8. EFFECT OF E. MACULATUS EXTRACT ON THE
HEPATOPROTECTIVE AND ANTIOXIDANT PROPERTIES
IN RATTUS NORVEGICUS

8.1 Introduction

The use of animals or its parts in the preparation of drug / lehyam
(sweet paste) for treating human ailments were known and practiced
traditionally. Many animals have been studied and used for the preparation
of drugs. Marine animals such as starfish, sponges and corals were studied
for anti-tumor activity. In class Osteichthyes (Bony fishes), Cod and
Halibut are important for their liver oils which contain Vitamin A& D and
Eicosapentaenoic acid (dietary supplement). In class Amphibia, dried and
powered toad skins contain cardio-active principles and were used for the
treatment of dropsy before the wide spread adoption of digitalis (Trease
and Evans, 2002). Digitalis is a drug made from the leaves of Digitatis
purpurea and used as a heart stimulant. Many mammals were also studied
for their medicinal values. But scientific studies on fresh water fishes
regarding medicinal values are still lacking. Generally fish and fish oil are
known for Ω3 fatty acid, which are antioxidant, also cure heart and hepatic
diseases. In order to understand similar pharmaceutical importance, this
study was undertaken. The liver is an organ of paramount importance,
which plays an essential role in the metabolism of foreign compounds
entering into the body. Human beings are exposed to these compounds
through environmental exposure, consumption of contaminated food or
exposure to chemical substances in the occupational environment etc. In
addition, human being consumes a lot of synthetic drugs during their
diseased conditions or for prophylaxis, which are alien to the body organs.
All these compounds produce a variety of effect including toxic
manifestations. The biochemical damage produced by reactive oxygen
species and other free radicals has emerged as a fundamental pathway of
liver injury. One of the functions of the liver is excretion of drugs, toxins,
cholesterol, bile pigments, heavy metals and protectives (B.D. Chawrasia’s, Human Anatomy – 2002). Under certain such condition liver tissue undergoes fibrosis and shrinks. This is called Cirrhosis of the liver. In the laboratory condition to induce liver damage, CCl₄ - a chemical often used in studies for the search of hepatoprotective agents (McCay et al 1984) and D-galactosamine produces liver lesions comparable to those found in viral hepatitis and peroxides. Active oxygen molecules such as the Superoxide radical play an important role in the inflammation process after intoxication by CCl₄ (Slater & Sawyer 1971a, b). These radicals which react with cell membrane and induce lipid peroxidation have been implicated as important pathologic mediators in many clinical disorders (Slater 1984). A major defense mechanism is the antioxidant enzymes especially Superoxide dismutase (SOD), Catalase (Cat) and Glutathione peroxidase (GPx), which convert active oxygen molecules into non-toxic compounds. In such liver damage, in serum the level of liver enzymes are raised and antioxidant levels are reduced to the extent of its control by the antihepatic drug under test is used for estimation.

Conventional drugs used in the treatment of liver disease are often inadequate. It is therefore necessary to search for alternative drugs for the treatment of liver disease to replace the currently used drugs of doubtful efficacy and safety.

India is well known for a plethora of medicinal plants. The medicinal use of many plants as hepatoprotectants, have been reported in the literature. But the use of animal substances, particularly the extract of fresh water fishes for treating liver disorder are lacking. It is known that the rural mass use E. maculatus for treating some diseases related to reproductive disorders. Despite the casual use of E. maculatus for treating the said diseases, no systematic studies have been reported.
To understand the pharmaceutical importance of *E. maculatus* in relation with hepato protective and antioxidant level, present study was undertaken.

8.2 Materials and methods

*E. maculatus* fishes were collected from the Pullumbadi channel and dried in an incubator at 105°C for 4 hours. After that the dried, entire fish was grinded well in a pestle and mortar to make a fine powder and ethanolic extract was prepared.

Uniformly sized 5 to 6 weeks old, (180 to 230 g) *Rattus novergicus* were procured and grouped into five with six animals each. The CCl₄ (1ml /kg ie.0.5ml CCl₄ &0.5ml Olive oil) was administered to all in 4 groups by back sub – cutaneous injection except group - I. Group - I, served as a control, receiving normal saline only (10ml / kg i.p). Group - II served as CCl₄ control group. Group – III and IV received the *E. maculatus* ethanolic extract through oral administration in two divided doses, 50 mg /kg and 100mg / kg respectively and the reference hepatoprotective drug Silymarin (25 mg/ kg i.p) was administered to group – V, daily once for 15 days after CCl₄ administration. All the animals were sacrificed at the end of 15th day after CCl₄ administration, blood was drawn from the carotid artery and serum was separated for different assays.

