Introduction
All forms of life must assimilate from their environment a continuous supply of nutrients and energy to sustain life and to maintain health. Among higher life forms like animals and humans, nutrients and energy are heterogeneously supplied from diets containing a great diversity of biomolecules such as carbohydrates, proteins, lipids and other micronutrients like vitamin and mineral supplements. Fat is an important dietary component affecting health and growth. Inter relationship between dietary fatty acids and membrane fluidity, absorption of fat soluble vitamins, cell signaling and gene transcription functions elucidate their significance as a dietary component. Among the dietary lipids, polyunsaturated fatty acids (PUFA) are shown to be indispensable for maintaining health. They render many biological effects, which are beneficial for maintaining health.

Since the present study aims at investigating the role of dietary lipids in D-Galactosamine (D-GaIN) induced hepatitis, we take a look into the nature of lipids, their classification and importance.

1.1 Dietary Lipids

Lipids are an extremely diverse group of organic compounds that are insoluble in water but are generally soluble in one or a mixture of several organic solvents. In general, lipids are the esters of fatty acids with glycerol or other alcohol. The function of lipid is to maintain structural integrity of membrane, supply energy for physiological activities, transport of various metabolites in and out of the cells and regulation of physiological functions.
through the production of secondary metabolites (Lehninger, 1984). Dietary lipids of significance include the mono-, di- and tri-glycerides, cholesterol, cholesterol esters, glycolipids, phospholipids and fatty acids.

1.1.1 Mono-, Di- and Triglycerides

![Figure a: Structure of glycerol and triglycerides](image)

The simplest form of lipid is an ester of fatty acid with glycerol. Esterification of a single fatty acid to the glycerol moiety is the monoglycerides. Esterification of additional fatty acids to the monoglyceride, results in the formation of diglycerides and triglycerides respectively (Figure a). Triglycerides are also named as fats or neutral lipids. They are nonpolar, hydrophobic molecules essentially insoluble in water. Those containing the same kind of fatty acid in all the three positions are called simple triglycerides and are named after the fatty acid they contain for e.g. tristearin, tripalmitin and triolein. On the other hand, most naturally occurring triglycerides are mixed as they contain two or three different fatty acids which are esterified at different hydroxyl groups of glycerol moiety. To name such mixed triacylglycerols, name and position of each fatty acid are specified.

Triglycerides, which are also known as triacylglycerols, mainly serve as the stored fuels. Fatty acids for further use are stored in the form of triglycerides.
In vertebrates, specialized cells - adipocytes or fat cells store large amounts of triglycerides as fat droplets. They are also stored as oils in the seeds of many plants providing energy and biosynthetic precursors during seed germination. In some animals, triglycerides stored under the skin not only serve as energy store but also provide insulation against low temperatures e.g. polar animals. In hibernating animals, triacylglycerides serve dual purpose of insulation and energy storage. The low density of triacylglycerides helps some aquatic animals to match their buoyancy with the surroundings.

1.1.2 Glycolipids

Glycolipids are the conjugates of mono, di or oligosaccharide moiety with lipid. They are classified into galactolipids and sphingolipids. In galactolipids, one or two galactose residues are connected by a glycosidic linkage to a diglyceride. Sphingolipids unlike galactolipids contain no glycerol backbone. They are composed of one molecule of amino alcohol (sphingosine) one molecule of long chain fatty acid and a polar head group i.e. sugar moiety joined by a glycosidic linkage (Figure b). When a fatty acid is attached in amide linkage to the –NH₂ on C-2 the resulting compound is a ceramide, which is structurally similar to a diglyceride.

$$\text{CH}_3-(\text{CH}_2)_{12}-\text{C}=\text{C}-\text{CH}-\text{CH}_2\text{OH}$$

$$\begin{align*}
\text{H} & \\
\text{H} & \\
\text{H} & \\
\text{OH} & \\
\text{NH}_2 & \\
\end{align*}$$

*Figure b: Structure of sphingosine*
There are three subclasses of sphingolipids; all are derivatives of ceramide but differing in their head groups: sphingomyelins, glycosphingolipids and gangliosides.

