

## Dietary fish oil as hepatoprotective agent in *Mus musculus*

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The administration of galactosamine in omega-3 polyunsaturated fatty acid (PUFA) supplemented mice resulted in lesser amount of damage in the liver tissue compared to the mice without prior supplementation of fish oil. Only 50% elevation in the plasma total bilirubin was detected in omega-3 supplemented mice injected with galactosamine over those supplemented with omega-3 without galactosamine injection. The results suggest that very long chain omega-3 PUFA like eicosapentaenoic and docosahexaenoic acid may act as preventive agent for hepatic cirrhosis in *Mus musculus*.

**Keywords:** Cirrhosis, Fish-oil, Galactosamine, PUFA

Lipid metabolism in animals can no longer be considered to be simply a matter of dietary fatty acids. Dynamics of membrane structure and function depend on the complex role of lipid and help the organisms to adapt to a new environment<sup>1</sup>. The interrelationship between the dietary fatty acids, membrane fluidity, membrane integrity and metabolic pathways in animals are evident. The state of lipid in animal is thus in a constant flux. Polyunsaturated fatty acids (PUFA), mainly omega-3 and omega-6 fatty acids are very essential for growth and development and also for the regulation of the cellular functions in the animals<sup>2,3</sup>. However, these two series of fatty acids work in antagonistic fashion<sup>4,5</sup> and hence the dietary ratio of omega-6 and omega-3 fatty acid is an important indicator to keep the animals in well being state<sup>6</sup>.

PUFA reduces mucosal damage as assessed by biochemical and histological examination of inflammation, thus exhibiting anti-inflammatory properties<sup>7</sup>. Moreover, the defense or antioxidant system is enhanced with the dietary intake of omega-3 PUFA<sup>8</sup>. Simultaneous supply of both omega-3 and omega-6 PUFA can be beneficial to improve the fatty acid status<sup>9</sup>. One of the reasons for decreased plasma levels of docosahexaenoic acid in both alcoholic and non-alcoholic liver cirrhosis was found to be due to the impaired omega-3 fatty acid in the cirrhotic liver<sup>10</sup>. Guarini *et al.*<sup>11</sup> reported a significant increase in membrane cholesterol, plasmatic and saturated fatty acids in the patients suffering from liver cirrhosis. The

severe liver diseases are associated with lower percentages of plasma fatty acids of omega-3 and omega-6 series. Long chain PUFA deficiency is often observed in patients with advanced liver cirrhosis<sup>12</sup>. Habitual fish intake may protect hepatic encephalopathy<sup>13</sup>. PUFA are known for their effect against the fighting liver cirrhosis. However, the route, dosage and safety of PUFA supplementation in these patients need extensive investigation<sup>14</sup>. In the present paper an attempt has been made to find out whether the dietary long chain omega-3 PUFA like eicosapentaenoic and docosahexaenoic acid can prevent the liver cirrhosis induced by galactosamine in the laboratory animal like *Mus musculus*.

### Materials and Methods

The necessary permission for the use of experimental animals was obtained from the animal's ethics committee of Goa University. The albino mice, *Mus musculus*, of uniformed weight group ( $22 \pm 0.5$  g) were selected for the experiment. The animals were first divided into two groups of 12 each. One group were supplemented with Max EPA oil capsules (Marketed by M/s., E. Merck India) at a dose of ½ capsule per mouse (0.5ml of oil) for 30 days along with the normal commercial feed and water. This oil capsule contains the fish oil enriched with long chain omega-3 polyunsaturated fatty acids like, eicosapentaenoic acid (180 mg) and docosahexaenoic acid (120mg) per g of fish oil along with other saturated, mono-unsaturated and omega-3 and omega-6 PUFA. While the other group was maintained with only the commercial feed and water. Six mice from both the groups were intraperitoneally injected with

