musty, spicy respectively. Out of all these volatile compounds, sotolon was reported to be found most predominantly in (5s)-enantiomeric form (95%) in fenugreek. A study was conducted on sweat of human after fenugreek ingestion and it has been concluded that compounds responsible for the strong maple-syrup odor present in sweat after fenugreek ingestion are due to the following components including the following: pinene; 3-octen-2-one, 2,5-dimethylpyrazine, β -; camphor; terpinen-4-ol; 4-isopropyl-benzaldehyde; neryl acetate and β -caryophyllene but it was observed that 2,5-dimethylpyrazine to be a major component responsible for sweat odor contributing.

CHAPTER – 2

REVIEW OF LITERATURE

2.1 STUDIES ON ZEBRAFISH:

Since the 1960s, the zebrafish (*Danio rerio*) has become increasingly important to scientific research. It has many characteristics that make it a valuable model for studying human genetics and disease. Zebrafish is a tropical fresh water fish native of southeast Asia and it measures about 2.5 cm to 4 cm in length. Zebrafish has similar genetic makeup as that of humans. They share 70% of genes with humans. 84% of genes are known to be associated with human disease have a zebrafish counterpart. As a vertebrate, the zebrafish has the same major organs as humans like blood, muscles, kidneys and eyes. The zebrafish genome has been sequenced which enabled researchers to create mutations in more than 14,000 genes to study their functions. Zebrafish has an unique ability to repair their heart muscle researchers working out to find out the specific factors involved in this process so that it can help humans with heart failures. The review throws light on the use of Zebrafish for various biomedical researches.(Spence R.et.al,2008)

Zebrafish have several advantages compared to other vertebrate models used in modeling human diseases, particularly for large-scale genetic mutant and therapeutic compound screenings, and other biomedical research applications. With the impactful developments of CRISPR and next-generation sequencing technology, disease modeling in zebrafish is accelerating the understanding of the molecular mechanisms of human genetic diseases. These efforts are fundamental for the future of precision medicine because they provide new diagnostic and therapeutic solutions. This review focuses on zebrafish disease models for biomedical research, mainly in developmental disorders, mental disorders, and metabolic diseases.(Tae-Young Choi,et.al,2021)The zebrafish model has recently emerged as a significant experimental system for developmental biology studies. In this review, we provide an introduction to the zebrafish model system by overviewing set-up and husbandry requirements, the genome, and associated bioinformatics infrastructure. We then detail the utility of zebrafish for forward, reverse, and chemical genetic studies of development. We also discuss using zebrafish for live cell imaging experiments. Finally, we provide an overview of zebrafish community resources.(Brian A.et.al,2008)Much of medical research relies on animal models to deepen knowledge of the causes of animal and human diseases, as well as to enable the development of innovative therapies. Despite rodents being the most widely used research model worldwide, in recent decades, the use of the zebrafish (*Danio rerio*) model has exponentially been adopted among the scientific community. This is because such a small tropical freshwater teleost fish has crucial genetic, anatomical and physiological homology with mammals. Therefore, zebrafish constitutes an excellent experimental model for

behavioral, genetic and toxicological studies which unravels the mechanism of various human diseases. Furthermore, it serves well to test new therapeutic agents, such as the safety of new vaccines. The aim of this review was to provide a systematic literature review on the most recent studies carried out on the topic. It presents numerous advantages of this type of animal model in tests of efficacy and safety of both animal and human vaccines, thus highlighting gains in time and cost reduction of research and analyzes.(Ricardo Lacava Bailone.et.al, 2020)

2.2 HYPERLIPIDEMIA

Hyperlipidemia is a term that encompasses various genetic and acquired disorders that describe elevated lipid levels within the body. It is a very common disorder, especially in the Western hemisphere, but also throughout the world. Hyperlipidemia itself does not typically lead to critical symptoms itself, however, having this underlying pathology will often lead to serious illnesses that may ultimately lead to death. To lower morbidity and mortality rates associated with this disorder, it is critical to establish an early diagnosis and prevent the progression of the disease. This activity reviews the etiology, pathophysiology, evaluation, and treatment guidelines associated with hyperlipidemia. It also highlights the role of interprofessional communication and teamwork when managing the diagnosis and the correct treatment of patients diagnosed with hyperlipidemia. Hyperlipidemia is a condition that incorporates various genetic and acquired disorders that describe elevated lipid levels within the human body. Hyperlipidemia is extremely common, especially in the Western hemisphere, but also throughout the world. Alternatively, a more objective definition describes hyperlipidemia as low-density lipoprotein (LDL), total cholesterol, triglyceride levels, or lipoprotein levels greater than the 90th percentile in comparison to the general population, or an HDL level less than the 10th percentile when compared to the general population. Lipids typically include cholesterol levels, lipoproteins, chylomicrons, VLDL, LDL, apolipoproteins, and HDL. Through a vast array of trials and studies, it has been consistently shown that elevated levels of LDL cholesterol increase a person's risk for the development of atherosclerotic plaques and subsequent vascular disease. In stark contrast, high-density lipoprotein (HDL) cholesterol assists in regulating cholesterol levels to prevent imbalances that would increase the risk of atherosclerotic vascular disease. Each patient's LDL cholesterol goal is conditional on their overall cardiovascular risk, and medical therapy should be independently tailored to the patient. Managing risk factors, such as hyperlipidemia, to diminish the risk for atherosclerotic cardiovascular disease is referred to as "primary prevention." The grounds for lowering LDL cholesterol derives from widespread epidemiologic data that reveals a positive, continuous correlation between LDL cholesterol levels, cardiovascular events, and patient mortality. Treatment of hyperlipidemia continues to evolve as we better conceptualize the underlying pathophysiology, and we concurrently improve on preceding medical therapies.(Marcus F. Hill; Bruno Bordini.et.al,2022)Elevated levels of blood lipids are well documented risk factors for cardiovascular disease. Current classification schemes and treatment levels for hyperlipidemia are based on the National Cholesterol Education Panel's (NCEP) Adult Treatment Program-3

(ATP guidelines. Statins are the preferred class of drugs to lower elevated low density lipoprotein cholesterol (LDL-C). There are other classes to augment or substitute for statins, such as ezetimibe, fibrates, niacin and dietary supplements. Extensive research over the last decade has raised the question whether or not ATP-III guidelines are sufficiently aggressive. New guidelines from ATP-IV are expected to be released in the near future, but in the meantime physicians are faced with uncertainty about how low to target LDL-C, whether to pharmacologically treat high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels and how best to achieve target goals.(Robert H.et.al,2013)

2.3 FENUGREEK

Fenugreek (*Trigonella foenum-graecum*) is a legume and it has been used as a spice throughout the world to enhance the sensory quality of foods. It is known for its medicinal qualities such as antidiabetic, anticarcinogenic, hypocholesterolemic, antioxidant, and immunological activities. Beside its medicinal value, it is also used as a part of various food product developments as food stabilizer, adhesive, and emulsifying agent. More importantly it is used for the development of healthy and nutritious extruded and bakery product.(Sajad Ahmad Wani.et.al, 2018) Fenugreek is a herb that is widely used in cooking and as a traditional medicine for diabetes in Asia. It has been shown to acutely lower postprandial glucose levels, but the long-term effect on glycemia remains uncertain. We systematically reviewed clinical trials of the effect of fenugreek intake on markers of glucose homeostasis.(NithyaNeelakantan.et.al, 2014) Various studies have shown that *Trigonella foenum-graecum* (fenugreek) supplementation has lipid-lowering activity. This meta-analysis was performed to evaluate the effect of fenugreek supplementation on human serum lipid profile. Data sources were PubMed, EMBASE, Scopus, and Coherence library which were searched systematically from January 2000 up to December 2019. Inclusion criteria were randomized clinical trial (RCT) study design, at least one of lipid profile components (triglyceride [TG], total cholesterol [TC], low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol) levels measured before fenugreek use and one of the lipid components level reported as result.(Kiyan Heshmat-Ghahdarjani.et.al,2020) Fenugreek seed are rich sources of various essential compounds and such components shows a significant role in the diseases cure and management through its anti-inflammatory, hepatoprotective, cardioprotective, neuroprotective, immunomodulatory, nephroprotective and anti-microbial activity. Moreover, fenugreek seed is a rich source of antioxidant and such potentiality inhibit the pathogenesis of diseases through free radical scavenging activity. Besides, fenugreek and its active compound shows role in tumour inhibition through modulating various genes activity including induction of apoptosis, tumour suppressor gene and inhibition of tumor necrosis factor. This review comprehensively summarizes the role of fenugreek seeds in health management through modulating various physiological and biochemical activities, along with special emphasis on *in vitro and vivo study*. (SA Almatroodi.et.al, 2021).