*Sphingomyelins-* contain phosphocholine or phosphoethanolamine as their polar head group. Sphingomyelins are present in the plasma membranes of animal cells and are especially prominent in myelin sheath of neurons. In sphingomyelin there is no sugar moiety and therefore it is also treated as a phospholipid.

*Glycosphingolipids-* contain head groups with one or more sugars. They are further classified into cerebrosides and globosides.

*Gangliosides-* contain oligosaccharide as their polar head groups with one or more residues of N-acetylneuraminic acid at the terminal end.

In general, sphingolipids are present at cell surfaces and act as sites of biological recognition.

1.1.3 Phospholipids

Phospholipids are the essential components of all biological membranes. They constitute about 65-85% of the total membrane lipids. A polar head group is joined to the hydrophobic moiety of the diglyceride, by phosphodiester

![Figure c: Structure of phospholipids](image-url)
linkage (Figure c). Addition of phosphoric acid as a polar head group to diglyceride provides the principal phospholipid nucleus i.e. phosphatidic acid. Substitution of ‘X’ as shown in the Figure c with different alcohol group gives rise to different phospholipids. Substitutions include ethanolamine (phosphatidyl ethanolamine), choline (phosphatidyl choline also called lecithins), serine (phosphatidyl serine), glycerol (phosphatidyl glycerol), myo-inositol (phosphatidyl inositol).

Sometimes in phospholipids, out of two acyl chains, one is either alkyl ether or alkenyl ether and linked to the glycerol molecule through ether linkage. These types of phospholipids are referred to as plasmalogens. They act as potent molecular signals which stimulate platelet aggregation and other physiological functions.

1.1.4 Cholesterol

These are structural lipids present in the cell membranes. In addition to their roles as membrane constituents, the sterols serve as precursors for a variety of products with specific biological activities. The nucleus of the cholesterol is made up of four fused isoprene rings (Figure d).

![Structure of cholesterol](image)
It is amphipathic in nature with a polar head group (hydroxyl group at C-3 position) and nonpolar hydrocarbon body (steroid nucleus and the hydrocarbon side chain at C-17). It can be obtained from the ingestion of food or can be actively synthesized from acetyl Co A and isoprene ring in liver, intestinal epithelium, adrenal glands and skin. Both dietary cholesterol and that synthesized de novo are transported through the circulation in the form of lipoprotein particles.

1.1.5 Fatty acids

Fatty acids are the building block components of most lipids. They are long chain organic aliphatic acids having 14-24 carbon atoms. They have a single carboxyl group and a long, non polar hydrocarbon tail. The fatty acids differ from each other in their chain length and presence (number and position) of double bonds. Fatty acids that contain no C=C are termed as saturated fatty acids; those that contain one or more C=C are unsaturated fatty acids. Unsaturated fatty acids are further classified as monounsaturated fatty acids (monoenoic) and polyunsaturated fatty acids (dienoic, trienoic, tetraenoic etc.). Polyunsaturated fatty acids (PUFA) are classified into two major groups viz. n-3 and n-6 depending upon the position of first double bond from methyl end. The fatty acid biosynthesis occurs in the cytoplasm through a multi step reaction catalyzed by a set of enzymes commonly termed as fatty acid synthase system. The fatty acids produced by fatty acid synthase system do not contain any double bond between carbon carbon atoms. The desaturation of the fatty acid occurs in the endoplasmic reticulum and catalyzed by a group of enzymes known as desaturase system. In any fatty acid chain, the first double bond is always inserted between Δ9 and Δ10 carbon atoms. Further desaturation of oleic (18:1)
Acetyl CoA $\rightarrow$ 14:0 $\rightarrow$ 16:0 $\rightarrow$ 18:0

$\Delta 9$-desaturase

18:1 n-9

$\Delta 12$-desaturase

Plants & microbes only

18:2n-6 & 18:3n-6 through diet

in animals

$\Delta 15$-desaturase

Plants & microbes only

$\Delta 6$-desaturase

18:3n-6

18:4n-3

$\Delta 5$-desaturase

COX / LOX

20:3n-6

20:4n-3

3-Series PG

20:4n-6

Eicosapentaenoic Acid (EPA)

20:5n-3

5-Series LT

COX / LOX

22:6n-3

Docosahexaenoic Acid (DHA)

Inflammation and immunity

Figure e: Outline of the pathway of biosynthesis and metabolism of PUFA in animal beings
Introduction

Acid is not possible in animal cells due to the lack of Δ-12 and Δ-15 desaturase enzymes. However, linoleic acid (18:2, n6) and alpha linolenic acid (18:3, n3) are the precursor molecules of long chain polyunsaturated fatty acids (Hander and Tocher, 1987). Long chain PUFA is the characteristic lipid from animal sources. Therefore, these two fatty acids are known as essential fatty acids.

