Anti-diabetic studies of PUFA extracts from *Sardinella longiceps* and *Sardinella fimbriata*

1.1 Introduction

Diabetes mellitus is a chronic metabolic disorder with a worldwide incidence of 5% in general, with a suffering population of over 246 million and its proliferance is increasing steadily with changing life styles (Anonymous 2006). It is generally associated with complications like hypercholesterolemia, hypertriglyceridemia, atherosclerosis, coronary heart disease, renal malfunctioning and hypertension (Simopoulos 1991).
Diabetes can arise from a failure of the pancreas to secrete insulin. Insulin makes possible adequate utilization of glucose by the tissues at normal blood sugar levels. When sufficient insulin is not secreted by the beta cells of the Islets of Langerhans in the pancreas, higher concentrations of blood sugar are produced and this partially restores a more normal rate of glucose utilization.

5.1.1 Lipids and Diabetes

Lipid is a name given to compounds that are insoluble in water, and this property means that a lipid has to be transported bound to other molecules, to enable it to be transported in an aqueous environment. There are four major groups of lipids in human body viz. Cholesterols, Triglycerides, Phospho-lipids and Fatty acids (Thomas 2000).

Cholesterol is present in the diet and is required by all cells. Cells can synthesize cholesterol, which is the precursor for the steroid hormones mostly made in the adrenal glands and the gonads. Triglyceride is the major lipid found in the diet and it is broken down to yield glycerol and fatty acids.

Lipid is transported in the blood in small particles called lipoproteins, synthesized in the liver and gut. These particles are a complex of triglyceride, cholesterol, phospholipids and proteins. There are five main types of lipoprotein, classified according to size and density. The five lipoproteins, in size order are Chylomicrons, Very low density lipoprotein (VLDL), Intermediate density lipoprotein (IDL), Low density lipo protein (LDL) and High density lipoprotein (HDL). Of which HDL, LDL and VLDL are of clinical significance.
VLDL is a medium sized particle, containing mainly triglyceride, are synthesized in liver. The main function of VLDL is to transport lipids synthesized in the liver to parts of the body which require triglyceride as an energy source or for storage.

LDL is a small particle rich in cholesterol derived from the metabolism of VLDL. They contain an important apolipoprotein called apo B-100, which is responsible for recognizing an LDL receptor on the surface of cells.

HDL is the smallest of the lipoproteins but the densest, and contains the highest protein concentration. The role of HDL is to remove cholesterol from peripheral cells and plasma, transporting the cholesterol to the liver for reprocessing or excretion.

The relative levels of LDL and HDL have been shown to be important in assessing the risk of developing atherosclerosis. There is much research showing links between the development of heart disease and the presence of lipid in the blood. The major findings have shown that increased LDL correlates with an increased risk of heart disease while increased HDL correlates with a decreased risk of heart disease.

5.1.2 Kidney Disease and Diabetes

Diabetic condition in a long term can result in kidney disease (Simopoulos 1991). Disease of the kidney often manifests itself with symptoms relating to biochemical changes that can be detected in the blood. The two important measures of renal function are the urea and creatinine
Anti-diabetic studies of PUFA extracts from Sardinella longiceps and Sardinella fimbriata

5.1.3 PUFA and Diabetes

Poly-unsaturated Fattyacids (PUFA), particularly eicosapentaenoic acid (EPA; 20:5n23) and docosahexaenoic acid (DHA; 22:6n23) present in marine sources, have been found to have healing effects against several of these complications (Simopoulos 1991). Fish oil or fish oil supplemented diets, PUFA extracts from fish oils or substantially pure EPA or/and DHA have all been used in several invivo experiments. Though the utility value of fish oils in the treatment of diabetes is univocal, there is considerable disparity between results of most of these studies. Disparity subsumes in several factors; response of fish oil or EPA/DHA on normal humans and humans suffering from ailments varies considerably. Responses of PUFA extracts on the lipid profile of mice are sometimes different from that of concentrations (Thomas 2000). Urea is the end product of amino acid metabolism, formed from the break down of proteins. It is very water soluble and is freely filtered by the glomerulus and passes into the kidney tubules. Creatinine is derived from phosphocreatine found in muscle. Creatinine is filtered by the glomerulus and passes through the nephron without reabsorption taking place. An increase in the amount of urea and creatinine shows impaired kidney function.