A. Serum enzymes level
B. Antioxidant level

Histological evaluation

After sacrificing the animals, the liver was rapidly excised and serially sectioned. The hepatic tissues were fixed in 10% buffered formalin and embedded in paraffin by employing the standard technique, 5μm thick sections were cut and stained with hematoxylin - eosin for histological examination.
8.3 Result

The results from the examination of the effects of *E. maculatus* extract on this liver injury model are summarized in Table – 24 and 25. The results indicate that after CCl₄ injection there were significant increases of Aspartate transaminase (AST) Alanine transaminase (ALT) Alkaline phosphatase (ALP), Acid phosphatase (ACP) and Bilirubin, compared with the control group. But the AST, ALT, ALP and ACP levels were significantly decreased (compared with the CCl₄ treated control) when the rats were given *E. maculatus* extract (50mg/kg and 100mg / kg) and Silymarin (Table – 24).

Table – 24. Effects of *E. maculatus* extract on Bio – chemical parameters in the serum of *Rattus norvegicus*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
<th>ACP (U/L)</th>
<th>Bilirubin (mg / 100ml of blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Nacl)</td>
<td>97.3 ± 1.18</td>
<td>35.08±0.2</td>
<td>15.92±0.72</td>
<td>10.5±0.064</td>
<td>0.39±0.04</td>
</tr>
<tr>
<td>CCl₄ treated control</td>
<td>186.7±1.82</td>
<td>136.9±1.94</td>
<td>98.3±7.9</td>
<td>38.6±2.9</td>
<td>0.89±0.76</td>
</tr>
<tr>
<td>1 ml / kg i.p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish extract 50 mg / kg</td>
<td>138.2±1.96*</td>
<td>79.3±5.9*</td>
<td>68.5±4.8*</td>
<td>21.3±0.78*</td>
<td>0.75±0.06</td>
</tr>
<tr>
<td>Fish extract 100mg/kg</td>
<td>109.6±5.78**</td>
<td>47.5±1.7**</td>
<td>40.5±2.8**</td>
<td>15.6±0.19**</td>
<td>0.58±0.04</td>
</tr>
<tr>
<td>Silymarin (25 mg / kg)</td>
<td>105.3 ±4.3**</td>
<td>49.4±3.6**</td>
<td>34.8 ±2.9**</td>
<td>16.2 ±1.2*</td>
<td>0.24 ±0.03*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E., n = 6  *P < 0.01.  **P < 0.001.
Table – 25. Effect of *E. maculatus* extract on the Antioxidant enzymes level in *Rattus norvegicus*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glutathione peroxidase (mg liver protein)(^{-1})</th>
<th>SOD (mg liver protein)(^{-1})</th>
<th>Catalase (mg liver protein)(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Nacl)</td>
<td>0.992±0.05</td>
<td>75.81±1.94</td>
<td>296.83±10.05</td>
</tr>
<tr>
<td>CCl(_4) treated control (1ml /kg i.p)</td>
<td>0.61±0.03</td>
<td>47.84±0.50</td>
<td>179.73±5.78</td>
</tr>
<tr>
<td>Fish extract 50 mg /kg</td>
<td>0.85±0.06*</td>
<td>68.93±0.94*</td>
<td>258.52±5.82*</td>
</tr>
<tr>
<td>Fish extract 100mg /kg</td>
<td>0.96±0.05*</td>
<td>88.73±0.79*</td>
<td>282.48±4.92*</td>
</tr>
<tr>
<td>Silymarin (25mg/kg)</td>
<td>0.95±0.03*</td>
<td>88.34±2.54*</td>
<td>268.27±6.46*</td>
</tr>
</tbody>
</table>

Data are expressed as Mean ± S.E., n = 6  *P < 0.01.

The activity of the antioxidant enzymes such as SOD, Glutathione peroxidase and Catalase were measured in the control and found a significant decrease in the antioxidant enzymes level in the CCl\(_4\) treated group, whereas the decrement was considerably recovered in the treatment of the extracts of *E. maculatus* and silymarin groups (Table-25).

Histopathological observations elucidates an histoarchitectural modulations of Massive fatty change, gross necrosis, broad infiltration of lymphocytes and Kupffer cells around the central vein and loss of cellular boundary in the livers of CCl\(_4\) - treated rats (Plate-20 and 20a).
Such damage were considerably recovered in the livers of rats administered with *E-maculatus* extract (50mg/kg and 100mg/kg) and silymarin (Plate-21,22 and 23).

