CHAPTER 6
IMMUNOSTIMULATORY EFFECT OF CUTTLEFISH LIVER OIL

6.1 INTRODUCTION

The interactions between immune and inflammatory cells are mediated in large part by proteins, termed interleukins (IL) that are able to promote cell growth, differentiation and functional activation. Tumour necrosis factor (TNF-\(\alpha\)) and IL-1 and IL-6 are the most important cytokines produced by monocytes and macrophages. Production of appropriate amounts of TNF, IL-1 and IL-6 is beneficial in response to infection, but in inappropriate amounts can be dangerous and these cytokines, especially TNF, are implicated in causing some of the pathological responses that occur in inflammatory conditions. They induce fever and the synthesis of acute phase proteins by the liver, activate T and B lymphocytes and endothelial cells and are involved in many other aspects of the acute phase response.

Results of animal and human studies support the hypothesis that omega-3 PUFA suppress cell mediated immune responses, in part at least by inhibiting antigen presenting-cell function, increase membrane fluidity and alter the expression of membrane proteins, possibly by influencing the vertical displacement of the proteins within the membrane. Kremer et al. (1985) measured neutrophil leukotriene B\(_4\) (LTB\(_4\)) production, which was decreased in the patients receiving fish oil. The prolonged suppression of LTB\(_4\) beyond the period of supplementation with fish oils most likely accounted for the continued clinical benefits observed after the period of discontinuation of fish oil. Prolonged effects on the immune system were subsequently reported in normal volunteers ingesting fish oil. Harbige (2003) reports that low intake of long chain \(\omega\)-3 fatty acids i.e., fish oils, enhances certain immune functions, whereas, high intakes are
inhibitory on a wide range of functions. In the present chapter, the immunomodulatory action in male albino Sprague Dawley rats fed a low dose of 1 % cuttlefish liver oil in diet is discussed.

6.2 MATERIALS AND METHODS

6.2.1 ANIMALS

As mentioned in section 5.2.1

6.2.2 PREPARATION OF CUTTLEFISH LIVER OIL

The cuttlefish liver oil was prepared as described in section 2.2.1

6.2.3 EXPERIMENTAL DESIGN

Similar to that described in section 5.2.3.

6.2.4 IMMUNOSTIMULATORY EFFECT

The immunostimulatory action was determined by assaying the Splenic T-lymphocyte mitogen response, bone marrow cell proliferation assay, plaque formation cell assay and circulating antibody titre.

6.2.4.1 Splenic T-lymphocyte mitogen response and bone marrow cell proliferation assay

At the end of the feeding study, the animals were sacrificed; spleen removed aseptically and made into single cell suspension. The cells from both the control and test animals were cultured (10^6 cells/ml) in the presence and absence of mitogen PHA (4μg/ml) in RPMI-1640 medium containing 10% FBS (final volume 1 ml) and antibiotics in a humidified atmosphere of 5 % CO₂ at 37 °C. After 48 h, ^3^H- Thymidine was added (1μCi/vial) and further incubated for 18 h, at the end of which, the DNA was precipitated using 0.8 M perchloric acid and the incorporated radioactivity was counted using Wallac 1409 Liquid Scintillation Counter (Mustafa, 1992).
6.2.4.2 Bone marrow cell proliferation assay

Bone marrow cell proliferation assay was carried out by following the method of Kumar et al. (1999). Total bone marrow cells were collected from control and test animals and made into single cell suspension in RPMI-1640 as described above. The cells ($10^6$ cells/ml) were cultured in the presence and absence of mitogen PHA ($4\mu$g/ml). The proliferation of bone marrow cells was determined from the amount of radioactive thymidine incorporated into the DNA.

6.2.4.3 Plaque formation cell assay

Modified slide technique of Jern’s Plaque assay was adopted for plaque formation cell assay (Mehrotra, 1992). At the end of experimental period the control and test animals were immunized with 1 ml of 5 % SRBC intraperitoneally. The spleen was collected from the sacrificed animals on the 9th day following immunization. A single cell suspension of the spleen cells was prepared in HBSS ($8\times10^6$ cells/ml). To 0.5 ml of 0.5% agarose prepared in HBSS, 50 $\mu$l of 10% SRBC and 50 $\mu$l of spleen cell suspension were added, mixed well and poured over a glass slide. The slides were allowed to solidify and then incubated with fresh guinea pig serum as a source of complement for 1 h at 37 °C. The plaques formed were counted using a colony counter and represented as plaque forming cells (PFCs /million spleen cells).