Once the parent essential fatty acids are provided through the diet, they are further desaturated and elongated in animal cells to produce long chain PUFA of respective series. Linoleic acid (18:2, Δ^9,12) undergo oxidation and add third double bond through Δ6 desaturase system and becomes γ-linolenic (18:3n-6). This fatty acid is further elongated by 2 carbon unit and becomes dihomo-γ-linolenic acid (20:3n-6) which again undergoes oxidation reaction and add fourth double bond to it by Δ5 desaturase system to produce arachidonic acid (20:4n-6). Similarly dietary linolenic acid (18:3, Δ^9,12,15) is also oxidized via Δ6 desaturase system to produce 18:4, n3 fatty acid and is elongated to 20:4, n-3. This fatty acid is further oxidized by Δ-5 desaturase system to produce eicoapentaenoic acid (20:5, n-3). Eicosapentaenoic acid through chain elongation as well as oxidation by Δ4 desaturase system adds another double bond to produce docosahexaenoic acid (22:6, n-3). Dihomo-γ-linolenic acid, arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid undergo further metabolism via cyclooxygenase (COX) and lipoxygenase (LOX) pathways to produce various eicosanoids/ docosanoids (Figure e). They are the signaling molecules made by oxidation of C-20 / C-22 fatty acids. Prostaglandins (PG) and leukotrienes (LT) are the various eicosanoids produced from long chain PUFA. Eicosanoids produced from omega-6 PUFA are pro-inflammatory, pro-aggregating, vasoconstricting and immuno-suppressing while those of omega-3 PUFA are...
anti-inflammatory, anti-aggregating, vaso-dilating, anti-arrhythmic and immuno-modulatory (Lands, 2000). Hence, a precise balance between these two series of PUFA is important in maintaining health. PUFA are very essential for growth and development and also for the regulation of the cellular functions in animals (Simopoulus, 1999; Zamaria, 2004). They are shown to be the membrane components and precursors of signaling molecules (Watts and Browse, 2002).

1.1 **Hepatitis- inflammation of the liver**

Hepatitis refers to any structural and functional distortion of liver causing its inflammation. It implies injury to the liver characterized by the presence of inflammatory cells in the tissue of the organ. The important symptoms of acute hepatitis may include fatigue, nausea, loss of appetite, fever, abdominal pain, jaundice, itching, dark colored urine and light colored stools. Chronic hepatitis usually causes no symptoms or may be noticeable as only a loss of energy and tiredness. There are several types of hepatitis as follows:

1.2.1 **Viral Hepatitis**

It may be caused by hepatitis virus A to E. Acute viral infections rapidly trigger the non-specific immune response, principally involving type I interferon secretion and natural killer cell activation (Thimme *et al.*, 2001). Hepatitis C virus alters the host defense and innate immunity early during infection through a variety of complementary mechanisms, thereby facilitating chronic infection (Pawlotsky, 2004). Hepatitis C virus core protein can regulate lipid accumulation by cells *in vitro* (Barba *et al.*, 1997).
1.2.2 Chemically Induced Hepatitis

It is due to alcohol consumption, drugs, and environmental toxins. Metabolism of hepatotoxic agents like alcohol, carbon tetrachloride, acetaminophen etc. can generate excessive reactive oxygen species thereby damaging the membrane structure and function activating the host defense mechanisms and leakage of some enzymes like aminotransferases (Kono et al., 2000; Chundong et al., 2002).

1.2.3 Inherited Forms of Hepatitis

Crohn’s disease or hemochromatosis is caused due to excess accumulation of iron. Usually occurs with the family history of cirrhosis, skin hyperpigmentation, diabetes mellitus, cardiomyopathy etc. due to signs of iron overload. Wilson’s disease is associated with an accumulation of excess copper in liver, brain, and other tissues. It is an autosomal recessive disorder.