CHAPTER-3

AIM AND OBJECTIVE OF THE STUDY

AIM

To identify the effects of fenugreek on hyperlipidemia in high cholesterol diet zebrafish.

OBJECTIVE

- ❖ To evaluate the role of fenugreek in hyperlipidemic zebrafish.
- ❖ To analyze the complete lipid profile of the model of zebrafish.
- ❖ And to analyze the biochemical parameters and biochemical assay in the model of zebrafish.

CHAPTER- 4

MATERIALS AND METHODS

4.1 ZEBRAFISH

This study was conducted using wild-type zebrafish (5 month adult danio rerio) were obtained from local suppliers. The fish were kept in a static system in aquariums under constant aeration, with weekly three days replacement of 20% of the water and cycles of light/ darkness of 14:10. The temperature and pH of the water were held constant at 25 °C and 7.4, respectively. Uneaten feed and waste were removed daily to avoid contamination. Goodwin et al. (2016) have shown that these strategies maintain the water quality parameters closer to the acceptable standards. The protocol for zebrafish care was approved by the Committee of Research and Ethics of the Faculty of Medicine and the IACUC of the Pontificia Universidad Javeriana. Sixty specimens were used, complying with the principle of the three Rs, which encourages minimizing the number of samples and optimizing their use.

4.2 DIET

The feed used for all experiments was a form of commercial flake tropical fish food having the following defined composition: 47% protein, 10% lipids, fiber 3%, and 6% moisture. First I have divided fish into three groups such as control (group-1), high cholesterol diet (HCD-group-2), fenugreek treated diet (group-3)

4.3 GROUP-1

For the first group I have chosen 20 adult zebrafish (5 month old male/female) for control. The first group received the normal diet for tropical commercial fish feed (standard fish feed) was given for 4 weeks.

4.4 GROUP-2

For the second group, 40 adult zebrafish (5 months old female/male) were selected. The second group received the high cholesterol diet was given for 4 weeks.

4.4.1 High cholesterol feed preparation;

4 % of cholesterol feed that is 0.4g of cholesterol, that was mixed with fish feed 9.6g, So totally 10g of cholesterol feed for given for 4 weeks ;

- Fish feed = 9.6g
- 4% cholesterol = 0.4g
- 100% Ethanol = 5ml

4.4.2 PROCEDURE

To prepare high cholesterol diet fish feed such as 4% of cholesterol feed that is, 9.6g of regular fish food can be mixed with 0.4g of 4% standard cholesterol powder, which is then soaked in 100% ethanol. This allows the cholesterol to

become fully absorbed by the fish food. After that the regular fish food and the standard cholesterol powder have been soaked in 100% ethanol to create cholesterol-rich feed, the mixture can be air-dried at room temperature until the excess ethanol has standard cholesterol powder have been soaked in 100% ethanol to create cholesterol-rich feed, the mixture can be air-dried at room temperature until the excess ethanol has evaporated and the feed is dry. This cholesterol-rich feed can be used to feed zebrafish providing a way to study the effects of cholesterol on their health and physiology. It is important to store the cholesterol feed in a cool and dry place to prevent it from spoiling.

4.5 GROUP-3

After 4 weeks, for the third group, 20 adult zebrafish(5 months old female/male) were selected from group-2(High cholesterol diet zebrafish). The third group received the fenugreek diet was given for 2 weeks.

4.5.1 Fenugreek feed preparation;

4 % of fenugreek feed that is 0.4g of fenugreek weighed and made a powder, that was mixed with fish feed 9.6g, So totally 10g of fenugreek feed was given for 2 weeks ;

- Fish feed = 9.6g
- Fenugreek =0.4g
- Water =5ml

4.5.2 PROCEDURE

To prepare fenugreek diet fish feed such as 4% of fenugreek feed that is,9.6g of regular fish feed can be mixed with 0.4g Of fenugreek weighed and made a powder, which is then soaked in 5ml of water. This allows the fenugreek to become fully absorbed by the fish food. After that the regular fish food and the fenugreek powder have been soaked in 5ml of water to create fenugreek feed, the mixture can be air-dried at room temperature until the excess water has evaporated and the feed is dry. This fenugreek feed can be used to feed zebrafish providing a way to study the effects of fenugreek on their health and physiology. It is important to store the fenugreek feed in a cool and dry place to prevent it from spoiling.

In three groups, daily cleaning of the aquariums was performed, consisting of vacuuming residue from the bottoms of the aquariums in order to prevent the accumulation of food and contamination.

4.6 DISSECTION AND SAMPLE COLLECTION

The collection of blood from the adult Zebrafish. I have collected the blood samples from group-1(control), group-2(HCD), group-3(FTD). It also provides instructions for obtaining serum for use in lipid profile test, such as tests for determining the total cholesterol and triglyceride, LDL, HDL levels, lipid peroxidation,

biochemical parameters, biochemical assay such as Alanine transaminase (SGPT), Aspartate transaminase (SGOT).



Figure : 1 Zebrafish Dissection Sample Collection

4.6.1 PROCEDURE

- ❖ Before collecting zebrafish blood, it is necessary to prepare anesthetizing water. Pour ~200 ml of aquarium water into a container with a 500-ml capacity. Add ~200 g of ice chips. The temperature should be about 4 °C. As the ice chips melt, it will be necessary to add more ice chips to maintain a constant temperature near 4 °C.
- ❖ When the anesthetizing water is ready, prepare the materials needed for blood collection. Do not allow the pipette tip to contact any sources of contamination.
- ❖ Cover a Petri dish with a piece of dry gauze. A steel blade and another piece of gauze should be placed in an easily accessible place.
- ❖ A centrifuge adapted for plastic tubes will be needed.
- ❖ When the aforementioned materials are prepared, capture the first zebrafish to be anesthetized with a fishing net and release it into the water that has been prepared for anesthesia. Zebrafish require 3-6 s in chilled water to be anesthetized, depending on the fish. Keep the fish in the cold water until it no longer responds to external stimuli.
- ❖ Using the fishing net, place the anesthetized fish on a prepared piece of gauze, leaving the tail off of the gauze. Fold the gauze over the fish's head and body leaving out only its tail. Put the fish covered with the gauze on the Petri dish.
- ❖ Use the steel blade to make a diagonal incision just between the anal fin and the caudal fin. The blood will start to come out. At this point, it is necessary to work quickly.
- ❖ Gently aspire the blood that comes out with the micropipette. The amount of blood that can be collected depends on the size of the fish and to what extent that it was anesthetized correctly. It usually varies from 5 to 20 μ l. When the blood stops coming out, gently transfer the aspirated blood into a tube.

- ❖ To avoid hemolysis, it is critical that the tube with blood in it be handled very carefully, without any drastic movements until it is placed into the centrifuge.
- ❖ To avoid hemolysis, it is also important that the blood sample be secured in the centrifuge within 10 minutes of blood collection.
- ❖ If necessary, it is possible to combine blood from more than one animal, making a pool. Pooled samples will work fine as long as the delay between blood collection from the first fish and centrifugation does not exceed 10 minutes.
- ❖ When the blood collection is done, centrifuge the blood for 10 minutes at 0.5 g (Eppendorf Centrifuge 5415D).
- ❖ Following centrifugation, the serum is at the top layer of the tube. With a pipette, aspirate the serum, making sure to get just the serum while keeping both layers well divided and stable.
- ❖ Transfer the serum into a new microtube and it is ready to be used in biochemical analyses. The serum can be stored in ice while it wait to start the biochemical analyses.
- ❖ If the serum will not be used immediately, it can be frozen at -18 °C for up to approximately 3 months.

4.7 CHOLESTEROL(CHOD/POD Method)

4.7.1 Principle:

Cholesterol esterase hydrolyses esterified cholesterol to free cholesterol. The free cholesterol is oxidised to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample. The total cholesterol level was quantified by using beacon reagent kit (pod method) at 505 nm in multi plate reader.

4.7.2 MATERIALS REQUIREMENTS

- Clean & Dry Glassware.
- Laboratory Glass Pipettes or Micropipettes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.
- Multi plate reader

4.7.3 CHEMICAL REQUIREMENTS

- Cholesterol Reagent Kit

4.7.4 Reagent composition

Reagent 1 : Cholesterol Enzyme Reagent

Reagent 2 : Cholesterol Standard 200 mg/dl.

Reagent 3 : Cholesterol Precipitating Reagent (Free)

Reagent 1 : Cholesterol Enzyme Reagent

Goods Buffer : > 80 mmol/L

Phenol : > 10 mmol/L

4-aminoantipyrine : > 0.3 mmol/L

Cholesterol esterase : > 250

Cholesterol oxidase : > 250

Peroxidase : > 2250

Reagent 2 : Cholesterol Standard 200 mg/dl.