The characteristic feature of diabetes mellitus is a diminished ability to utilize glucose at ordinary blood sugar levels. This produces a diminished glucose tolerance with a raised blood sugar, which is often well above the renal threshold for glucose. There is also increased mobilization of fat. More fat is used for supplying the energy requirements of the body. As a result, blood fat and cholesterol are also increased.
Anti-diabetic studies of PUFA extracts from *Sardinella longiceps* and *Sardinella fimbriata*

Humans (De Caterina et al. 2007). It is also established that EPA and DHA have divergent effects on total cholesterol and triglycerides with exact nature of their action still unknown (Hansena et al. 1998). However, in all these studies, profiling of blood glucose, total, LDL and HDL cholesterol and triglycerides seems to be the most widely used strategy to prove beneficial or adverse effects.

Studies have also shown that Omega-3 fattyacids offer a direct or indirect reno-protective effect in diabetes patients (Holm et al. 2001, van der Heide et al. 1993). Diet supplemented with Omega-3 fattyacids from plant sources is known to prevent diabetic renal injury and can even reverse kidney damage in mice subjects (Garman et al. 2009). Hence, two additional parameters serum urea and serum creatinine were considered worth monitoring.

Hypocholesterolemic effect of fish oil from *S. longiceps* has also been reported (Sen et al. 1977). However, there has not been any PUFA estimation done for the equally prolific *S. fimbriata*. The purpose of this study was to determine and compare the hypolipidimic and anti-diabetic properties of PUFA extracts from these two widely available sardines in Cochin coast obtained from the same area in its range. A comparison of their recovery profile is also attempted.
5.2 Materials and Methods

5.2.1 Extract Preparation and Determination of PUFA Composition:
Freshly caught samples of the fishes were subjected to the procedure documented in Chapter 2 to obtain a mixture of substantially pure PUFA. The composition of PUFAs in the above mixture was directly analysed by Gas Chromatography (GC) adopting the fatty acid methyl ester (FAME) method mentioned in Chapter 3 (3.3) and individual fatty acids were expressed as a percentage of total fatty acids.

5.2.2 Experimental Animals

Adult male albino mice (230-260 g) were obtained from the animal house of College of Veterinary and Animal Sciences, Mannuthy and housed at 22±2 in an air-conditioned chamber. Animals were maintained throughout the study at 24-28 °C, were fed a standard laboratory rat diet and water *ad libitum* and maintained in specious polypropylene cages and well ventilated animal house with 12 hr dark and light cycle. The experimental protocol has been approved by the animal ethics committee.

5.1.3 Induction of experimental diabetes

Alloxan tetra hydrate (Sigma) was dissolved in sterile distilled water. Diabetes was induced in 18 mice by intra-peritoneal injection of 185 mg/kg (5%) of Alloxan tetra hydrate (Kavitha *et al.* 2007). The mice were fasted 12hrs before and after the alloxan injection. The mice with blood glucose above 250 mg/dl, which lasted for at least one week, were selected for the experiment.
5.1.4 Study design

The mice were randomly divided into four groups of eight numbers each and the groups were labeled I-IV as follows.

Group I : **Standard Control (C).** Normal mice with no extra diet components.

Group II : **Diabetic Control (DC).** Mice induced with diabetes with no extra diet components.

Group III : **Sardinella longiceps Group (SL).** Diabetic mice orally administered with PUFA extract of *Sardinella longiceps* (1ml) daily using intra gastric tube for 28 days.

Group IV : **Sardinella fimbriata Group (SF).** Diabetic mice orally administered with PUFA extract of *Sardinella fimbriata* (1ml) daily using intra gastric tube for 28 days.

