Dosage Fixation
The male Wistar rats weighing 100-150 gm were divided into 4 groups each group comprising about 12 animals. The animals were administered isoproterenol hydrochloride dissolved in 0.1 ml of normal saline by subcutaneous injection consecutively for two days at an interval of 24 hrs. The details of the dosage given was as below:

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>60 mg/kg body wt.</td>
</tr>
<tr>
<td>II</td>
<td>80 mg/kg body wt.</td>
</tr>
<tr>
<td>III</td>
<td>100 mg/kg body wt.</td>
</tr>
<tr>
<td>IV</td>
<td>120 mg/kg body wt.</td>
</tr>
</tbody>
</table>

12 hrs after the second injection the animals were inspected for mortality rate and then the surviving animals were sacrificed by cervical decapitation method. The blood was collected without any anticoagulant and serum was separated.

The enzymes CK and LDH enzymes were estimated in serum. The mortality rate was moderate in Groups I & II and very high in Groups III & IV. The LDH and CK values were significantly elevated in serum of all the groups. Therefore we have decided to carry out further studies with 60 mg and 80 mg/kg body weight of isoproterenol because at these doses the mortality rate is low at the same time LDH, CK values were high which is a sign of myocardial infarction.
Effect of Isoproterenol treatment on mortality rate (top) and LDH and CK activities in serum (bottom) of rats

Fig. 2.1

Values are expressed as mean ± S.D. for 6 animals in each group
Having fixed the dosage for isoproterenol we proceeded for fish oil dosage fixation and treatment period. We have taken three dosages for the study based on the literature which is comparable to human consumption - 0.05 ml, 0.1 ml and 0.15 ml. So the animals were divided into 3 groups each group comprising around 36 animals. The first group of animals were given 0.05 ml of fish oil orally by gastric intubation. The second group of animals were given 0.1 ml of fish oil orally by gastric intubation. The third group of animals were given fish oil 0.15 ml orally by gastric intubation.

To fix the period of treatment again the animals in each group were divided into 4 groups. First group animals were treated with fish oil for 15 days. Second group animals were treated with fish oil for 30 days. Third group animals were treated with fish oil for 45 days and fourth group animals were treated with fish oil for 60 days. At the end of the treatment period of fish oil the animals were administered isoproterenol hydrochloride 60 mg/kg body weight by subcutaneous injection for two days consecutively at an interval of 24 hrs. For comparison purpose we included two groups of animals in each treatment period i.e., control and IPH alone treated animals.

12 hrs after the second injection the animals were sacrificed by cervical dislocation. Blood was collected and serum separated. LPO, CK and LDH assays were carried out in serum.
Effect of fish oil pretreatment on Serum LPO value at 60 mg/kg body weight (top) 80 mg/kg body weight (bottom) of isoproterenol treated rats

Fig. 2.3

Period of fish oil treatment in days

Fig. 2.4

Period of fish oil treatment in days

Values are expressed as mean ± S.D. for 6 animals in each group
Effect of fish oil pretreatment on Serum creatine kinase values at 60 mg/kg body weight (top) and 80 mg/kg body weight (bottom) of isoproterenol treated rats

![Graph showing the effect of fish oil pretreatment on Serum creatine kinase values.](image)

**Fig. 2.5**

Values are expressed as mean ± S.D. for 6 animals in each group
Effect of fish oil pretreatment on LDH values in serum at 60 mg/kg body weight (top) and 80 mg/kg body weight (bottom) of isoproterenol treated rats

![Graph showing the effect of fish oil pretreatment on LDH values in serum at 60 mg/kg body weight (top) and 80 mg/kg body weight (bottom) of isoproterenol treated rats.](img)

**Fig. 2.7**

![Graph showing the effect of different doses of fish oil on LDH values in serum at 60 mg/kg body weight.](img)

**Fig. 2.8**
Again the fish oil treatment schedule was repeated with isoproterenol administration at the dosage of 80 mg/kg body weight. The results of the fish oil treatment schedule at the doses 60 mg/kg body weight and 80 mg/kg body weight of isoproterenol were discussed as below:

(The oral treatment with fish oil at 0.05 ml dosage showed significant increase in the serum values of LPO, LDH and CK at 15 days treatment period and then induction with IPH at both doses i.e., 60 mg of IPH/kg body weight and 80 mg of IPH/kg body weight. At the same dose of fish oil treatment for 30 days and subsequent induction of MI with IPH resulted in comparatively less values of serum LPO, LDH and CK compared to 15 days period of treatment but still the values are very high compared to control values.) 45 days treatment with fish oil at the dosage of 0.05 ml resulted in a significant decrease in the serum LPO, LDH and CK values after IPH administration compared to the values of animals treated with IPH alone at both the doses of IPH i.e. 60 mg/kg body weight and 80 mg/kg body weight.