Ready to use.

Reagent Preparation

Reagent is liquid, ready to use.

4.7.5 Stability and Storage

All Reagents are ready to use and are Stable till the expiry date mentioned on the label, when stored at 2 - 8 °C.

4.7.6 Assay procedure

Wavelength : 505nm

Cuvette :1cm

4.7.7 PROCEDURE

Pipette into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T):

Addition	Sequence	Blank	Standard	Control	Test 1	Test 2
Reagent	1	100 µl	100 µl	100 µl	100 µl	100 µl
Standard			2 µl			
Sample				10 µl	10 µl	10 µl
Distilled Water		10 µl				

Mix well and incubate at 37°C for 5mins. Measure absorbance of the Standard (Abs.S) and Test (Abs.T) against Reagent Blank at 505 nm.

4.7.8 CALCULATION

$$\text{Cholesterol (mg/dl)} = \text{Abs. T} / \text{Abs.S} \times 200$$

4.8 LIPID PEROXIDATION METHOD

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides are unstable and decompose to form a complex series of compounds including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) upon decomposition. The measurement of MDA and HAE has been used as an indicator of lipid peroxidation (1). This method is designed to assay either MDA alone (in hydrochloric acid) or MDA in combination with HAE (in methanesulfonic acid.)

4.8.1 Principle

This Lipid Peroxidation Assay is based on the reaction of a chromogenic reagent, N-methyl-2- phenylindole (R1), with MDA and HAE at 45°C. One molecule of either MDA or HAE reacts with 2 molecules of Reagent R1 to yield a stable chromophore with maximal absorbance at 586 nm. The lipid peroxidation level was quantified by using multi plate reader at 530 nm.

4.8.2 MATERIALS REQUIREMENTS

- Clean & Dry Glassware.
- Laboratory Glass Pipettes or Micropipettes & Tips.
- Eppendorfs tupe.
- Multi plate reader.
- Centrifuge,Water bath.
- Distilled Water.

4.8.3 CHEMICAL REQUIREMENTS

- 10mm Feso4 (27.8mg) = 10ml
- 10mm KH2PO4 (13.6mg) =10ml
- 10mm H2O2 (120ml) = 10ml
- 0.15m Tris Hcl (3.63g) = 200ml (PH-7.4)
- 1% TBA (Thiobarbituric acid)(1g) =100ml

4.8.4 PROCEDURE

	BLANK	CONTROL	TEST -1	TEST -2
TRIS BUFFER	1.6 ml	1.4 ml	1.4 ml	1.4 ml
KH2PO4	0.2 ml	0.2 ml	0.2 ml	0.2 ml
FESO4	-	0.2 ml	0.2 ml	0.2 ml
H2O2	-	0.2 ml	0.2 ml	0.2 ml

- All solutions are keep it in shaker for 20 minutes.
- Then add 1.5 ml of 1% Thiobarbituric acid in all solutions.

- And then the 1% TBA added solutions are kept in a boiling water bath for 15 minutes and read at 530nm in a multi plate reader.

4.8.5 CALCULATION

$T/0.015 \times 1 \times 1/0.2 \times 1/\text{mg ptn}$ (moles of MDA formed / mg ptn)

4.9 ALANINE TRANSAMINASE (SGPT)

SGPT – Serum Glutamic Pyruvic Transaminase

The alanine aminotransferase (ALT) test is a blood test that checks for liver damage. Your doctor can use this test to find out if a disease, drug, or injury has damaged your liver. This enzyme is found mainly in your liver. Smaller amounts of ALT are in your kidneys and other organs, too. Your body uses ALT to break down food into energy. Normally, ALT levels in the blood are low. If your liver is damaged, it will release more ALT into your blood and levels will rise. (ALT used to be called serum glutamic-pyruvic transaminase, or SGPT). Doctors often give the ALT test along with other liver tests. ALT stands for alanine transaminase. It is an enzyme found mostly in the liver. An ALT test measures the amount of ALT in the blood. When liver cells are damaged, they release ALT into the bloodstream. High levels of ALT in your blood may be a sign of a liver injury or disease. Some types of liver disease cause high ALT levels before you have symptoms of the disease. So, an ALT blood test may help diagnose certain liver diseases early.

Other names: Alanine aminotransferase (ALT), Serum Glutamic-Pyruvic Transaminase (SGPT), GP.

4.9.1 PRINCIPLE

SGPT (ALT) catalyzes the transfer of amino group between Alanine and α -Ketoglutarate to form Pyruvate and Glutamate. The Pyruvate formed reacts with NADH in the presence of Lactate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGPT(ALT) activity in the sample. The Alanine transaminase level was quantified by using a multi plate reader at 540 nm.

4.9.2 MATERIALS REQUIREMENTS

- Clean & Dry Glassware.
- Laboratory Glass Pipettes or Micropipettes & Tips.

- Eppendorfs tupe.
- Multi plate reader.
- Centrifuge.
- Distilled water.

4.9.3 CHEMICAL REQUIREMENTS

- Alanine =
- Alpha-Ketoglutarate = 1.2 mg
- Phosphate buffer = 4 ml
- Disodium hydrogen po4 = 0.04748 mg
- Sodium hydrogen po4 = 0.03628 mg
- H₂O = 10 ml
- 2,4-Dinitrophenylhydrazine = 1mg
- 1 N HCl = 0.4165 ml
- 0.4 N Sodium Hydroxide (NaOH) = 0.4 g

4.9.4 SOLUTION PREPARATION

1) Substrate ;

Aanine + 1.2 mg of Alpha-Ketoglutarate + 4 ml of Phosphate buffer.

2) Po4 Buffer : PH- 7.4

Sol A : 0.04748 mg of Disodium hydrogen po4 / 4ml H₂O

Sol B : 0.03628 mg of Sodium hydrogen po4 / 4ml H₂O

Working = 3.36 ml of Sol A + 0.64 ml of Sol B = 4 ml of PO₄ buffer.

3) DNPH – 1 mg + 0.4165 ml of 1N HCL + 5 ml of H₂O

4) 0.4 N NaoH – 0.4 g + 25 ml of H₂O

4.9.5 PROCEDURE

	BLANK	CONTROL	TEST - 1	TEST - 2	TEST - 3
SUBSTRARE	-	1 ml	1 ml	1 ml	1 ml
SAMPLE	-	-	50 µl	50 µl	50 µl
Incubate at 37°C for 10 minutes					
DNPH	1 ml	1 ml	1 ml	1 ml	1 ml
WATER	1.5 ml	500 µl	450 µl	450 µl	450 µl
Incubate at 37°C for 20 minutes					
NAOH	2 ml	2 ml	2 ml	2 ml	2 ml

- ❖ The substrate solution were added , then Incubate at 37°C for 10 minutes.
- ❖ Then added serum sample and Incubate at 37°C for 10 minutes.
- ❖ Then added 1 ml of DNPH in Blank, Control and Test solutions, then added 1.5 ml of distilled water in Blank solution, 500 µl of distilled water in Control solution and added 450 µl of distilled water in Test solution.
- ❖ Then to incubate at 37°C for 20 minutes.
- ❖ And finally 2ml of NAOH added in blank, control and test samples.

4.9.6 CALCULATION

$$T / 0.1 \times 0.1 \times 1/0.43 \times 7.2$$

4.10 ASPARATATE TRANSAMINASE (SGOT)

SGPT – Serum Glutamic Oxaloacetic Transaminase

Aspartate transaminase (AST) is an enzyme that is released when your liver or muscles are damaged. Although AST is found mainly in your liver and heart, AST can also be found in small amounts in other muscles. AST, aspartate aminotransferase, serum glutamic oxaloacetic transaminase test, SGOT. This blood test is used to diagnose liver damage. Aspartate transaminase (AST) is an enzyme that is released when your liver or muscles are damaged. Although AST is found mainly in your liver and heart, AST can also be found in small amounts in other muscles. It makes a fluid called bile that helps your body digest food. It also removes waste products and other toxins from your blood. It produces proteins, as well as substances that help your blood clot. Alcohol or drug use and diseases such as hepatitis can damage your liver and keep it from doing these jobs. AST is an enzyme your liver makes. Other organs, like your heart, kidneys, brain, and muscles, also make smaller amounts. AST is also called SGOT (serum glutamic- oxaloacetic transaminase). Normally, AST levels in your blood are low. When your liver is damaged, it puts more AST into your blood, and your levels rise. A high AST level is a sign of liver damage, but it can also mean you have damage to another organ that makes it, like your heart or kidneys. That's why doctors often do the AST test together with tests of other liver enzymes.