5.1.5 Blood sampling

The blood was collected from orbital plexus and serum was separated by immediate centrifugation of blood samples using semi ultra cooling centrifuge at 3000 rpm for 5 minutes at room temperature. This was repeated on the 0\(^{th}\), 7\(^{th}\), 14\(^{th}\), 21\(^{st}\) and 28\(^{th}\) day of the experiment from each individual mouse in the set. The following bio-chemical parameters, viz. glucose, total cholesterol, HDL cholesterol, LDL Cholesterol, triglycerides, urea, creatinine, were estimated for each of the samples.
5.1.6 Analysis of Biochemical Parameters

Fasting blood glucose was estimated by glucose oxidase-peroxidase method (Marks 1959). Serum was analysed and estimated for total cholesterol (Zak 1957), HDL (Burstein and Scholnick 1972) and LDL Cholesterol (Warnick et al. 1990) levels and triglycerides (Rice 1970). Urea and Creatinine levels were estimated using procedures of Chaney and Marbach (1983) and Jaffe Reaction (John and Keith 1994) respectively. For each day, all parameters were expressed as a Mean ± SD across 5 samples in each set.

**Estimation of Blood Glucose (Marks 1959)**

To 1.1ml of 0.9% NaCl, 0.4ml of 5% ZnSO4·7H2O solution and 0.4ml of 0.3N NaOH were added. To this 0.1ml of blood was added, mixed well, centrifuged and separated the supernatant. 1ml of the supernatant was transferred to a test tube. Into other two test tubes were added 1ml of water (blank) and 1ml of standard glucose solution. 3ml of glucose oxidase reagent was added to each at half minute intervals. This was mixed gently for not more than ten seconds and absorbance read in a Shimadzu-UV spectrophotometer-1601 at 625nm. The values were expressed as mg glucose/dl.
Estimation of Total Cholesterol (Zak 1957)

0.1ml serum was added to 10ml of the Ferric chloride- acetic acid reagent in a glass stoppered centrifuge tube, mixed well and kept for fifteen minutes for the proteins to flocculate. This was centrifuged and 5ml of the clear supernatant was transferred to a glass stoppered centrifuge tube. To two other tubes, 5ml of cholesterol standard & 5ml of ferric chloride- acetic acid reagent were added separately. 3ml of sulphuric acid was added to all the three tubes, stoppered tightly and mixed by repeated inversion. The stopper was loosened carefully and kept for twenty minutes and then absorbance read at 560nm. The values were expressed as mg /dl.

Estimation of Total Triglycerides (Rice 1970)

0.1ml of serum, standard and distilled water (blank) are added in 3 stoppered centrifuge tubes. 3.9ml isopropanol was added to each test tube, mixed well and then 400mg activated alumina added. This was shaken in a vortex mixer for 15min and centrifuged at 4000rpm for 5min. 2ml of supernatants were transferred to another 3 test tubes. 0.6ml of saponification reagent (5.0g of potassium hydroxide dissolved in 60ml distilled water and 4.0ml isopropanol) was added, stoppered and incubated at 60ºC for 15 minutes and then cooled and 1ml metaperiodate solution added. This was mixed and 0.5ml acetyl acetone reagent added, incubated at 50ºC for 30minutes, cooled and absorbance read at 405nm. The value of triglyceride in plasma was expressed as mg per dl.
Estimation of high-density lipoprotein fraction (HDL) (Burstein and Scholnick 1972)

To 0.2ml of serum, 0.09 ml of heparin-manganous chloride reagent was added and mixed well. This was allowed to stand at RT for 10 minutes and then centrifuged at 4000 rpm for 10 minutes. The supernatant represented HDL fraction. 0.1ml of supernatant was taken for the estimation of cholesterol.