60 days treatment period also resulted in a significantly low serum values of LPO, LDH and CK.

The oral treatment with fish oil at the dose of 0.1 ml per day for 15 days and then induction with IPH at both doses of IPH i.e., 60 mg/kg body weight and 80 mg/kg body weight did not show significant decrease in serum values of LPO, LDH and CK when compared to IPH alone treated animals.
At the same dose of fish oil treatment for 30 days and subsequent induction of MI with IPH at the doses of 60 mg/kg body weight and 80 mg/kg body weight resulted in comparatively less values of serum LPO, LDH and CK compared to the values of 15 days treatment period. But still the values are significantly high when compared to control values.

45 days treatment with fish oil at the dose of 0.1 ml and then IPH administration at the doses of 60 mg/kg body weight and 80 mg/kg body weight resulted in a significant reduction in the serum LPO, LDH and CK values when compared to animals treated with IPH alone.

60 days treatment with fish oil at this dosage and then induction with IPH at the dosages mentioned above also resulted in a similar decrease in the values of serum LPO, LDH and CK.

A similar trend was observed with fish oil treatment at the dose of 0.15 ml for different periods of treatment and then induction of MI with IPH at the doses of 60 mg/kg body weight and 80 mg/kg body weight.

15 days period of treatment had no significant effect on serum LPO, LDH and CK values when compared to animals treated with IPH alone.

30 days treatment period showed a moderate but not highly significant reduction in the serum values of LPO, LDH and CK when compared to IPH treated animals.
45 days treatment period and 60 days treatment period showed significant reduction in the values of serum LPO, LDH and CK values when compared to IPH treated animals at both doses of IPH i.e., 60 mg/kg body weight and 80 mg/kg body weight.

A careful observation of the above results suggest that the fish oil offers protection at 45 days and 60 days treatment periods i.e., 45 days treatment period is the minimum and effective period for cardio protection. Therefore we have decided to carry out further studies at this treatment period.

As for the effective dosage of fish oil we studied the values of serum LPO, LDH and CK at the three dosages i.e., 0.05 ml per day, 0.1 ml per day and 0.15 ml per day for 45 days period of treatment and subsequent induction of MI with IPH at two dosages i.e., 60 mg/kg body weight and 80 mg/kg body weight. The values of serum LPO, LDH and CK were comparatively low at 0.05 ml of fish oil per day with gradual rise in these values at the 0.1 ml and 0.15 ml of fish oil per day doses. Hence, 0.05 ml of fish oil/day was taken for the present study.

After 45 days pre treatment with various doses of fish oil, two different doses of IPH i.e., 60 mg/kg body weight and 80 mg/kg body weight were given and the serum values of LPO were observed. It was found that animals treated with 80 mg IPH/kg body weight showed higher values of LPO at all the doses of fish oil than animals treated with 60 mg IPH/kg body weight. Hence, 60 mg IPH/kg body weight was taken as an effective dose to induce MI with low LPO levels.
Lipid peroxides are believed to inhibit the production of prostacyclin (Gwenda Mark, 1994) this explains why fish oil at increasingly higher doses offers lesser protection. The n-3 fatty acids namely EPA & DHA of fish oil being highly unsaturated fatty acids which gets incorporated in the membrane phospholipids offer protection only at low doses. Myocardial protection with fish oil containing 0.06 gm/kg per day of eicosapentaenoic acid for 6 weeks in a canine model was reported by Helgi J Oskarsson et al. (1993). Our study confirms their finding since 0.05 ml of mehaden fish oil also contains approximately the same amount of EPA. Therefore based on the results observed we carried out further studies at the dose of 0.05 ml of fish oil for 45 days pre treatment period and 60 mg/kg body weight of isoproterenol.