galactosamine (0.5 mg/g body weight in 1.5 ml of physiological saline) for two consecutive days<sup>15</sup> to develop the liver cirrhosis. Rest of the mice received only saline injection. After a gap of two days, the blood samples were collected using heparinized syringe (for plasma) or non-heparinized syringe (for serum). The liver tissues from mice were also removed and washed properly in phosphate buffer saline and stored in formalin for histopathology. Besides, a group of mice were also supplemented with 0.5 ml of sunflower oil (enriched with omega-6 PUFA) along with the food and water and maintained for 30 days. Plasma protein, urea and total and direct bilirubin were measured using routine analytical methods<sup>16</sup>. Besides, the activities of serum alkaline phosphatase (SALP, EC 3.1.3.1), serum glutamate pyruvate transaminase (SGPT, EC 2.6.1.2), and serum glutamate oxaloacetate transaminase (SGOT, EC 2.6.1.1) were estimated following the methods described by Godkar<sup>16</sup>. After fixing the tissue, the paraffin block was prepared. Uniform sections (10 µm thick) of liver were prepared using the cryo microtome (at 4°C) and slides were stained with hematoxylin and eosin. Results were tabulated as mean values of 6 observations and their SE. The obtained data were treated with Student's *t* test and one way ANOVA for comparing the results among various groups<sup>17</sup>.

## Results and Discussion

The importance of dietary intake of fatty acids in health and diseases is well known. Healthy cellular membrane perform several important physiological functions including the free exchange of waste products, nutrients and electrolytes in and out of the cell, structural ability of the cell membrane and reception of cellular messenger for cell to cell communication and recognition. Immune function, inflammatory response and platelet aggregation were greatly affected in rat when the animals were supplemented with 1% cuttlefish liver oil enriched

with eicosapentaenoic and docosahexaenoic fatty acids<sup>18</sup>. About 100% increase ( $P < 0.01$ ) in the plasma urea concentration along with 10-fold augmentation ( $P < 0.01$ ) of the plasma total and direct bilirubin and 20% decrease ( $P < 0.05$ ) in the plasma protein concentration in mice, *Mus musculus*, after receiving the galactosamine injections at a dose of 0.5 mg/g body weight for two consecutive days (Fig. 1) over the control group indicate changes in the metabolism causing damages to the liver. These plasma biochemical changes also reflected in the activity of liver function enzymes like SGOT, SGPT and SALP (Table 1). Anandan *et al.*<sup>19</sup> reported similar kind of changes due to galactosamine injection in the rat, *Ratus norvegicus*. Besides, the histological observation of the liver tissue confirmed the induction of liver cirrhosis due to galactosamine injection. Increase in the number of clusters of inflammatory cells, ballooning, hemorrhage and congestion was noticed in the cross section of liver of treated mice over the same of control mice (Fig. 4). It is reported that due to impaired absorption of vitamin K, leading to prolonged coagulation time, formation of 'purpura', a tendency to hemorrhage, takes place<sup>20</sup>.

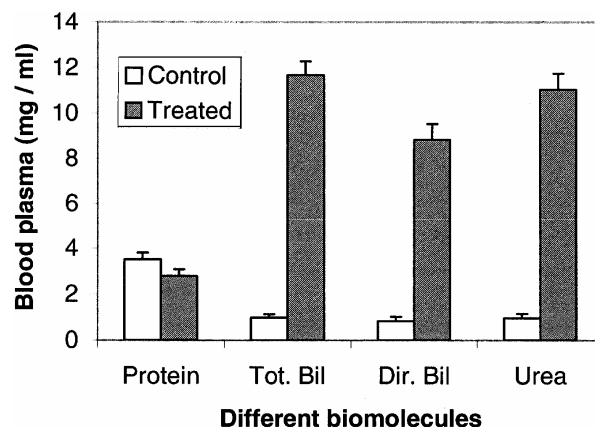


Fig. 1 — Changes in some biochemical parameters of blood plasma in liver cirrhosis induced *M. musculus* by galactosamine. [Values are mean  $\pm$  SE from 6 observations].