Estimation of low-density lipoprotein (LDL) Friedewald equation (Warnick et al 1990)

- \[ \text{LDL} = \text{Total cholesterol} - \text{HDL} - \text{VLDL} \]
- \[ \text{VLDL} = \text{Triglyceride}/5 \]

Estimation of Urea (Chaney and Marbach 1983)

To 0.02ml of serum, 0.2 ml of urease enzyme reagent was added. Urease reagent, 0.20 ml was added to two other test tubes, standard and blank and 0.02ml of urea solution was added to the standard. This was kept in a water bath at 37ºC for 15minutes. 5ml of the phenol-nitroprusside solution was added, mixed, and followed with 5ml of hypochlorite reagent in all the three test tubes. This was kept at 37 ºC for 15minutes and then absorbance read at 630nm. The values were expressed as mg per dl.

Estimation of Creatinine – Jaffe Reaction (John and Keith 1994)

To 4.5ml of tungstic acid reagent 0.5ml of serum was added. This was allowed to stand for 5mts and centrifuged at 3000rpm. To three test
tubes, 3ml of supernatant (protein free solution), 0.1ml creatinine standard and 3ml distilled water were added separately. One ml creatinine reagent and 0.5ml NaOH were added to all the 3 test tubes. This was allowed to stand for 5mts and absorbance read at 540nm. The values were expressed as mg/dl.

5.1.7 Statistical Procedure and Analysis

The results were analyzed using pair-wise 1-way ANOVA against diabetic control and p<0.01 was considered as significant (Zar 1984). Recovery percentage of biological parameters were calculated using the formula

\[
\text{Recovery \%} = \frac{(\text{Diabetic Control} - \text{Recovered Value})}{(\text{Diabetic Control} - \text{Standard Control})} \times 100
\]

5.3 Results

Anti-diabetic effects of PUFA on various parameters of blood are summarized in Table 9; values obtained for each parameter in each set across 5 samples are expressed as Mean±SD. With the possible exception of the means of the response of serum glucose for both the fish extracts, all the other means were found to be different with significance of p < 0.01 (See Appendix)
### Table 10: Bio-chemical parameters analysed for all the sets

<table>
<thead>
<tr>
<th>Set</th>
<th>Measurement</th>
<th>Start (mg/dl)</th>
<th>End (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control Set</strong></td>
<td>Glucose</td>
<td>81.20±0.84</td>
<td>80.60±0.55</td>
</tr>
<tr>
<td></td>
<td>Total Cholesterol</td>
<td>72.00±1.22</td>
<td>71.20±0.45</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>82.00±1.22</td>
<td>81.00±1.00</td>
</tr>
<tr>
<td></td>
<td>Urea</td>
<td>39.20±0.84</td>
<td>39.20±0.45</td>
</tr>
<tr>
<td></td>
<td>Creatinine</td>
<td>0.24±0.05</td>
<td>0.26±0.05</td>
</tr>
<tr>
<td></td>
<td>HDL Cholesterol</td>
<td>39.60±0.55</td>
<td>39.60±0.55</td>
</tr>
<tr>
<td></td>
<td>LDL Cholesterol</td>
<td>16.00±1.12</td>
<td>15.40±0.73</td>
</tr>
<tr>
<td><strong>Diabetic Control Set</strong></td>
<td>Glucose</td>
<td>322.60±2.51</td>
<td>320.00±1.00</td>
</tr>
<tr>
<td></td>
<td>Total Cholesterol</td>
<td>181.20±0.84</td>
<td>180.80±0.84</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>251.60±1.34</td>
<td>250.80±0.84</td>
</tr>
<tr>
<td></td>
<td>Urea</td>
<td>128.00±1.22</td>
<td>128.00±1.00</td>
</tr>
<tr>
<td></td>
<td>Creatinine</td>
<td>3.02±0.08</td>
<td>2.96±0.05</td>
</tr>
<tr>
<td></td>
<td>HDL Cholesterol</td>
<td>20.00±1.00</td>
<td>19.60±0.55</td>
</tr>
<tr>
<td></td>
<td>LDL Cholesterol</td>
<td>110.88±1.39</td>
<td>111.04±0.95</td>
</tr>
<tr>
<td><strong>Diabetic Set administered with PUFA extract from <em>Sardinella longiceps</em></strong></td>
<td>Glucose</td>
<td>321.40±2.07</td>
<td>311.00±1.00</td>
</tr>
<tr>
<td></td>
<td>Total Cholesterol</td>
<td>182.00±1.22</td>
<td>129.00±1.87</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>251.00±1.52</td>
<td>183.60±0.89</td>
</tr>
<tr>
<td></td>
<td>Urea</td>
<td>129.20±0.84</td>
<td>122.40±0.55</td>
</tr>
<tr>
<td></td>
<td>Creatinine</td>
<td>2.96±0.05</td>
<td>2.44±0.05</td>
</tr>
<tr>
<td></td>
<td>HDL Cholesterol</td>
<td>19.80±1.00</td>
<td>30.40±1.00</td>
</tr>
<tr>
<td></td>
<td>LDL Cholesterol</td>
<td>111.88±1.96</td>
<td>61.88±1.23</td>
</tr>
<tr>
<td><strong>Diabetic Set administered with PUFA extract from <em>Sardinella fimbriata</em></strong></td>
<td>Glucose</td>
<td>322.00±2.45</td>
<td>310.40±0.89</td>
</tr>
<tr>
<td></td>
<td>Total Cholesterol</td>
<td>182.20±0.84</td>
<td>117.60±2.51</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td>251.80±0.84</td>
<td>166.40±0.55</td>
</tr>
<tr>
<td></td>
<td>Urea</td>
<td>128.60±0.55</td>
<td>120.40±0.55</td>
</tr>
<tr>
<td></td>
<td>Creatinine</td>
<td>2.98±0.08</td>
<td>2.28±0.08</td>
</tr>
<tr>
<td></td>
<td>HDL Cholesterol</td>
<td>20.00±1.00</td>
<td>34.00±1.00</td>
</tr>
<tr>
<td></td>
<td>LDL Cholesterol</td>
<td>111.84±1.61</td>
<td>50.32±1.62</td>
</tr>
</tbody>
</table>
5.3.1 Effects on Serum Glucose