6.2.4.4 Circulating antibody titre

Blood was collected from the immunized animals on the 9th day following immunization. The blood was allowed to clot and serum was separated by centrifugation. Two-fold serial dilution of the serum samples were made in physiological saline and mixed (1:1) with 1% SRBC
in physiological saline. Agglutination was noted after incubation at room temperature for 3 h (Nelson and Davey, 1992).

6.3. RESULTS

The results (Table-6.1) on the effect of feeding 1% cuttlefish liver oil to rats for a period of 45 days showed a 2-fold increase in the thymidine uptake by spleen cells stimulated with PHA, when compared to the control group receiving no cuttlefish liver oil. In the absence of mitogen, there was no significant difference in the thymidine uptake between the control and the test group.

The results also showed that there was a significant increase in the proliferation of bone-marrow cells of rats fed on 1% cuttlefish liver oil compared to the control. Mitogen treatment did not affect the proliferation of these cells showing the absence of any mature T-cells.

There was a significant increase in the number of plaque forming cells in rats fed cuttlefish liver oil. There was a 16 times increase in the circulating antibody titre in the serum of test animals (Table 6.2).

6.4. DISCUSSION

Several studies have suggested that EPA rich fish oils boost immune function primarily by mediating eicosanoid production by decreasing those that are pro-inflammatory and increasing those that are anti-inflammatory. It is also reported that low intake of long chain ω-3 fatty acids i.e., fish oils, enhance certain immune functions, whereas, high intakes are inhibitory on a wide range of functions (Harbige, 2003).

In the cuttlefish liver oil treated animals, the spleen cell proliferation was found to be stimulated in the presence of mitogen as seen from the increased $^3$H Thymidine incorporation.
The T-cells respond to plant mitogens like PHA by rapid blastogenesis (Mustafa, 1992). The ability of T-cells to get transformed has been shown to bear correlation with in vivo parameters of cell mediated immunity status of the individual.

Enhanced proliferation of bone marrow cells were also observed in treated animals compared to control. This indicates induction of proliferation of bone marrow stem cells either directly or indirectly, stimulating the release of factors that are involved in the regulation of hemopoiesis. The treated animals also showed an increase in number of plaque forming cells in the spleen and antibody titre in the circulation, which are the functions of B-cells.

The fatty acid composition of inflammatory and immune cells is sensitive to change according to the fatty acid composition of the diet. In particular, the proportion of different types of PUFAs in these cells is readily changed and this provides a link between dietary PUFA intake, inflammation and immunity (Calder, 2001).

Animal studies have shown that dietary fish oils results in altered lymphocyte function and in suppressed production of proinflammatory cytokines by macrophages (Calder, 2001). Lymphocytes are involved in both the beneficial and detrimental effects of the immune system. Both the level of fat and the types of fatty acid present in the diet can affect lymphocyte functions (Calder, 2003). The present study reveals that feeding cuttlefish liver oil at a level of 1% in the diet for 45 days stimulates immune function in rats. The ω-3 PUFAs especially EPA present in CFLO maybe responsible for this. The finding is in agreement with the report that low intake of long chain ω-3 PUFAs enhances immune function (Harbige, 2003).
Table 6.1 Effect of feeding cuttlefish liver oil on Splenic T-lymphocyte mitogen response and bone marrow cell proliferation in rats

<table>
<thead>
<tr>
<th>Type of cells</th>
<th>Spleen cells</th>
<th>Bone-marrow cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No mitogen</td>
<td>PHA (4 μg/ml)</td>
</tr>
<tr>
<td>Control</td>
<td>3279.5 ±146.68</td>
<td>5303.5 ± 421.88</td>
</tr>
<tr>
<td>Test</td>
<td>3495.87 ± 68.38</td>
<td>11909.16 ± 402.79 *</td>
</tr>
</tbody>
</table>

*P < 0.001 (Values are mean ± SD of 6 different estimations)

Table 6.2 Effect of feeding cuttlefish liver oil on plaque forming cells and circulating antibody titre

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No of PFCs / million spleen cells</th>
<th>Circulating antibody titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Mean ± SD)</td>
<td>(Mean of 6 different estimations)</td>
</tr>
<tr>
<td>Control</td>
<td>165.166 ± 9.516</td>
<td>4</td>
</tr>
<tr>
<td>Test</td>
<td>381.5 ± 20.334 *</td>
<td>64</td>
</tr>
</tbody>
</table>

*P < 0.001 (Values are mean ± SD of 6 different estimations)
SUMMARY AND CONCLUSION