Table 1 — Changes in the activity of some liver functions enzymes. [Values are mean  $\pm$  SE from 6 observations in each group]

Enzyme	Control mice	Galactosamine injected mice	Max EPA supplemented mice	Sunflower oil supplemented mice	Max EPA supplemented & galactosamine injected mice
SLP (IU/ min/ mg of protein)	17.31 $\pm$ 2.1	30.53 $\pm$ 2.3 <sup>a</sup>	20.36 $\pm$ 2.47	16.34 $\pm$ 2.17 <sup>b</sup>	23.74 $\pm$ 1.38 <sup>a</sup>
SGOT (K/ min/ mg of protein)	117.25 $\pm$ 6.12	236.55 $\pm$ 8.35 <sup>a</sup>	90.47 $\pm$ 2.87 <sup>a</sup>	98.02 $\pm$ 1.58 <sup>ab</sup>	86.62 $\pm$ 4.85 <sup>a</sup>
SGPT (K/ min/ mg of protein)	13.77 $\pm$ 2.1	30.53 $\pm$ 2.3 <sup>a</sup>	12.64 $\pm$ 1.44	15.22 $\pm$ 1.87	11.72 $\pm$ 1.02

<sup>a</sup>The changes over the control group are statistically significant ( $P < 0.01$ ).

<sup>b</sup>The change over the Max EPA supplemented group is statistically significant ( $P < 0.05$ ).

Dietary supplementation of omega-3 polyunsaturated fatty acids in the form of 0.5ml of Max EPA or of omega-6 polyunsaturated fatty acids in the form of 0.5ml of sunflower oil for 30 days leads to 18-35 % enhancement ( $P < 0.01$ ) in plasma protein

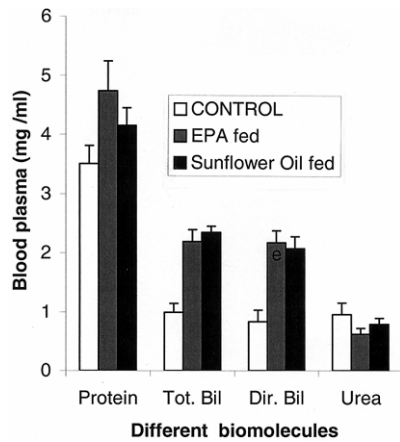


Fig. 2 — Effect of dietary supplementation of Max EPA and Sunflower oil in some biochemical parameters of blood plasma on *M. musculus*. [Values are mean  $\pm$  SE from 6 observations].

concentration and 17-35% decrease ( $P < 0.005$ ) in plasma urea concentration (Fig. 2) and 15-20% reduction ( $P < 0.05$ ) of SGOT activities (Table 1) which in turn indicate the better health status of the mice due to dietary supplementation of polyunsaturated fatty acid irrespective of their composition. Several other workers working on different animal model system<sup>21</sup> have reported

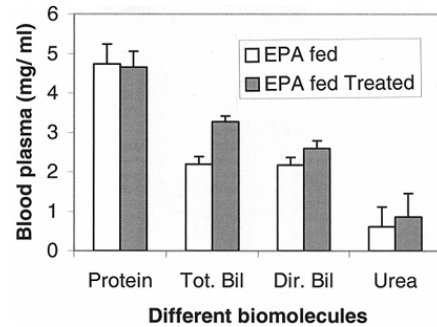


Fig. 3 — Effect of galactosamine injection for induction of liver cirrhosis in Max EPA supplemented *M. musculus*. [Values are mean  $\pm$  SE from 6 observations].