Other names: Aspartate aminotransferase (AST), Serum Glutamic-Oxaloacetic Transaminase (SGOT), GP.

4.10.1 PRINCIPLE

Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from L-aspartate to α -ketoglutarate to yield oxaloacetate and L-glutamate. Malate dehydrogenase (MDH) catalyzes the reduction of oxaloacetate with simultaneous oxidation of NADH⁺ to NAD. The resulting rate of decrease in absorbance at 340 nm is directly proportional to the AST activity. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate which is normally present in serum.

4.10.2 MATERIALS REQUIREMENTS

- Clean & Dry Glassware.
- Laboratory Glass Pipettes or Micropipettes & Tips.
- Eppendorfs tupe.
- Multi plate reader.
- Centrifuge.

-Distilled water.

4.10.3 CHEMICAL REQUIREMENTS

- Asparatic acid = 5.32 mg
- Alpha-Ketoglutarate = 0.06 mg
- Phosphate buffer = 4 ml
- Disodium hydrogen po4 = 0.047504 mg
- Sodium hydrogen po4 = 36.312 mg
- H₂O =10 ml
- 2,4-Dinitrophenylhydrazine = 1mg
- 1 N HCl = 0.4165 ml
- 0.4 N Sodium Hydroxide (NaOH) =0.4 g

4.10.4 SOLUTION PREPARATION

1) Substrate ;

5.32 mg of Asparatic acid + 0.06 mg of Alpha-Ketoglutarate + 4 ml of Phosphate buffer.

2)Po4 Buffer : PH- 7.4

Sol A : 0.047504 mg of Disodium hydrogen po4 / 4ml H₂O

Sol B : 36.312 mg of Sodium hydrogen po4 / 4ml H₂O

Working = 3.36 ml of Sol A + 0.64 ml of Sol B =4 ml of PO₄ buffer

3)DNPH – 1 mg + 0.4165 ml of 1N HCL + 5 ml of H₂O

4) 0.4 N NaOH – 0.4 g + 25 ml of H₂O

4.10.5 PROCEDURE

	BLANK	CONTROL	TEST - 1	TEST - 2	TEST - 3
SUBSTRATE	-	1 ml	1 ml	1 ml	1 ml
SAMPLE	-	-	50 µl	50 µl	50 µl
Incubate at 37°C for 10 minutes					
DNPH	1 ml	1 ml	1 ml	1 ml	1 ml
WATER	1.5 ml	500 µl	450 µl	450 µl	450 µl
Incubate at 37°C for 20 minutes					
NAOH	2 ml	2 ml	2 ml	2 ml	2 ml

- ❖ The substrate solution were added , then Incubate at 37°C for 10 minutes.
- ❖ Then added serum sample and Incubate at 37°C for 10 minutes.
- ❖ Then added 1 ml of DNPH in Blank,Control and Test solutions, then added 1.5 ml of distilled water in Blank solution, 500 µl of distilled water in Control solution and added 450 µl of distilled water in Test solution.
- ❖ Then to incubate at 37°C for 20 minutes.
- ❖ And finally 2ml of NAOH added in blank, control and test samples.

4.10.6 CALCULATION

$$T / 0.1 \times 0.1 \times 1/0.47 \times 5.8$$

CHAPTER – 5

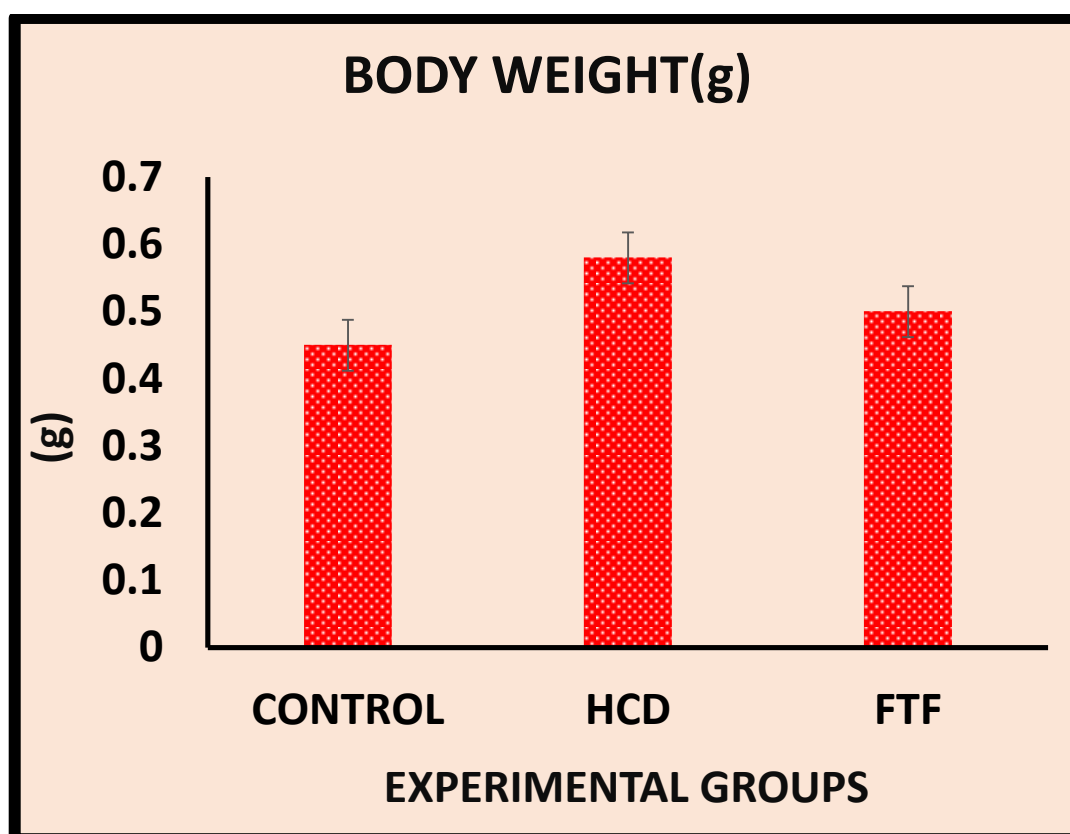
RESULT AND DISCUSSION

5.1 CHANGES IN BODY WEIGHT AND BODY IMAGE IN ADULT ZEBRAFISH

The body weight of adult zebrafish has been recorded between the control, High Cholesterol diet, and Fenugreek treated animals. **GRAPH-1** Changes in body weight between control, HCD and FTF.

CONTROL	HCD	FTF
0.45 g	0.58 g	0.50 g

The body weight has been increased in High Cholesterol diet zebrafish, and decreased in fenugreek treated fish. GRAPH-1 Changes in body weight between control, HCD, and FTF.



**WEIGHT VARIATION IN ZEBRAFISH IMAGE BETWEEN
CONTROL,HIGH CHOLESTEROL DIET AND (HCD) AND
FENUGREEK TREATED FISH (FTF)**



CONTROL



HCD



FTF

5.2 LIPID PROFILE ANALYSES

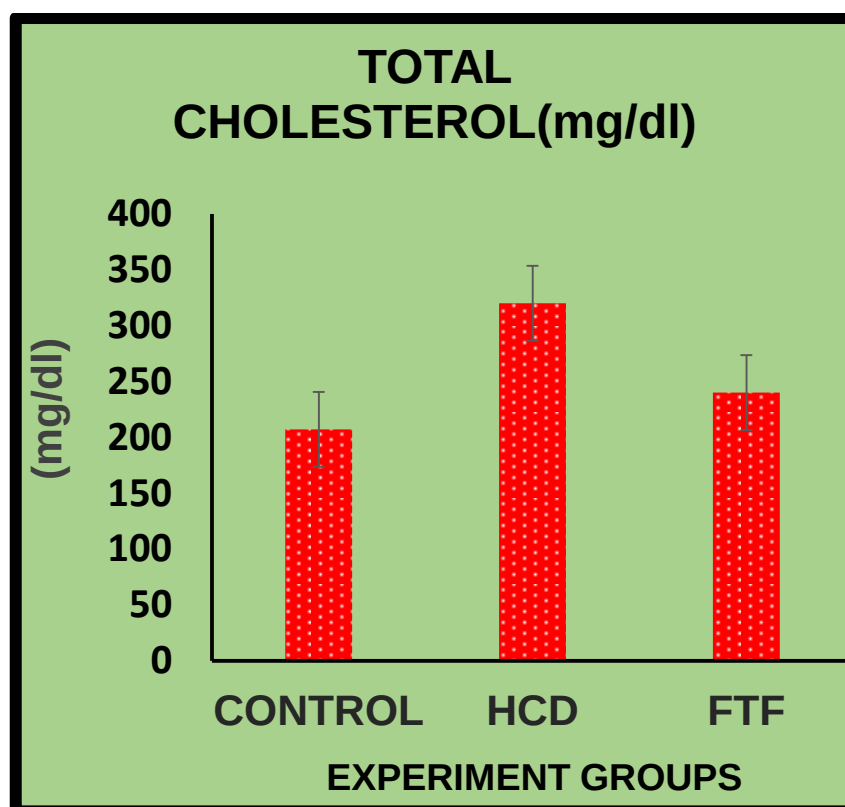
5.2.1 CHANGES IN TOTAL CHOLESTEROL LEVEL BETWEEN CONTROL, HCD AND FENUGREEK TREATED ANIMALS

The Total cholesterol level in adult zebrafish has been recorded between the control, High Cholesterol diet, and Fenugreek treated animals.

