CHAPTER 4
EFFECT OF CUTTFISH LIVER OIL IN ISOPROTERENOL ADMINISTERED RATS

4.1 INTRODUCTION

Myocardial infarction (MI) due to prolonged total occlusion of the artery causing infarction or death of some of the heart muscle is the second major feature of coronary heart disease (CHD), the first being angina pectoris. In India, the number of patients being hospitalized for heart attack is increasing over the past 35 years and male patients have shown a striking increase (Krishnaswami, 1998). MI induced by isoproterenol [L-β-(3,4-dihydroxy phenyl)-2-isopropylaminoethanol hydrochloride], a β-adrenergic agonist, has been reported to show many metabolic and morphologic aberrations in the heart tissue of the experimental animals similar to those observed in human MI (Nirmala and Puvanakrishnan, 1996a). These changes are hyperglycemia, hyperlipidemia, loss of membrane integrity, increased activities of serum creatinine phosphokinase (CPK), lactate dehydrogenase (LDH), glutamate oxaloacetate transaminase (GOT), and increase in corticosteroids, blood urea & nitrogen. The mechanism of action of isoproterenol in inducing myocardial necrosis is a multiple step process (Ravichandran et al., 1990). The primary disturbance of isoproterenol-induced MI has been reported to be, enhanced adenylate cyclase activity resulting in increased cAMP formation, which in turn would lead to the higher lipid accumulation in the myocardium (Subhash et al., 1978). Evidence also suggests that reactive oxygen-derived free radicals play a crucial role in the pathogenesis of isoproterenol-induced MI (Nirmala and Puvanakrishnan, 1996b).
Independent inverse association between base-line fish consumption and the 30-year risk of fatal myocardial infarction from MI has been reported by Martha et al. (1997). A significant inverse relation between fish consumption and the risk of death from coronary heart disease have also been reported by other studies (Kromhout et al., 1985; Norell et al., 1986; Kromhout et al., 1995; Albert et al., 1996). In this chapter, we report the study carried out to assess the cardioprotective effect of cuttlefish liver oil (CFLO) on isoproterenol induced MI in rats.

4.2 MATERIALS AND METHODS

4.2.1 ANIMALS

Male albino Sprague Dawley rats weighing 80-110 g body weight, purchased from Small Animal Breeding Centre, Kerala Agricultural University (KAU), Thrissur, were used for the study.

4.2.2 PREPARATION OF CUTTLEFISH LIVER OIL

The cuttlefish liver oil was prepared as described in section 2.2.1

4.2.3 EXPERIMENTAL DESIGN

The animals were housed in groups of six in polypropylene cages with a 12:12 light/dark cycle. Sufficient number of control groups and test groups were maintained so that at least 6 animals were available for each assay. The animals were grouped as 1a & 1b- fed on normal diet (purchased from College of Veterinary and Animal Sciences, KAU, Thrissur); Groups-2a & 2b which were fed on normal diet + 1 % cuttlefish liver oil. They were provided with food and water ad libitum for a period of 45 days.
4.2.4 CARDIOPROTECTIVE EFFECT

At the end of feeding study myocardial infarction was produced in animals of 1b (fed on normal diet) and in 2b (fed on normal diet + 1% CFLO) by subcutaneous injection of isoproterenol [6mg (dissolved in physiological saline) per 100g body weight] twice at an interval of 24h. Simultaneously, the control animals (1a and 2a) were injected with physiological saline alone. Animals surviving the 2nd injection were sacrificed at 36h after 1st injection. Blood was collected in ice cold containers and the serum was separated for the determination of diagnostic marker enzymes.

4.2.5 BIOCHEMICAL ASSAYS

Activities of serum enzymes such as lactate dehydrogenase (LDH), Creatine phosphokinase (CPK), Glutamate oxaloacetate transferase (GOT), Glutamate pyruvate transferase (GPT) and Creatine kinase-MB (CK-MB) were determined using commercial diagnostic kit supplied by AGAPPE Diagnostics, Mumbai, India. The principle of the estimations carried out is as given below:

(i) LDH

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L- lactate} + \text{NAD}^+$$

(ii) CPK

$$\text{Creatine phosphate} + \text{ADP} \rightarrow \text{Creatinine} + \text{ATP}$$

$$\text{ATP} + \text{glucose} \rightarrow \text{ADP} + \text{G-6-P}$$

$$\text{G-6-P} + \text{NADP} + \text{G-6-P-DH} \rightarrow 6 \text{PG} + \text{NADPH} + \text{H}^+$$
(iii) GOT

\[
\text{L-aspartate + 2 \text{ oxyglutarate} \rightarrow \text{L-glutamate + oxaloacetate}}
\]

\[
\text{GOT}
\]

\[
\text{Oxaloacetate + NADH + H} \rightarrow \text{L-malate + NAD}^+
\]

\[
\text{MDH}
\]

(MDH – malate dehydrogenase)

(iv) GPT

\[
\text{L-Alanine + 2- \text{oxoglutarate} \rightarrow \text{L-glutamate + Pyruvate}}
\]

\[
\text{GPT}
\]

\[
\text{Pyruvate + NADH + H}^+ \rightarrow \text{L-lactate + NAD}^+
\]

\[
\text{LDH}
\]

(v) CK-MB

Procedure involves measurement of CK- activity in the presence of antibody to CK-M monomer. This antibody completely inhibits the activity of CK-MM and half the activity of CK-MB & CK-BB. Then CK method is used to determine CK-B activity.

