Effects of dried fish on antioxidant levels in rat liver

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Short-term feeding studies were carried out to investigate the effect of ingestion of salted dried fish on alterations in tissue lipid peroxidation and modulation of the activities of detoxification enzymes in liver in order to study the induction of oxidative stress. Rats were fed diets with either 5, 10 and 20% dried mackerel for 4 weeks and levels of antioxidants in liver were estimated. The results showed that the fish intake at 10 and 20% dietary level reduced glutathione with a reciprocal increase in thiobarbituric acid reactive substances and a concomitant decrease in antioxidant vitamins A and C contents in liver. A significant decline in the activities of hepatic glutathione peroxidase and glutathione reductase were also observed at these levels of fish consumption. Kidney \(\gamma\)-glutamyl transpeptidase activity on the other hand was increased abnormally at 20% fish intake. The results suggested that the dried fish consumption at higher concentrations (at 10 and 20%) for a short period caused lowering of the activities of antioxidative enzymes thereby inducing oxidative stress in rat liver.

Salted dried mackerel, prepared by treating gutted fish with sea salt and drying under sunlight, is widely consumed in the coastal regions of India especially in seasons when the catch becomes low. The salt cured fish is very rich in biogenic amines. In presence of nitrite used as a preservative/contaminant or formed from nitrate in the body, the amines have been shown to form mutagens and carcinogens. Kumar et al. observed that lipid oxidation along with browning was the main cause of spoilage of salt-dried mackerel. There are reports of toxic effects of long term consumption of salted fish in man and animals. Nasopharyngeal cancers have been found commonly in people of Southern China who regularly consumed salted fish. However, data on the effect of consumption of salted dried fish on oxidative stress as a possible source of toxicity in man or animals are lacking. It was, therefore, felt worthwhile to carry out feeding studies in rat model using salted dried mackerel, generally consumed by people in southern parts of India. Earlier studies revealed cytotoxicity in rats on short-term feeding of salted dried mackerel. This communication describes alterations in tissue lipid peroxidation and modulation of biotransformation/detoxification enzymes in liver of rats fed salted-dried mackerel for four weeks.

Materials and Methods

Diet and animals—Salted dried mackerel (Rastrelliger kanagurta cuvier) was purchased locally. The dried fish, about three months old, freed from heads and fins was washed, cooked in boiling water for 2 min and deboned. The flesh portion was dried at 50°C to a moisture content of 5% and powdered. The powdered fish was sealed in paper-aluminium foil-polyethylene pouches and stored at -20°C until used for incorporation in diets for feeding of animals.

Male Wistar rats, bred in the laboratory and weighing 150-180g, were randomly divided into 4 groups. Group I rats served as control and were fed ad libitum on a standard casein diet for 4 weeks (Table 1). Groups II, III and IV were fed ad libitum on fish diets, that is, casein diet in which corn starch was partly

<p>| Table 1—Composition of the diet (g/100g) |</p>
<table>
<thead>
<tr>
<th>Composition</th>
<th>Control diet (g)</th>
<th>Experimental diets (g)</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>24.0</td>
<td>24.0</td>
<td>24.0</td>
<td>24.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Corn starch</td>
<td>65.8</td>
<td>60.8</td>
<td>55.8</td>
<td>45.8</td>
<td>45.8</td>
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<tr>
<td>Vitamin mixture</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>(USP XIV)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL Methionine</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Groundnut oil</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Shark liver oil</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Fish, salted and dried</td>
<td>0.0</td>
<td>5.0</td>
<td>10.0</td>
<td>20.0</td>
<td></td>
</tr>
</tbody>
</table>

*USP: United States Pharmacopia XIV (1950)
substituted with dried fish to the level of 5, 10 and 20% respectively and fed for the same period. At the end of the experimental period, rats were sacrificed under mild anaesthesia (nembutal, ip at 50 mg/kg body weight) and liver and kidney were quickly excised and frozen in liquid nitrogen until analysed.

Chemical analyses—Lipid content and peroxide value of diets were estimated according to standard AOAC procedures. Lipid peroxidation in the liver was determined as thiobarbituric acid reactive substances (TBARS) using the method of Girotti and Deziel. Hepatic glutathione (GSH), vitamin A, ascorbic acid and total tocopherols, catalase activity, glutathione peroxidase (GSH-Px) and glutathione reductase (GSSGR) activities and glutathione S-transferase (GST) activity and kidney γ-glutamyl transpeptidase activity were estimated.

Statistical analysis—Data are presented as mean ± SD for 6 rats per group.