Serum Glucose levels quadrupled in alloxan-induced mice at the start of the experiment and remained so throughout the experimental period. However, groups administered with both fish extracts showed a small decrease in levels of blood glucose from the 7th day sample itself (Figure. 14). Though the decrease was statistically significant (P < 0.01), the percentage decrease does not indicate a recovery worth further assessment. Hence, both fish extracts do not largely impact the serum glucose level in diabetes induced animals.

5.3.2 Effect on Cholesterol and Triglycerides

Total Cholesterol levels more than doubled and triglyceride levels tripled in alloxan-induced mice at the start of the experiment and remained so throughout the experimental period. However, groups administered with both fish extracts showed a significant recovery in total cholesterol and triglycerides (Figure. 16, 17). About 10-15% of improvement of total cholesterol and triglycerides was noted after every seven day interval when the sampling was done.

In terms of its components, LDL Cholesterol level (Figure. 19) also reduced significantly across the experiment for groups administered with extracts. Recovery values shot up by 15% after the 7th day of administration of the drug for both the species but after that recovery with S. longiceps extract was slower by about 5% as compared to that with S. fimbriata extract for every next 7th day of sampling. HDL Cholesterol (Figure. 18) which came down drastically in diabetic control improved significantly towards the end of the experiment. This also showed a remarkable improvement after the first 7 days itself with about 20% recovery for both
species. Thereafter, *S. fimbriata* extract showed a slightly better recovery than *S. longiceps* extract.

Recovery plots for all four showed that sets treated with extracts from *S. fimbriata* was recovering better than the ones treated with extracts from *S. longiceps* and this became more apparent towards the end of the experiment (Fig. 20,21,22,23).

LDL and HDL Cholesterol levels almost recovered 60% in one month after being treated with the extract of *S. fimbriata*. Triglycerides and total cholesterol levels recovered by 50% for this particular fish extract. Recovery was obvious in all these parameters from the first collection after drug administration (7th day) itself. A minimum of 35-40% recovery in all these parameters were observed with both species of fishes after a month and recovery curves indicated that the sets were still improving with good chances of reaching total normalcy. In summary, it can be concluded that there is considerable positive impact on cholesterol and triglycerides of diabetic mice subjects due to the administration of the fish extracts.