1.2.4 Non-Alcoholic Fatty Liver Disease (NAFLD)

One of the most common causes of chronic hepatitis is accumulation of excess fat in the liver. It is the occurrence of fatty liver in people who have no history of alcohol use. It may be associated with diabetes and obesity etc.

1.2.5 Autoimmune Hepatitis

Reasons are not fully understood for autoimmune hepatitis but here the body’s immune system targets and attacks the liver itself.

1.2.6 Cirrhosis

It is a condition in which the liver slowly deteriorates and malfunctions due to chronic injuries that are characterized by replacement of liver tissue by fibrous scar tissue as well as regenerative nodules. Cirrhosis is most commonly
caused by alcoholism, hepatitis B and C and fatty liver diseases. Usually years of chronic injury are required to cause cirrhosis.

1.3 D-Galactosamine induced hepatotoxicity

![Structure of D-Galactosamine](image)

**Figure f: Structure of D-Galactosamine**

D-Galactosamine is a hexosamine derived from galactose with the molecular formula C₆H₁₃NO₅ with a molar mass of 179.171 KD. Its IUPAC name is 2-Amino-2-deoxy-D-galactose (Figure f). This amino sugar is a constituent of some complex polysaccharides and also of some hormones such as follicle stimulating hormone and leuteinizing hormone. It is a known hepatotoxic agent and thus used to create liver damage model in animals.

Viral hepatitis in mice (Margolis *et al.*, 1968) is different from human viral hepatitis in its histological appearance. Liver damage caused by toxic chemicals (Kroner and Staib, 1967) does not resemble viral hepatitis either. D-Galactosamine administration caused morphologic and functional features similar to those found in acute human viral hepatitis (Lim *et al.*, 2000).

Among the early biochemical events which occur following the injection of Galactosamine are an accumulation of galactosamine1-phosphate, UDP-
hexosamines, and UDP-N acetylhexosamines, a marked decrease in the concentrations of UTP+UDP, UDP-glucose and UDP galactose (Keppler et al., 1970). Hepatic UTP deficiency has been suggested as the cause for an inhibition of RNA and Protein synthesis induced by galactosamine (Decker et al., 1973). D-GaIN is known as a specific hepatotoxic transcriptional inhibitor that leads to an acute cytokine dependent liver inflammation (Sass, 2002). A high dose of D-GaIN causes necrosis of the liver by UTP depletion and inhibition of protein synthesis, although D-GaIN is often used in combination with lipopolysaccharide or tumor necrosis factor (Sun et al., 2001). Accumulation of UDP-sugar nucleotides (Endo et al., 1992; Manabe et al., 1996) may contribute to the changes in the rough endoplasmic reticulum and to the disturbance in the protein metabolism. Further, intense galactosamination of membrane structure is thought to be responsible for loss in the activity of ionic pumps. The impairment in the calcium pump, with consequent increase in the intracellular calcium is considered to be responsible for cell death (Tsai et al., 1997).

In recent years, apart from the well documented inhibition of protein synthesis, it has been suggested that reactive oxygen species produced by activated macrophages might be the primary cause in D-GaIN induced liver damage (Hu and Chen 1992).

1.4 Role of PUFA in health and diseases

PUFA have important effects on the structure and physical properties of localized membrane domains. They modulate enzyme activities, act as carriers
and help in membrane receptors production, signal transduction and the activation of nuclear transcription factors (Spector, 1999).

PUFA help in regulating the proper functioning and development of various tissues like retina and brain etc by controlling their membrane fluidity (Simopoulos, 1991). PUFA are also related to the neonatal growth and development (Patrix and Gerard, 2000). The n-3 and n-6 series of fatty acids play a significant role to maintain physiological homeostasis in animals. PUFA are shown to be essential for the intellectual growth of the brain (Broadhurst et al., 2002). PUFA play an important role in the brain and vascular development and in the normal course of pregnancy, as well as in a number of conditions as fetal growth retardation (Matorras et al., 1999).