Results and Discussion

The purpose of the study was to gain an insight into the oxidative and antioxidative forces acting on the hepatic tissues by examining the modulation of bio-transformation/detoxification enzymes and the associated alterations in the precursors/metabolites caused by short-term feeding of rats with salt cured mackerel. Analyses of results revealed interesting findings.

The data on the hepatic glutathione levels revealed that it remained unchanged when the fish content was 5%, however, it showed a significant reduction to more or less half the value of the control, when the dietary fish content was raised from 5 to 10 and then to 20% (Table 2). This was followed by a reciprocal rise in the TBARS, when the incorporation of the fish in the diet was above 5%; the increase in the values reached almost twice that of the control in the 20% fish group. GSH represents an important defence mechanism and its role as the main intracellular interceptor of reactive electrophiles in protecting the cells against oxygen free radicals is well-established. Depletion of hepatic glutathione in vivo enhances the susceptibility of tissues to lipid peroxidation; on the contrary, its presence in excess scavanges the electrophilic moieties produced by xenobiotics by conjugation to lesser toxic products.

The rise in TBARS in liver could be ascribed to the higher intake of lipid peroxides from the fish. Dried salted fish contains higher amount of lipid oxidation products. Ingestion of oxidised lipids promotes peroxidation of membrane lipids in liver. Rise in TBARS is considered to be the indicator of the onset of oxidative stress from reduced species of molecular oxygen including hydrogen peroxide, superoxide radical and reactive hydrogen radical. It appears from these data that the higher consumption of salted dried fish (at or above 10% level) has adversely affected the ability of the animals to maintain hepatic GSH within control levels, thereby predisposing the tissues to oxidative stress.

The antioxidant vitamins determined in the liver indicated a steady decrease in the levels of vitamin A and C with the increase in the fish content of the diet, however vitamin E level did not change much (Table 2). The decrease in vitamins A and C became significant only at 20% level of fish intake. Ascorbic acid is the principal antioxidant in extracellular fluids and traps peroxyl radicals before they can initiate lipid peroxidation. Vitamin E has also been shown to be an efficient inhibitor of lipid oxidation in vivo and in vitro and a major antioxidant in cell membranes. It reacts with and neutralises peroxyl radicals generated from poly unsaturated fatty acids (PUFA). Thus, these vitamins have a role in preven-

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>GSH (nmol/g)</th>
<th>TBARS (nmol/g)</th>
<th>Vitamin C (mg/g)</th>
<th>Vitamin E (mg/g)</th>
<th>Vitamin A (IU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.12 ± 0.012</td>
<td>1.24 ± 0.12</td>
<td>2.67 ± 0.47</td>
<td>18.03 ± 1.07</td>
<td>58.58 ± 5.92</td>
</tr>
<tr>
<td>Fish</td>
<td>0.14 ± 0.020</td>
<td>1.22 ± 0.12</td>
<td>2.83 ± 0.40</td>
<td>18.72 ± 1.70</td>
<td>55.23 ± 7.26</td>
</tr>
<tr>
<td>5%</td>
<td>0.04 ± 0.013</td>
<td>2.06 ± 0.117</td>
<td>2.32 ± 0.45</td>
<td>19.21 ± 1.16</td>
<td>53.89 ± 5.55</td>
</tr>
<tr>
<td>10%</td>
<td>0.07 ± 0.011</td>
<td>2.31 ± 0.117</td>
<td>1.97 ± 0.40</td>
<td>18.67 ± 1.03</td>
<td>46.99 ± 5.88</td>
</tr>
<tr>
<td>20%</td>
<td>0.07 ± 0.011</td>
<td>2.31 ± 0.117</td>
<td>1.97 ± 0.40</td>
<td>18.67 ± 1.03</td>
<td>46.99 ± 5.88</td>
</tr>
</tbody>
</table>

Note: Values not sharing the same superscript in the column are significantly different (P<0.05).
Table 3—Effects of fish on hepatic GSH and antioxidant/detoxifying enzyme activities in liver and kidney

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH-Px units*</th>
<th>GSSGR units*</th>
<th>GST units**</th>
<th>GGT units†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.39 ± 0.45a</td>
<td>0.48 ± 0.05a</td>
<td>16.19 ± 2.46a</td>
<td>1.0 ± 0.05a</td>
</tr>
<tr>
<td>Fish 5%</td>
<td>2.40 ± 0.41a</td>
<td>0.50 ± 0.06a</td>
<td>17.60 ± 2.61ab</td>
<td>1.3 ± 0.03b</td>
</tr>
<tr>
<td>Fish 10%</td>
<td>1.89 ± 0.43b</td>
<td>0.39 ± 0.05b</td>
<td>17.41 ± 2.28ab</td>
<td>1.3 ± 0.05b</td>
</tr>
<tr>
<td>Fish 20%</td>
<td>1.78 ± 0.29b</td>
<td>0.40 ± 0.06b</td>
<td>19.47 ± 2.50b</td>
<td>5.35 ± 0.18b</td>
</tr>
</tbody>
</table>