![Glucose](image)

*Fig. 14: Glucose Variation in the four experimental groups*
Anti-diabetic studies of PUFA extracts from Sardinella longiceps and Sardinella fimbriata

Chapter 5

Fig. 15: Urea Variation in the four experimental groups

Fig. 16: Total Cholesterol Variation in the four experimental groups
**Anti-diabetic studies of PUFA extracts from**

**Sardinella longiceps and Sardinella fimbriata**

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**Chapter 5**

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**Fig. 17: Triglycerides Variation in the four experimental groups**

**Fig. 18: HDL Cholesterol Variation in the four experimental groups**
Anti-diabetic studies of PUFA extracts from *Sardinella longiceps* and *Sardinella fimbriata*

Fig. 19: LDL Cholesterol Variation in the four experimental groups

Fig. 20: Recovery of Total Cholesterol in *S. longiceps* and *S. fimbriata* extract treated groups
Anti-diabetic studies of PUFA extracts from *Sardinella longiceps* and *Sardinella fimbriata*

Chapter 5

Fig. 21: Recovery of Triglycerides in *S. longiceps* and *S. fimbriata* extract treated groups

Fig. 22: Recovery of LDL Cholesterol in *S. longiceps* and *S. fimbriata* extract treated groups
5.3.3 Effects of PUFA Extracts on Urea and Creatinine

Urea levels tripled in alloxan-induced mice while Creatinine levels shot up 12 times after inducing alloxan into the mice. The levels remained unchanged throughout the experiment. Urea levels in sets administered with fish extracts showed a small but statistically significant ($P < 0.01$) improvement (Fig. 15).

However, creatinine levels improved significantly right from the first collection after drug administration ($7^{th}$ day) but further recovery was slow (Figs. 24, 25) and did not show signs of reaching full normalcy.

Sets administered with $S$. fimbriata extract recovered marginally better as compared to that of $S$. longiceps extract. In summary, it can be
concluded that there is an impact on renal parameters, specifically creatinine, of diabetic subjects due to the administration of these fish extracts.

Table 11 summarises the recovery (%) of various parameters which showed a significant variation.

**Table 11: Recovery in bio-chemical parameters in S. longiceps extract and S. fimbriata extract treated groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Recovery (%) after 28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. fimbriata</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>58</td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>63</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>72</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>50</td>
</tr>
<tr>
<td>Creatinine</td>
<td>25</td>
</tr>
</tbody>
</table>

**Fig. 24: Creatinine Variation in the four experimental groups**
5.3.4 GC Analysis

The PUFA extracts were analyzed by GC to identify the fatty acids present in the extract. The major compounds identified were unsaturated fatty acids ranging from C20 to C24 with a preponderance of C22:6 (DHA) and C20:5 (EPA) PUFA. GC analyses of the PUFA from the fish *S. fimbriata* showed a DHA content of 65.82% and an EPA content of 24.02%, an EPA-DHA Ratio of 3:8. The GC analyses of the PUFA from the fish *S. longiceps* gave a much lower DHA figure 32.52% while an EPA content of 55.54%, an EPA-DHA Ratio of 3:2.

5.4 Discussion

The purpose of this experiment was to determine effects of two sardine oil extracts on the diabetic condition of mice. PUFA extracts used are rich in Omega-3 fatty acids but with different ratio of DHA and EPA as clear from GC analysis.