4.2.6 RESULTS

The results are given in Table 4.1. There was a significant rise in the levels of diagnostic marker enzymes (LDH, CPK, GOT, GPT and CK-MB) in the serum of Group 1b isoproterenol-administered rats compared to that of group 1a control rats. The administration of 1% CFLO along with feed to the Group 2b animals prior to isoproterenol administration decreased the activities of these marker enzymes as compared to group 1b isoproterenol-injected rats fed on normal diet alone.
Table 4.1 – Activities of LDH, CPK, GPT, GOT and CK-MB in serum

<table>
<thead>
<tr>
<th>Group</th>
<th>LDH</th>
<th>CPK</th>
<th>GPT</th>
<th>GOT</th>
<th>CKMB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Units / litre</td>
<td>Units / litre</td>
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<td>Units / litre</td>
<td>Units / litre</td>
</tr>
<tr>
<td>1a</td>
<td>400.6 ± 34</td>
<td>365.2 ± 31</td>
<td>21.2 ± 1.4</td>
<td>73.3 ± 3.7</td>
<td>172.6 ± 15.8</td>
</tr>
<tr>
<td>1b</td>
<td>1218.3 ± 110#</td>
<td>1093.5 ± 92#</td>
<td>68.3 ± 3.4#</td>
<td>220.7 ± 18.3#</td>
<td>518.8 ± 48.7#</td>
</tr>
<tr>
<td>2a</td>
<td>392.3 ± 27</td>
<td>353.4 ± 29</td>
<td>18.1 ± 1.2</td>
<td>68.5 ± 3.2</td>
<td>166.4 ± 13.4</td>
</tr>
<tr>
<td>2b</td>
<td>783.4 ± 71*</td>
<td>705.5 ± 72*</td>
<td>45.8 ± 2.5*</td>
<td>142.4 ± 14.3*</td>
<td>390.5 ± 38*</td>
</tr>
</tbody>
</table>

Group 1a and 2a, normal controls, rats fed on normal diet and normal diet +1 % CFLO, respectively, for a period of 45 days; Group 1b and 2b, myocardial infarction was induced by isoproterenol administration after 45 days of feeding with normal diet and normal diet +1% CFLO respectively.

Values are mean ± SD of six values. *Significant at 5% level as compared to Group 1b.

# Significant at 5% level as compared to Group 1a
4.2.7 DISCUSSION

The administration of isoproterenol to group 1b rats (6mg/100g body wt., twice) resulted in the induction of myocardial infarction as is evident from the increased levels of marker enzymes namely LDH, CPK, GPT, GOT and CK-MB. Raised values of serum CPK as well as the higher activities of LDH, GPT, GOT and CK-MB in the blood are indicative of myocardial damage. During myocardial infarction condition, these enzymes are released from the damaged heart tissue into the blood stream. This finding is in accordance with earlier studies (Ithayarasi et al., 1996; Sathish et al., 2002). Geetha et al., (1990) have reported that the concentration of enzymes is directly proportional to the number of necrotic cells present in the cardiac tissue.

In the present study, the prior administration of 1% CFLO along with feed was found to significantly prevent the isoproterenol-induced elevation in the levels of these marker enzymes in serum of Group 2b animals as compared to Group 1b isoproterenol-injected rats. Shekelle et al. (1985) reports inverse correlation between CHD mortality and consumption of poly unsaturated fatty acids (PUFAs). Meittinen et al. (1982) and Riemersma et al. (1986), report the low levels of PUFAs in blood and adipose tissue to be associated with subsequent risk of CHD. PUFAs, viz. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have powerful antithrombogenic effect and are suggested to act by inhibiting the conversion of arachidonic acid (AA) to thromboxane A$_2$ (TXA$_2$) and facilitating production of prostacyclin (PGI$_3$), an inhibitor of platelet aggregation (Rao et al., 1983; Lagard, 1990). Long term dietary fish oil supplementation significantly reduced myocardial infarct size whereas short term supplementation had no effect. The higher activities of the marker enzymes in the control 1b (administered normal diet) compared to that of 2b (1% CFLO treated group) highlights the cardio protective effect of $\omega$-3 fatty acids of CFLO. The 1% CFLO level seems to be effective in
the cardioprotection supporting the finding that EPA and DHA of fish oil being highly
unsaturated get incorporated in the membrane phospholipids and offer protection through
stabilizing the membrane and / or inhibiting the conversion of AA to TXA₂ and facilitating the
production of prostaglandin (PGI₃), an inhibitor of platelet aggregation. Also, the ω-3 PUFAs
present in CFLO may protect myocardial membrane against oxidative damage as they are known
inhibitors of free radical generation.
CHAPTER 5
ANTI-INFLAMMATORY AND PLATELET AGGREGATION INHIBITING ACTIVITIES OF CUTTFLEFISH LIVER OIL