TABLE – 2 Changes in total cholesterol level between control, HCD and FTF.

CONTROL	HCD	FTF
207 mg/dl	320 mg/dl	240 mg/dl

The total cholesterol level has been increased in High Cholesterol diet zebrafish, and decreased in fenugreek treated fish. **GRAPH – 2** Changes in total cholesterol level between control, HCD and FTF.



5.2.2 CHANGES IN TRIGLYCERIDES LEVEL BETWEEN CONTROL, HCD AND FENUGREEK TREATED ANIMALS

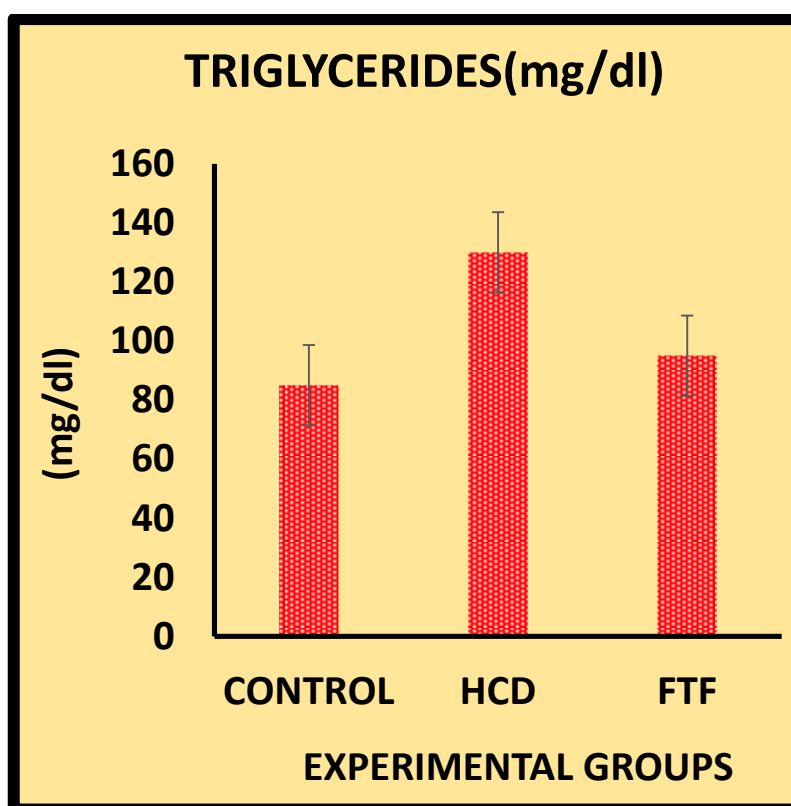
The Triglycerides level in adult zebrafish has been recorded between the control, High Cholesterol diet, and Fenugreek treated animals.

TABLE – 3 Changes in Triglycerides between control, HCD and FTF.

CONTROL	HCD	FTF
85 mg/dl	130 mg/dl	95 mg/dl

The triglycerides level has been increased in High Cholesterol diet zebrafish, and decreased in fenugreek treated fish.

GRAPH – 3 Changes in Triglycerides between control, HCD and FTF.



5.2.3 CHANGES LOW DENSITY LIPOPROTEIN(LDL) LEVEL BETWEEN CONTROL, HCD AND FENUGREEK TREATED ANIMALS

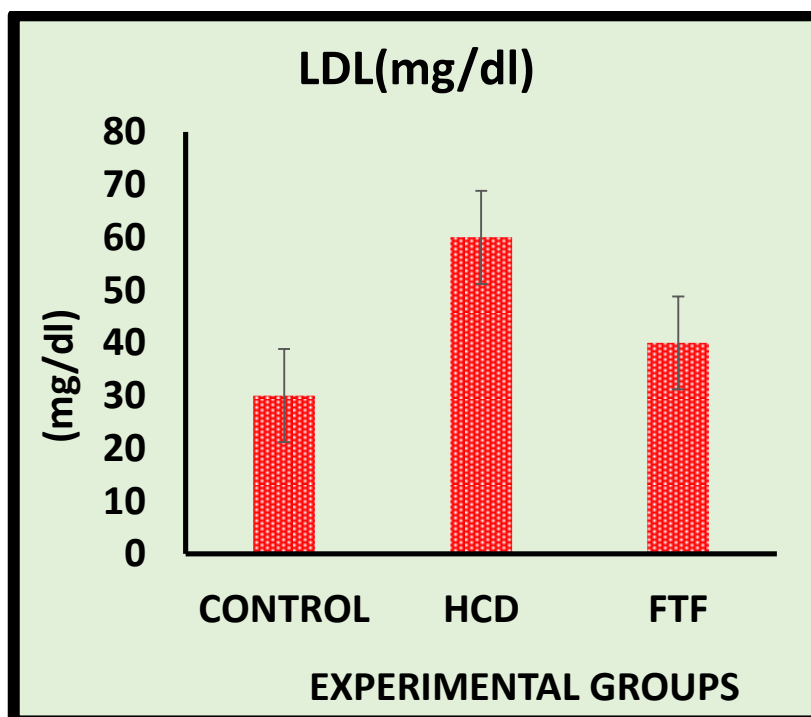
The Low Density Lipoprotein (LDL) level in adult zebrafish has been recorded between the control, High Cholesterol diet, and Fenugreek treated animals.

TABLE – 4 Changes in LDL level between control, HCD and FTF.

CONTROL	HCD	FTF
30 mg/dl	60 mg/dl	45 mg/dl

The Low Density Lipoprotein (LDL level has been increased in High Cholesterol diet zebrafish, and decreased in fenugreek treated fish.

GRAPH – 4 Changes in LDL level between control, HCD and FTF.



5.2.4 CHANGES HIGH DENSITY LIPOPROTEIN(HDL) LEVEL BETWEEN CONTROL, HCD AND FENUGREEK TREATED ANIMALS

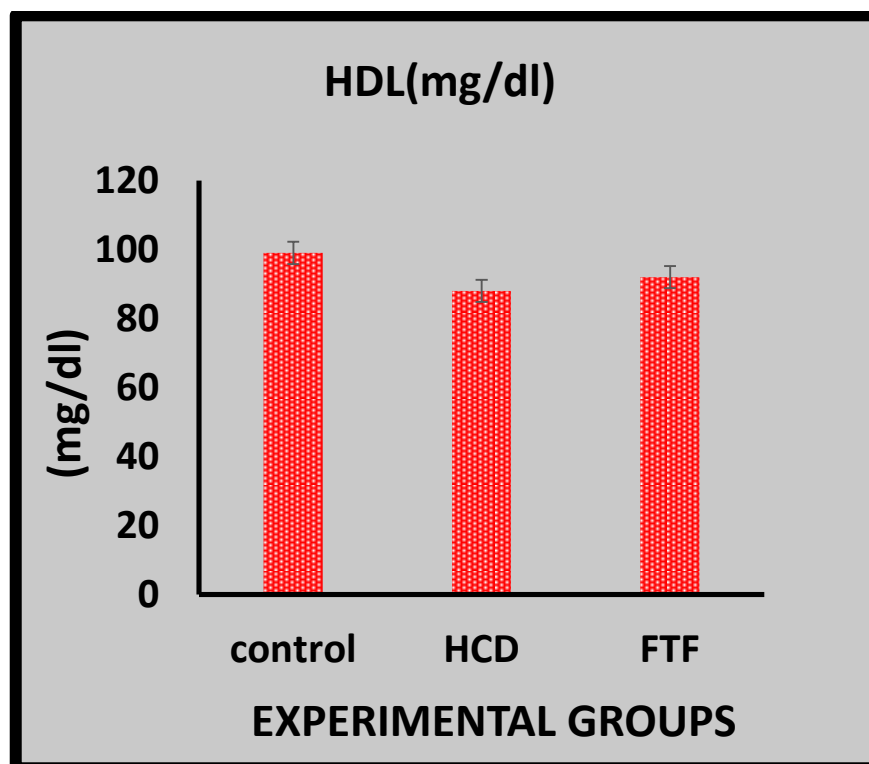
The High Density Lipoprotein (HDL) level in adult zebrafish has been recorded between the control, High Cholesterol diet, and Fenugreek treated animals.