*μ moles NADPH oxidized/min/g liver
**μ moles of conjugate formed/min/g liver
†μ mole p-nitroanilide formed/min/g kidney
ab(Values not sharing the same superscript in the column are significantly different (P<0.05)

tion of oxidation of PUFA. (Vitamins A, E and C in the fish were estimated to be 12 μg/g, 40 mg/g and 1.85 mg/100g respectively. These levels seem to be highly insufficient in inhibiting the oxidation of PUFA present in the fish as seen from the TBARS and PV content (TBARS: 651 x 10^2, 129 x 10^2, 257 x 10^2 % PV; 183, 262 and 433% of control in 5, 10 and 20% fish supplemented diets). The decreased hepatic levels of vitamins A and C observed in the present study could be the result of increased demand for cellular antioxidants due to over-production of reactive oxygen species and/or decreased efficiency of free-radical scavenging systems. The lack of observed change in hepatic α-tocopherol content could be the result of recycling of tocopheroxyl radical to tocopherol achieved by reaction with ascorbic acid as noted by Tappel. In this process ascorbic acid is thought to be utilised in liver of rats exposed to higher amount of fish diets and thus gets depleted. The α-tocopherol thus regenerated is able to offset its increased utilisation and maintain its level in the liver.

Effect on biotransformation/detoxifying enzymes in liver

Analysis of glutathione-related enzymes indicated lowering of the activity of glutathione peroxidase and glutathione reductase in the liver of rats in 10 and 20% fish groups as compared to the controls (Table 3). The reduced GSH-Px activity is perhaps due to a lowered GSH content in liver and could be an adaptive mechanism attempting, though unsuccessfully, to maintain GSH level in liver. The decline of GSH-Px activity leads to inefficient disposal of peroxides and reduced formation of oxidised glutathione. The latter phenomenon is attributed to the drop in GSSGR activity observed at higher intakes of salt-cured fish. This enzyme system works in association, in the regulation of cellular GSH concentration and modulates the detoxification reaction.

Another important enzyme system identified with detoxification processes is glutathione transferases. In the present study hepatic GST has not altered in any significant extent in its activity, when the animals ingested varying levels of fish in the diet (Table 3). GSTs are a group of enzymes that catalyse the binding of various electrophiles to GSH to form conjugates which are rapidly eliminated by excretion. They are thought to play a major role in the detoxification of xenobiotics. Regulation of GSH levels in vivo must be looked into in terms of the entire organism while liver synthesizes and exports large amounts of GSH into the blood, the kidney takes up GSH from the plasma and breaks it down via glutamyl transpeptidase reaction. Gama glutamyl transpeptidase determined in the kidney homogenate was found to increase more than five-folds in the animals fed 20% fish diet compared to the control fed the fish-free diet (Table 3). Abnormally high levels of GGT in cells is an indicator of a greater xenobiotic metabolism. It is considered as a marker of preneoplastic lesions and cytotoxicity. GSTs and GGTs are multifunctional enzymes that perform several roles in the detoxification of a broad spectrum of electrophilic reactive substances, drugs, carcinogens and metabolites.

The oxidative damage of tissues is an important mechanism that contributes to degenerative diseases such as cardiovascular diseases, cataract and cancer. However, cells have evolved a number of protective mechanisms to reduce the damage by prooxidants. Enzymes such as catalase, superoxide dismutase, glutathione peroxidase/reductase system, glutathione-s-
transferase etc, all function to inactivate potentially damaging prooxidants. The major responsibility of cellular protection against oxygen mediated toxicity rests upon modulation by these enzyme systems.

Notwithstanding the protective mechanism existing in the cell against chemical toxicity in situ, a tilt in the balance in favour of the deactivating forces caused by salted fish might have lead to appearance of toxic symptoms.

The results of the present study demonstrate that high intake of salt-dried mackerel for short duration induced lipid peroxidation in vivo exposing the animals to oxidative stress leading to toxicity. This was evidenced by the depletion of thiols and antioxidant vitamins in liver and reduction of activities of glutathione related detoxification enzymes in liver and an abnormal rise in kidney GGT. This observation is important from the point of view of nutrition and health of certain section of the population and calls for an in depth study.

Acknowledgement
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References
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