Fig. 25: Recovery of Creatinine in *S. longiceps* and *S. fimbriata* extract treated groups
5.4.1 Hypoglycemic Effect

Omega-3 fattyacids and fish oils are not known to affect the blood glucose levels in animals or humans. In reviews on dietary and pharmaceutical applications of Omega-3 fattyacids and fish oils (Simopoulos 1991, Kris-Etherton et al. 2002), there has been no major studies cited that favourably increased blood glucose levels after the administration of fish oils or Omega-3 fattyacids. In contrast, there is a reported moderate worsening of glycemia noticeable in patients with impaired glucose tolerance and diabetes with levels > 3g/day of Omega-3 fattyacids (Kris-Etherton et al. 2002). It is generally accepted that the application of fish oils and Omega-3 fattyacids in anti-diabetic pharmacology is mainly in arresting the associated disorders like hypercholesterolemia and hypertriglyceridemia (Simopoulos 1991). This is very much in accordance with the results of current study on mice subjects where a 28 day administration of two fish oil extracts with differing ratios of EPA and DHA did not significantly decrease the blood glucose levels with recovery percentage being a mere 2-3%.

However, in a 60-day study on low-dose streptozotocin-induced diabetic mice subjects, a decrease in blood glucose level was recorded when fed with an Omega-3 enriched diet (Linn et al. 1989). Rubin, D (1991) extracted substantially pure free fattyacids by urea complexing from sardine oil claimed to have found this method to be more effective (52% recovery) in treating diabetes in humans as compared to the fish oil in its natural form (12%). It is unclear on why scattered studies like the above reported a hypoglycemic effect of fish oils and Omega-3 fattyacids. However, there are
several studies on plant extracts and α-linoleic acid that have a positive hypoglycemic effect (Konrad et al. 2001). Hence the lack of hypoglycemic effects for Omega-3 fatty acids may perhaps restricted only to EPA and DHA. However, a more recent study reported that colon-specific delivery of DHA and EPA on mice subjects observed substantial insulin release and subsequent glucose reduction (Morishita et al. 2008).

5.4.2 Hypolipidemic Effects

It is well known that in uncontrolled diabetes mellitus, there will be an increase in total cholesterol, triglycerides and LDL cholesterol associated with decrease in HDL cholesterol (Arvind et al. 2002). This was in accordance with the start of the experiment in current study when alloxan induced mice tested high levels of total cholesterol, triglycerides and LDL cholesterol while HDL cholesterol decreased significantly. Patients with diabetes are at increased risk of Coronary Heart Disease (CHD) and to a clustering of risk factors for CHD, including excess weight, hypertension, dyslipidemia, and unfavorable hemostatic changes. Though there has been discordant views on the effect of Omega-3 fatty acids on CHD, evidence of Omega-3 enriched diet showing a positive correlation to reduce CHD is more overwhelming than scattered evidence of no or negative correlation (Kris-Etherton et al. 2002). Dietary Omega-3 fatty acids have been shown to be effective in reducing triglycerides and increasing HDL Cholesterol in patients with diabetes (Simopoulos 1991, De Caterina et al. 2007, Landgraf-Leurs et al. 1990). n-3 PUFA (EPA and DHA in excess of 65%) administered on myocardial rats significantly improved the cholesterol and triglyceride levels specifically increasing the levels of HDL Cholesterol and decreasing
the levels of LDL Cholesterol (Anandan et al. 2007). In the current study, Triglycerides, LDL and Total Cholesterol decreased markedly during the 28 day course of the experiment in both PUFA extracts. Levels of HDL Cholesterol showed a sustained improvement and levels went up to 50-60% of normalcy in 28 days. These results are in accordance with similar experiments with extracts or diets rich in Omega-3 fatty acids; both in animals and in humans.

There are several studies which evidenced DHA to be a comparatively stronger hypocholesterolemic n-3 fatty acid as compared to EPA. Childs et al. 1990, in their experiments on normal lipidemic men with three different concentrations of EPA and DHA, concluded that LDL and total Cholesterol were significantly lower in DHA rich diets but did not get affected by diets rich in EPA rich. However, level of triglycerides decreased significantly in all diets. They also concluded that HDL concentrations are better maintained with oil rich in DHA than EPA. Invivo mice studies have also reported specifically that DHA reduced total cholesterol significantly as compared to EPA. However, these studies also established that EPA reduces triglycerides better than DHA (Ikeda et al. 1993, Kobatake et al. 1984, Willumsen et al. 1993). In the current experiment, extracts from S. fimbriata fared better over the extracts from S. longiceps in both total cholesterol and triglycerides. S. fimbriata is DHA rich and this could clearly explain the effect on total cholesterol. However, the higher response to triglycerides for the same extract cannot be explained directly in terms of the relative concentrations of these n-3 fatty acids. Perhaps, the ratio of DHA and EPA in the extract also has a role to play in the recovery of triglycerides in diabetes induced mice. However, a hypotriglyceridimic effect for DHA was
shown in healthy human subjects (Nelson et al. 1997) and in patients with combined hyperlipidemia (Davidson et al. 1997). Another study reported a slightly better triglyceride lowering effect in humans for DHA than EPA (Grimsgaard et al. 1997). In mildly hyperlipidemic men, it was also found that triglycerides levels come down better with DHA than EPA (Mori et al. 2000).