TABLE – 5 Changes in HDL level between control, HCD and FTF.

CONTROL	HCD	FTF
99 mg/dl	88 mg/dl	92 mg/dl

The High Density Lipoprotein (LDL) level has been decreased in High Cholesterol diet zebrafish, and increased in fenugreek treated fish.

GRAPH– 5 Changes in HDL level between control, HCD and FTF.



5.3 BIOCHEMICAL ASSAY

5.3.1 CHANGES IN LIPID PEROXIDATION LEVEL BETWEEN CONTROL, HCD AND FENUGREEK TREATED ANIMALS

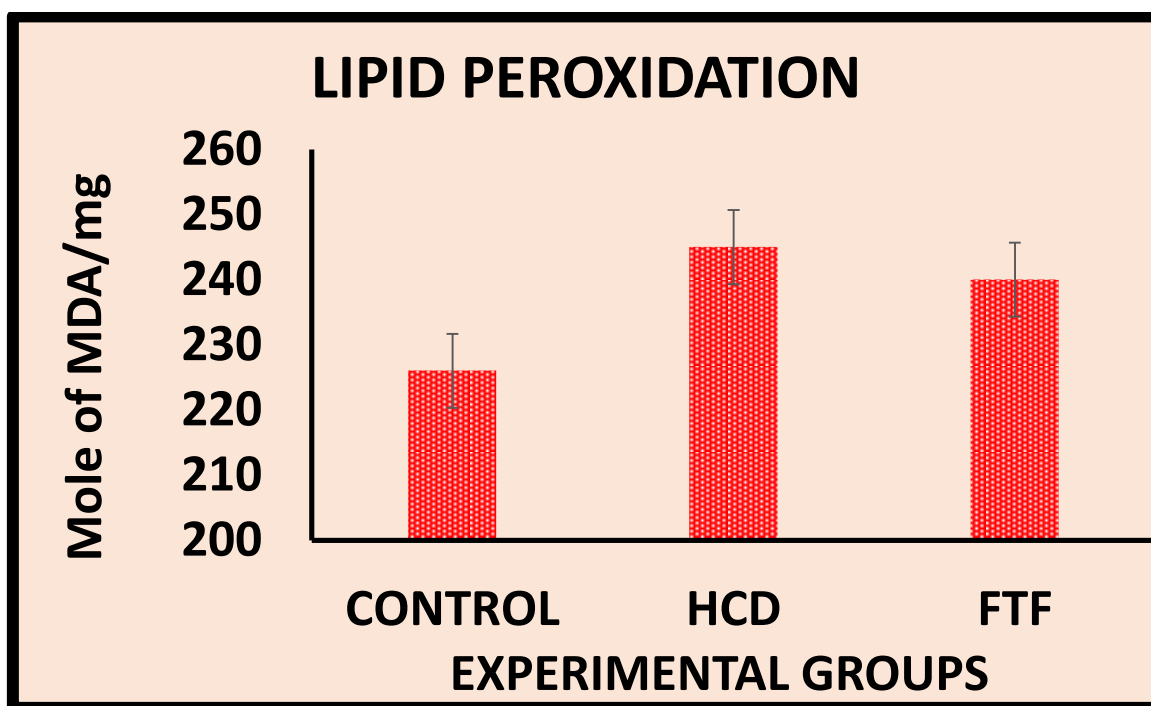
The Lipid peroxidation level in adult zebrafish has been recorded between the control, High Cholesterol diet, and Fenugreek treated animals.

TABLE – 6 Changes in LPO between control, HCD and FTF.

CONTROL	HCD	FTF
226 (mole of MDA / mg)	245 (mole of MDA / mg)	240 (mole of MDA / mg)

The Lipid peroxidation level has been increased in High Cholesterol diet zebrafish, and decreased in fenugreek treated fish.

GRAPH – 6 Changes in LPO between control, HCD and FTF.

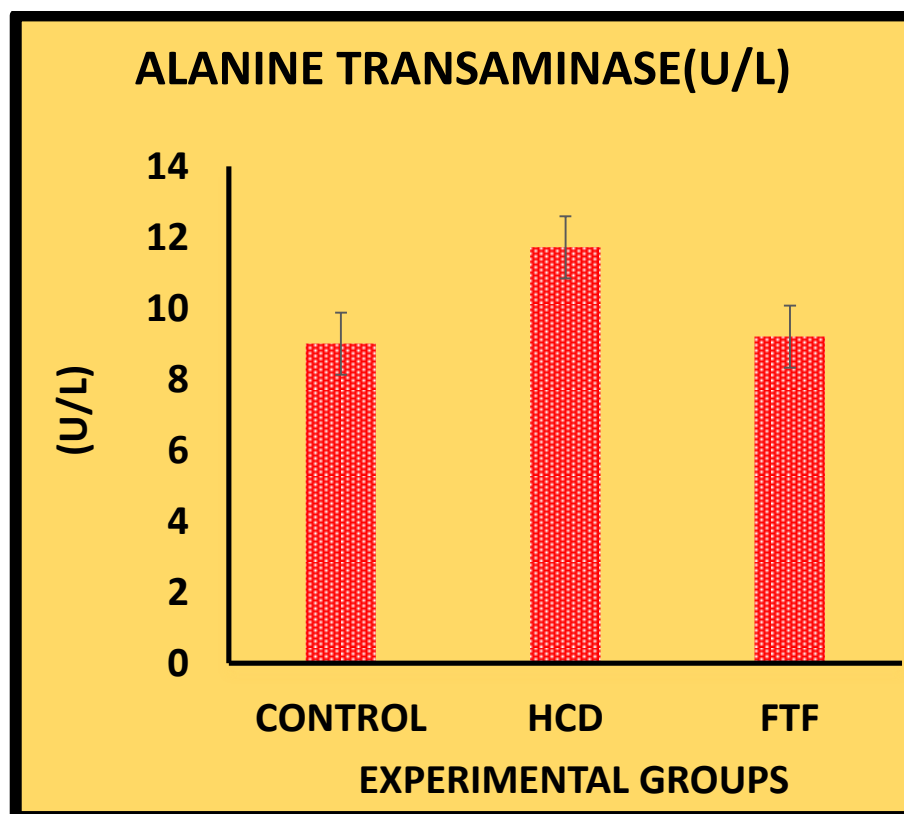


5.3.2 CHANGES IN ALANINE TRANSAMINASE LEVEL BETWEEN CONTROL, HCD AND FENUGREEK TREATED ANIMALS

The Alanine Transaminase (Serum Glutamic Pyruvic Transaminase)level in adult zebrafish has been recorded between the control,High Cholesterol diet, and Fenugreek treated animals.**TABLE-7** Changes in Alanine Transaminase between control,HCD,and FTF.

CONTROL	HCD	FTF
9.0 (U/L)	11.72 (U/L)	9.2 (U/L)

The Alanine Transaminase (Serum Glutamic Pyruvic Transaminase) level has been increased in High Cholesterol diet zebrafish,and decreased in fenugreek treated fish.**GRAPH-7** Changes in Alanine Transaminase between control,HCD and FTF.