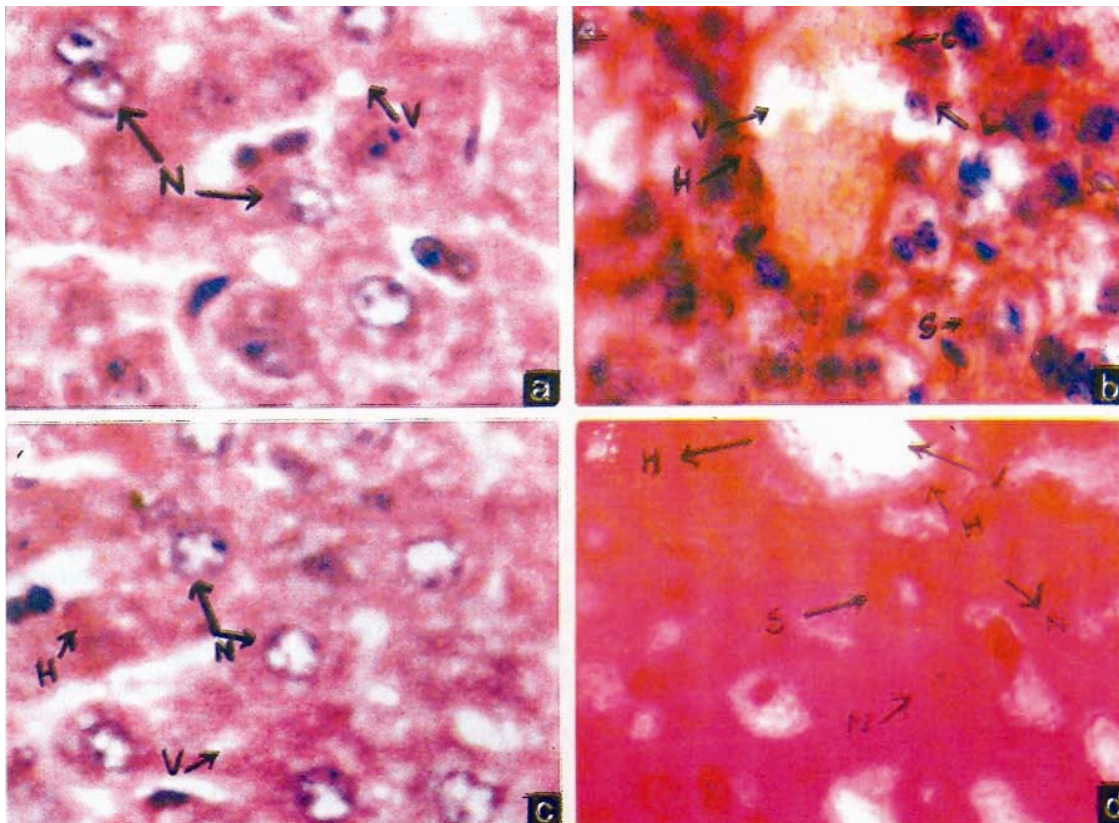


Fig. 4 — T. S. of liver tissue of *M. musculus* (a) Control mouse, (b) galactosamine injected mouse, (c) Max EPA supplemented mouse but no galactosamine injection, (d) Max EPA supplemented and galactosamine injected mouse. [C= congestion, H= hemorrhage, L= lysed cell, N= normal hepatocyte, S= swollen cells, V= vein; oil immersion, 100 $\times$ ].

similar results. However, about 2.5 fold increase in the plasma bilirubin, ( $P < 0.01$ ) level indicates some metabolic changes leading to damage in the liver by prolonged intake of polyunsaturated fatty acid. Castillo *et al.*<sup>22-24</sup> reported that omega-3 enriched PUFA is much more beneficial than omega-6 enriched PUFA to keep the animals in healthy state. Present results once again confirm the beneficial effect of fish oil (omega-3 PUFA) over the sunflower oil (omega-6 PUFA).

Pre treatment with Max EPA results in only 50% elevation ( $P < 0.01$ ) of plasma total bilirubin concentration and non significant changes in plasma protein, plasma direct bilirubin and plasma urea concentration in *M. musculus* due to injection of galactosamine for 2 consecutive days (Fig. 3). These elevations are comparatively lower than that of the mice injected with galactosamine without any pre-treatment of Max EPA. Besides, pre treatment with Max EPA does not show any changes in the activity of SGOT, SPT and SALP (Table 1). The damage caused by galactosamine in liver like, ballooning, hemorrhage congestion and inflammation reduced with the pre-treatment of Max EPA (Fig. 4). The protective effect of Max EPA may be due to depletion of reduced glutathione that results in enhanced lipid peroxidation and protects the mitochondrial membrane from damaging action of oxidase<sup>19</sup>. The present results suggest a hepato-protective effect of Max EPA in experimental animal like *Mus musculus*. However, the effective dose of Max EPA, the duration of dietary supplementation and the molecular mechanisms of the protection need to be investigated thoroughly.

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