Early study indicated that fish oil from *Sardinella longiceps* demonstrate a pronounced hypocholesterolemic effect but it was not clear whether the effect was due to EPA or DHA (Sen et al. 1977). Since there has been no similar studies on DHA rich *S. fimbriata* till date, this current study gains importance as extracts from *S. fimbriata* seems more potent as a hypercholesterolemic agent and result tallies well with earstwhile studies that proved a similar effect for DHA.

### 5.4.3 Effects on Renal Functioning

Diabetes is associated with several renal disorders and abnormal levels of serum urea and serum creatinine (Simopoulos 1991). The diabetic hyperglycemia induced by alloxan produce elevation in plasma levels of urea and creatinine in animals, which are considered significant markers of renal dysfunction. Action of chemically induced alloxan on animals is not specific to pancreas but also affects organs like kidney (Sabu & Kuttan 2002). A 30 week study on streptozotocin-induced diabetic mice demonstrated that n-3 fatty acids are superior to n-6 fatty acids in renal functioning by controlling urine albumin excretion, glomerulosclerosis and tubulointerstitial fibrosis (Garman et al. 2009). It has also been shown that fish oils prolong the survival in mice that develop *lupus nephritis* (Kelley...
et al. 1985). Studies reported that Omega-3 fatty acids improve renal functioning in patients who undergo heart and kidney transplants (Holm et al. 2001, van der Heide et al. 1993, Stoof et al. 1989, Urakaze et al. 1989). Urakaze et al. (1989) reviews several studies done on the effect of omega-3 fatty acids on human subjects with renal disease by assessing serum creatinine among other factors and concludes that two studies reported a statistically significant improvement in serum creatinine when treated with fish oil. The current study on diabetic mice reports a recovery of 15-20% in serum creatinine over a period of 28 days, recovery peaking within 7 days and remaining more or less steady. This reduction suggests potential utility of these fish extracts in diabetes associated complications. It is also established that normal subjects do not show any change in renal function even when given pharmacologic doses of fish oil, which is encouraging from the safety standpoint (Dosing et al. 1987).

Moreover, it also known that the beneficial effects on renal function is partly dependent on an increase in EPA and DHA (Holm et al. 2001). The mechanism involved is unknown, but experimental studies have shown that omega-3 fatty acids may increase thromboxane A3 formation, coinciding with a fall in thromboxane A2 and a significant increase in total prostacyclin levels (von Schacky et al. 1985). It is also not clear whether EPA or DHA has a greater effect. In the present study, DHA rich S. fimbriata showed a marginally better recovery as against the EPA rich S. longiceps perhaps indicating that DHA has a greater role in maintaining creatinine levels and hence renal functioning.
5.4 Conclusion

In conclusion, widely available marine fishes like sardines serve as a rich source of DHA and EPA and is an excellent nutritional source for human subjects having hyperlipidemia and renal disorders associated with diabetes. Though there is no significant positive influence on the blood sugar levels, the positive influence on associated disorders of these compounds creates an opportunity to be used as a supplement to the main drug. Hence these natural sources have the potential to be an excellent source of pharmaceuticals that target these disorders. Fish oil extracts from *Sardinella fimbriata* have higher concentrations of DHA than EPA and hence seem to have greater hypolipidemic and renal effects.

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