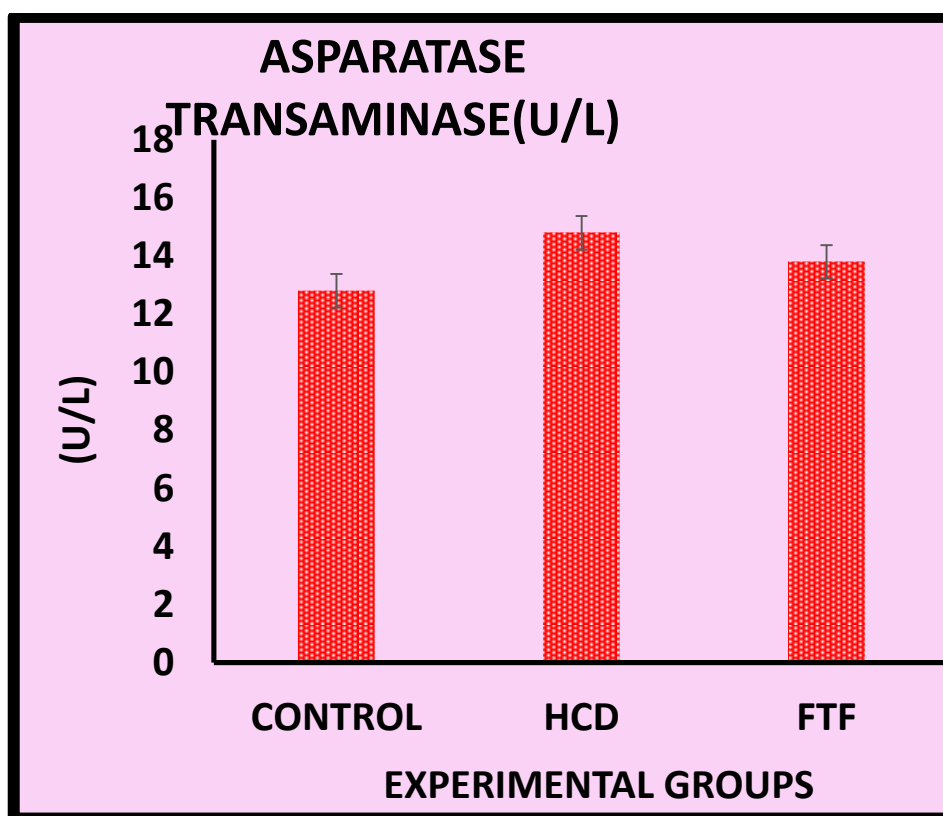


5.3.3 CHANGES IN ASPARATATE TRANSAMINASE LEVEL BETWEEN CONTROL, HCD AND FENUGREEK TREATED ANIMALS

The Asparatate Transaminase (Serum Glutamic Oxaloacetic Transaminase) level in adult zebrafish has been recorded between the control, High Cholesterol diet, and Fenugreek treated animals. **TABLE – 8** Changes in Asparatate Transaminase level between control, HCD and FTF.

CONTROL	HCD	FTF
12.8 (U/L)	14.8 (U/L)	13.8 (U/L)

The Asparatate Transaminase (Serum Glutamic Oxaloacetic Transaminase) level has been increased in High Cholesterol diet zebrafish, and decreased in fenugreek treated fish. **GRAPH– 8** Changes in Asparatate Transaminase level between control, HCD and FTF



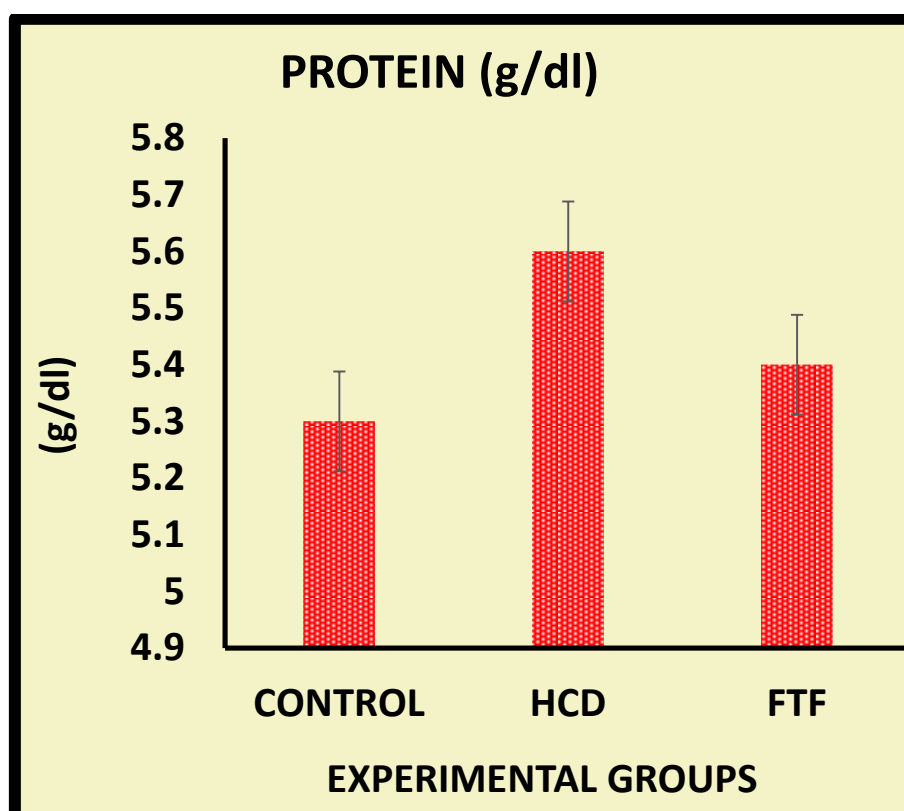
5.4 BIOCHEMICAL PARAMETERS

5.4.1 CHANGES IN PROTEIN LEVEL BETWEEN CONTROL, HCD AND FENUGREEK TREATED ANIMALS

The Protein level in adult zebrafish has been recorded between the control, High Cholesterol diet, and Fenugreek treated animals. **TABLE – 9** Changes in Protein level between control, HCD and FTF.

CONTROL	HCD	FTF
5.3 (g/dl)	5.6 (g/dl)	5.4 (g/dl)

The Protein level has been increased in High Cholesterol diet zebrafish, and decreased in fenugreek treated fish. **GRAPH-9** Changes in Protein level between control, HCD and FTF.



5.4.2 CHANGES IN GLUCOSE LEVEL BETWEEN CONTROL, HCD AND FENUGREEK TREATED ANIMALS

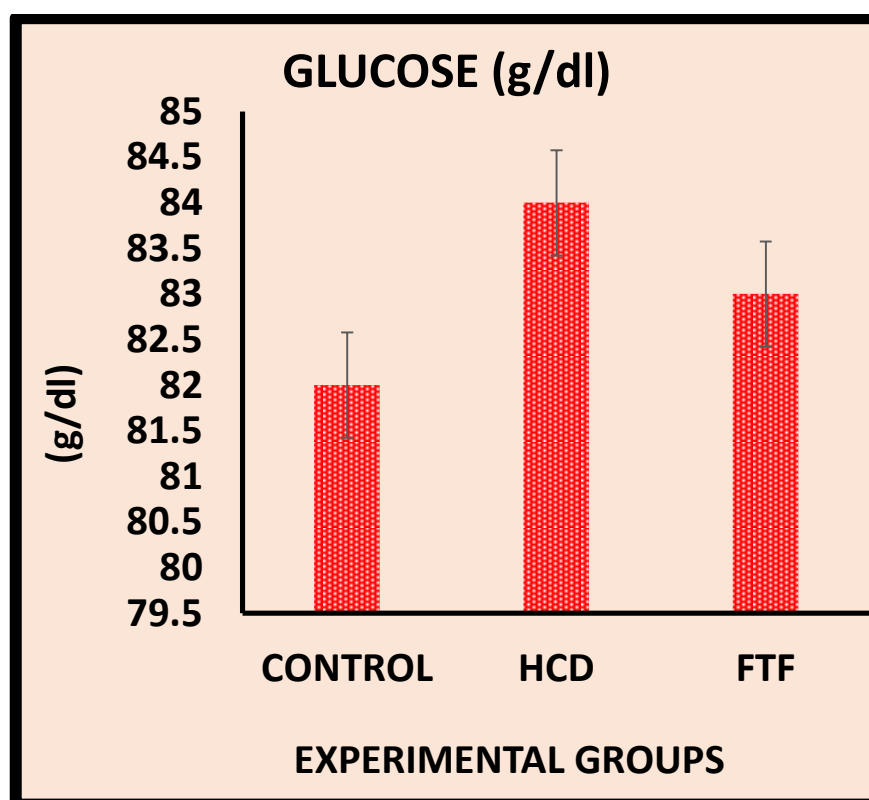
The Glucose level in adult zebrafish has been recorded between the control, High Cholesterol diet, and Fenugreek treated animals.

TABLE – 10 Changes in Glucose level between control, HCD and FTF.

CONTROL	HCD	FTF
82 (g/dl)	84 (g/dl)	83 (g/dl)

The Glucose level has been increased in High Cholesterol diet zebrafish, and decreased in fenugreek treated fish.

GRAPH – 10 Changes in glucose level weight between control, HCD and FTF.