Animal studies show that increasing the availability of n-3 polyunsaturated fatty acids in the diet results in a decreased proportion of arachidonic acid and an increased proportion of n-3 fatty acids in immune cell phospholipids (Calder, 1998). When fish oil is provided in human diet the proportion of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in immune cells are significantly elevated, probably in a dose dependent manner (Calder, 2001). The incorporation of the long chain n-3 fatty acids is largely at the expense of arachidonic acid (Yaqoob et al., 2000). Eicosanoids derived from arachidonic acid and eicosapentaenoic acids have opposing metabolic properties making it important to ensure a balanced dietary intake of n-6 and n-3 PUFAs (Gopalan and Patnaik, 2002).

PUFA are essential for human development in utero and in infancy and are likely to have a role throughout life. It has been reported that polyunsaturated fatty acids especially n-3 PUFA play an important role in the modulation and
prevention of various human diseases. The unique properties of these fatty acids in coronary heart disease first became apparent in the investigations of the health status of Greenland Eskimos who consumed diets very high in fat from seals, whales and fish and yet had a low rate of coronary heart disease events (Bang and Dyerberg, 1973). Thrombosis is a major complication of coronary atherosclerosis that can lead to myocardial infarction. The n-3 fatty acids from fish oil have powerful antithrombotic actions. EPA inhibits the synthesis of thromboxane A2 from arachidonic acid in platelets (Goodnight et al. 1982). One of the most important effects of n-3 EPA and DHA is their ability to inhibit ventricular fibrillation and consequent cardiac arrest (Albert, 2002).

The effects of both n-6 and n-3 fatty acids on rheumatoid arthritis have been reported (Simopoulos, 2002). Clinical improvements in tender joint scores and morning stiffness have been reported with the fish oil (Guesens, 1994). Daily supplementation of gamma linolenic acid for a period of 24 weeks was reported to reduce the number of tender and swollen joints (Leventhal et al., 1993). James and Cleland (1997) have also reported the beneficial effects of fish oil supplementation in rheumatoid arthritis patients.

Epidemiological studies have demonstrated that dietary fat consumption modulates the risk of several types of cancer, especially breast, prostate and colorectal cancer (Willet, 1994). It is reported that essential fatty acids and their metabolites can reverse and/or inhibit the growth of tumor. The level of α-linolenic acid is inversely related to the risk of developing metastases in breast cancer (Bougnoux, 1999). Additionally, the decreased consumption of fish and increased intake of vegetable oils rich in n-6 fatty acids among Japanese women
during the past decades have been accompanied by increased breast cancer rates (Lands et al., 1990).

Increasing the content of polyunsaturated fatty acids in the cell membrane enhances the insulin receptor number and binding and insulin action while saturated fat decreases binding and transport (Field et al., 1990). Decreased content of long chain polyunsaturated fatty acids, in particular arachidonic acid, and the total percentage of C20-C-22 polyunsaturates are associated with decreased insulin sensitivity (Pelikanova et al., 1989). Another study suggested that hyperinsulinemia and insulin resistance are inversely associated to the amount of 20 and 22 C PUFA in muscle cell membrane phospholipids in patients with coronary heart disease and in normal volunteers (Borkman et al., 1993).

Δ-6 desaturase activity is somewhat reduced in atopic eczema and bypassing this enzyme by giving gamma linoleic acid in the form of evening primrose oil led to a partial normalization of fatty acid and phospholipids composition thus producing clinical improvement (Bordoni et al., 1987). In psoriasis, arachidonic acid metabolism is altered. Proinflammatory leukotrienes like leukotriene B4 i.e. LTB4 are markedly produced in the psoriatic lesions. Consumption of fish oil along with the standard treatment produces further improvement and a decrease in LTB4 (Oliwiecki et al., 1990). Thus dietary supplementation of PUFA can be an adjunct protocol for the management of psoriasis and inflammatory skin disorders with negligible side effects (Ziboh, 1991).

Patients with chronic intestinal disorders or severe malabsorption often develop essential fatty acid deficiency. Intravenous lipids can correlate the
deficiency, but essential fatty acid abnormalities persist. Stimulation of n-6 PUFA biosynthesis that could lead to an inhibition of n-3 PUFA elongation has been observed (Chambrier et al., 2002).