CHAPTER – 6

CONCLUSION

From the above study the hypelipdemia condition is major risk factor for cardiac disease. hypelipidemia can be treated by recent drugs and diet food chart, home remedies, regular physical exercise. If attentive care is given to diet maintenance and fitness may reduce the risk of hyperlipidemic, CVD and many other disease. The zebrafish (*Danio rerio*) is a new research model for determining the Hyperlipidemia of high cholesterol diet and fenugreek treated diet. The use of zebrafish as a study model (lipid profiles, biochemical assay and biochemical parameters) for this experimental research provides a significant advantage in determining the lipid profiles, biochemical assay and biochemical parameters level of hyperlipidemia-causing substances that are widely present in the environment and have an impact on human health. Zebrafish adult were also allocated to three diet groups such normal diet, high cholesterol diet, fenugreek treated diet and over 4 weeks were fed a high-cholesterol diet. Another group of high cholesterol diet adult zebrafish was fed with 4% fenugreek powder supplementation for 2 weeks. Zebrafish fed the high-fat diets had increased the body fat, lipid profiles, biochemical assay and biochemical parameters, and the fenugreek treated diet adult zebrafish had decreased the body fat, lipid profiles, biochemical assay and biochemical parameters. All of these results are similar to those found in mammals, suggesting that zebrafish can serve as a suitable animal model in research into obesity induced by a diet rich in fat.

CHAPTER – 7

REFERENCE

1. Faxon DP, Fuster V, Libby P, Beckman JA, Hiatt WR, Thompson RW, Topper JN, Annex BH, Rundback JH, Fabunmi RP, Robertson RM, Loscalzo J, American Heart Association Atherosclerotic Vascular Disease Conference: Writing Group III: pathophysiology. *Circulation*. 2004.
2. Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *JAMA* 1986.
3. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, Hoes AW, Jennings CS, Landmesser U, Pedersen TR, Reiner Ž, Riccardi G, Taskinen MR, Tokgozoglul L, Verschuren WMM, Vlachopoulos C, Wood DA, Zamorano JL, Cooney MT., ESC Scientific Document Group. 2016.
4. Nissen SE, Stroes E, Dent-Acosta RE, Rosenson RS, Lehman SJ, Sattar N, Preiss D, Bruckert E, Ceška R, Lepor N, Ballantyne CM, Gouni-Berthold I, Elliott M, Brennan DM, Wasserman SM, Somaratne R, Scott R, Stein EA., GAUSS-3 Investigators. Efficacy and Tolerability of Evolocumab vs Ezetimibe in Patients With Muscle-Related Statin Intolerance: The GAUSS-3 Randomized Clinical Trial. *JAMA*. 2016.
5. Ford I, Murray H, McCowan C, Packard CJ. Long-Term Safety and Efficacy of Lowering Low-Density Lipoprotein Cholesterol With Statin Therapy: 20-Year Follow-Up of West of Scotland Coronary Prevention Study. *Circulation*. 2016.
6. Franklin BA, Brubaker P, Harber MP, Lavie CJ, Myers J, Kaminsky LA. The Journal of Cardiopulmonary Rehabilitation and Prevention at 40 yr and Its Role in Promoting Preventive Cardiology: Part 2. *J Cardiopulm Rehabil Prev*. 2020.
7. Álvarez C, Ramírez-Campillo R, Lucia A, Ramírez-Vélez R, Izquierdo M. Concurrent exercise training on hyperglycemia and comorbidities associated: Non-responders using clinical cutoff points. *Scand J Med Sci Sports*. 2019.

8. Bakker EA, Lee DC, Sui X, Eijssvogels TMH, Ortega FB, Lee IM, Lavie CJ, Blair SN. Association of Resistance Exercise With the Incidence of Hypercholesterolemia in Men. *Mayo Clin Proc.* 2018.
9. Bakker EA, Lee DC, Sui X, Eijssvogels TMH, Ortega FB, Lee IM, Lavie CJ, Blair SN. Association of Resistance Exercise With the Incidence of Hypercholesterolemia in Men. *Mayo Clin Proc.* 2018.
10. Abdel-Barry, J. A., Abdel-Hassan, I. A., Jawad, A. M., & Al-Hakiem, M. H. (2000). Hypoglycaemic effect of aqueous extract of the leaves of *Trigonella foenum-graecum* in healthy volunteers. *Eastern Mediterranean Health Journal.*
11. Annida, B., & Stanely Mainzen Prince, P. (2004). Supplementation of fenugreek leaves lower lipid profile in streptozotocin-induced diabetic rats. *Journal of Medicinal Food.*
12. Begg, C. B., & Mazumdar, M. (1994). Operating characteristics of a rank correlation test for publication bias. *Biometrics.*
13. Benjamin, E. J., Muntner, P., Alonso, A., Bittencourt, M. S., Callaway, C. W., Carson, A. P., et al. (2019). Heart disease and stroke statistics. Update: A Report.
14. Boca, C., Gross, R., Petit, P., Sauvaire, Y., Manteghetti, M., Tournier, M., et al. (1999) 4-Hydroxyisoleucine: experimental evidence of its insulinotropic and antidiabetic properties. *American Journal of Physiology.*
15. Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *JAMA* 1986.
16. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, Hoes AW, Jennings CS, Landmesser U, Pedersen TR, Reiner Ž, Riccardi G, Taskinen MR, Tokgozoglu L, Verschuren WMM, Vlachopoulos C, Wood DA, Zamorano JL, Cooney MT., ESC Scientific Document Group. 2016.
17. Nissen SE, Stroes E, Dent-Acosta RE, Rosenson RS, Lehman SJ, Sattar N, Preiss D, Bruckert E, Ceška R, Lepor N, Ballantyne CM, Gouni-Berthold I, Elliott M, Brennan DM, Wasserman SM, Somaratne R, Scott R, Stein EA., GAUSS-3 Investigators. Efficacy and Tolerability of Evolocumab vs Ezetimibe in Patients With Muscle-Related Statin Intolerance: The GAUSS-3 Randomized Clinical Trial. *JAMA.* 2016.
18. Ford I, Murray H, McCowan C, Packard CJ. Long-Term Safety and Efficacy of Lowering Low-Density Lipoprotein Cholesterol With Statin Therapy: 20-Year Follow-Up of West of Scotland Coronary Prevention Study. *Circulation.* 2016.

19. Franklin BA, Brubaker P, Harber MP, Lavie CJ, Myers J, Kaminsky LA. The Journal of Cardiopulmonary Rehabilitation and Prevention at 40 yr and Its Role in Promoting Preventive Cardiology: Part 2. J Cardiopulm Rehabil Prev. 2020.
20. Álvarez C, Ramírez-Campillo R, Lucia A, Ramírez-Vélez R, Izquierdo M. Concurrent exercise training on hyperglycemia and comorbidities associated: Non-responders using clinical cutoff points. Scand J Med Sci Sports. 2019.
21. Bakker EA, Lee DC, Sui X, Eijssvogels TMH, Ortega FB, Lee IM, Lavie CJ, Blair SN. Association of Resistance Exercise With the Incidence of Hypercholesterolemia in Men. Mayo Clin Proc. 2018.
22. Bakker EA, Lee DC, Sui X, Eijssvogels TMH, Ortega FB, Lee IM, Lavie CJ, Blair SN. Association of Resistance Exercise With the Incidence of Hypercholesterolemia in Men. Mayo Clin Proc. 2018.
23. Abdel-Barry, J. A., Abdel-Hassan, I. A., Jawad, A. M., & Al-Hakim, M. H. (2000). Hypoglycaemic effect of aqueous extract of the leaves of *Trigonella foenum-graecum* in healthy volunteers. Eastern Mediterranean Health Journal.
24. Annida, B., & Stanely Mainzen Prince, P. (2004). Supplementation of fenugreek leaves lower lipid profile in streptozotocin-induced diabetic rats. Journal of Medicinal Food.
25. Begg, C. B., & Mazumdar, M. (1994). Operating characteristics of a rank correlation test for publication bias. Biometrics.
26. Benjamin, E. J., Muntner, P., Alonso, A., Bittencourt, M. S., Callaway, C. W., Carson, A. P., et al. (2019). Heart disease and stroke statistics. Update: A Report.
27. Boca, C., Gross, R., Petit, P., Sauvaire, Y., Manteghetti, M., Tournier, M., et al. (1999) 4-Hydroxyisoleucine: experimental evidence of its insulinotropic and antidiabetic properties. American Journal of Physiology.
28. Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA 1986.
29. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, Hoes AW, Jennings CS, Landmesser U, Pedersen TR, Reiner Ž, Riccardi G, Taskinen MR, Tokgozoglu L, Verschuren WMM, Vlachopoulos C, Wood DA, Zamorano JL, Cooney MT., ESC Scientific Document Group. 2016.

- 30.** Nissen SE, Stroes E, Dent-Acosta RE, Rosenson RS, Lehman SJ, Sattar N, Preiss D, Bruckert E, Ceška R, Lepor N, Ballantyne CM, Gouni-Berthold I, Elliott M, Brennan DM, Wasserman SM, Somaratne R, Scott R, Stein EA., GAUSS-3 Investigators. Efficacy and Tolerability of Evolocumab vs Ezetimibe in Patients With Muscle-Related Statin Intolerance: The GAUSS-3 Randomized Clinical Trial. JAMA. 2016.