Abnormalities in linoleic acid and n-3 fatty acid profiles in erythrocytic lipid may exist in multiple sclerosis patients. Increased turnover of arachidonic acid, the major substrate for the synthesis of the pro-inflammatory eicosanoids, which could contribute to the pathology and clinical picture seen in cystic fibrosis (Christophe and Robberecht, 2001).

Docosahexaenoic acid (DHA) is the major fatty acid of neurological and retinal membranes. Low levels of circulating DHA might be a significant risk in the development of Alzheimer dementia (Kyle et al., 1999). The inability to maintain a high level of DHA may be due to a reduced Δ-6 desaturase activity. Alterations in phospholipids which are structural components of all cell membranes in the brain may induce changes in membrane fluidity and consequently, in various neurotransmitter systems, which are thought to be related to the pathophysiology of major depression (Hibbeln and Salem, 1995). Depletion of n-3 fatty acid levels in red blood cell membranes of depressive patients has been reported (Peet et al., 1998). The abnormalities in n-3 PUFA may play a critical role among schizophrenic patients (Assies et al., 2001).

1.5 Objectives of the present study

PUFA are essential for growth and development (Simopoulos, 1999; Patrix and Gerard, 2000; Broadhurst et al., 2002) and are also shown to possess anti inflammatory and immunomodulatory properties by modulating enzyme activities, signal transduction and nuclear factor activation (Spector, 1999).
They are used in the prevention of several physiological disorders (Zamaria, 2004; Riediger et al., 2009). A large number of studies have shown positive health benefits associated with the consumption of n-3 polyunsaturated fatty acids (PUFA) on infant development (Gopalan and Patnaik, 2002) and combating various diseases (Willet 1994; Hibbeln and Salem, 1995; Kyle et al., 1999; Albert, 2002, Simopoulos, 2002). Dietary PUFA have been shown to suppress proinflammatory cytokine production (James et al., 2000) and inhibit lymphocyte proliferation (Thies et al., 2001). Cabre and Gassul (1996) reported that habitual fish intake protects against hepatic encephalopathy. On the other hand a few studies have also suggested that the total dietary fat intake is linked to an increased risk of obesity and diabetes (Astrup et al., 2008). Long chain PUFA deficiency is often associated with advanced liver cirrhosis (Okita et al., 2002). Acute severe deterioration of liver function is associated with essential fatty acid deficiency (Clemmesen et al., 2000). Thus, some of the most common medical disorders are characterized by the altered levels of fatty acids or their metabolites (Ferrara et al., 2001).

**We hypothesize that the consumption of dietary fatty acids especially n-3 PUFA might be associated with the amelioration of hepatitis.**

The anti inflammatory and immunomodulatory properties of the diets depend upon its PUFA composition. High saturated fatty acid content present in the diet may counteract the beneficial effects of PUFA. Diets rich in n-3 PUFA or n-6 PUFA have different metabolic fates that lead to contrasting health effects. Hence, a precise dietary balance between n-6 and n-3 PUFA is required to maintain a better health status (Allen and Dandorth, 1988).
High intake of marine fish, which is a rich source of PUFA, is common in the state of Goa. Today in Goa, the number of liver cirrhosis and other liver disease cases reported per year is increasing at an alarming rate (Goa Messenger, 2005). Correlation between dietary fat intake and hepatitis has not been thoroughly studied before. Hence, it is relevant to know if the dietary intake of lipids can have a preventive effect on hepatitis. We have used D-GaIN induced hepatitis model as this immunological liver injury model is often used to evaluate the efficacy of various substances on hepatitis. Besides it is very similar to human viral hepatitis in its morphological and functional features (Lim et al., 2000).

Considering the background and relevance of the topic, assumed Ph.D. work was based on the following broad objectives.

1. **What is the effective dose of dietary lipid that would be the most beneficial for mice?**

2. **What are the path physiological responses to D-galactosamine induced hepatitis in mice model?**

3. **To find out whether the dietary lipid acts as a preventive measure for hepatitis or it further aggravates the disease.**

We selected two sources of dietary fatty acids namely fish oil, rich in n-3 PUFA and other unsaturated fatty acids with unsaturation index 0.7 and meat oil rich in saturated fatty acid and moderately higher amount of n-6 PUFA with unsaturation index 2.3 for our present study (Table A).