8.4 Discussion

Reactive oxygen species (ROS) such as Superoxide, hydroxyl radical, iron – oxygen complexes, hydrogen peroxide are considered as cytotoxic agents (Jamieson, 1989) because of their ability to induce lipid peroxidation within the cell membrane (Jones et al., 1979). ROS can be used for the purpose of intracellular signaling (Schreck et al., 1992). They are generated by several reactions; including metabolism of triplet oxygen molecules; one – electron reduction of oxygen; catalytic decomposition of hydrogen peroxide and lipid hydroperoxides by metal ions, attack of metal or of metal – oxygen complexes; light and x-ray irradiation; intake of exogenous radicals (Fridovich, 1976).

These radicals react with biological molecules such as DNA, proteins and phospholipids and eventually damage membranes and other tissue (Vuillaume, 1987). Aerobic organisms employ a battery of defense mechanisms such as antioxidant enzymes (Superoxide Dismutase, Catalase and Glutathione- Peroxidase) to prevent or mitigate oxidative tissue damage (Halliwell and Gutteridge, 1989). Superoxide dismutase removes the superoxide radical to prevent formation of the hydroxyl radical. Catalase deals especially effectively with the large amounts of hydrogen peroxide generated in the peroxisomes. Glutathione -peroxidase is capable not only of utilizing hydroperoxides but also of metabolizing hydrogen peroxide in both the cytosolic and mitochondrial compartments. At other sites, intake of compounds which induce antioxidant enzyme activity or scavenge free radicals, both prevent oxidative damage (Hochstein and Atllah, 1988).
Hepatic cells appeared to participate in a variety of enzymatic metabolic activities and CCl₄ produced marked damage at the given doses as expected (Roderick et al; Kenneth et al., 1992).

CCl₄ is metabolized by the mixed function oxidase (MFO) system in the endoplasmic reticulum of the liver. Cleavage of the carbon -chloride bond results in the formation of free trichloromethyl radicals (CCl₃) which are highly unstable and thus immediately react with membrane components (Recknagel and Glende, 1973). They form covalent bonds with unsaturated fatty acids or abstract a hydrogen atom from the unsaturated fatty acids of membrane lipids, resulting in the production of chloroform and lipid radicals which react with molecular oxygen; this initiates peroxidative decomposition of phospholipids in the endoplasmic reticulum. The peroxidation process results in the release of soluble products that affect other membranes, such as cell membranes. (Pecker et al 1978). It has been found that microsomal oxidation of chloroform results in the formation of phosgene (colourless poisonous gas such as carbonyl chloride). It is thought that a secondary metabolite causes cell death (Shah, et al 1979). Thus protective agents against CCl₄ induced liver injury exert their action by impairing CCl₄ mediated lipid peroxidation by inhibiting the generation of free radical derivatives (Castro et al 1974) or as a result of antioxidant activity of the protective agent itself. (Yasuda et al 1980).

In the current work, histological changes observed in the CCl₄ treated group (Plate-20 and 20a) were fatty change, cell necrosis, lymphocyte infiltration and increase in Kupffer cells. Treatment with increasing doses of *E- maculatus* extract (Plate-21 and 22), reduced the fatty change, cell necrosis, lymphocyte infiltration and Kupffer – cell proliferation. Also of interest was that treatment with *E- maculatus* extract reversed the decrease in rat liver Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Catalase (Cat) activity induced by CCl₄ treatment.
with 50mg/kg and 100mg/kg of *E-maculatus* extract resulted in levels that were higher than that of CCl₄ treated control group (Table 25). On the basis of the results the activities of all the antioxidant enzymes were declined by the treatment with CCl₄, results demonstrated that *E-maculatus* extract restore the activity of Glutathione peroxidase, Superoxide dismutase and Catalase.

Rats treated with CCl₄ developed significant hepatic damage as observed from elevated serum enzyme (AST, ALT,ALP&ACP) levels (Table - 24) and significant decreases in the antioxidant enzymes (Table - 25). The extract of *E.maculatus* 50 mg/kg and 100mg/kg produced a significant reduction in Carbon tetra chloride induced increases in serum enzyme levels and also significant decreases in the antioxidant enzymes.

Histopathological studies of the liver in the CCl₄ treated shows fatty change, cloudy appearance(Plate-20a), lymphatic infiltration, necrosis and increase in Kupffer cells (plate-21).In the treated groups (Group – II and III), necrosis which is more severe form of injury, is markedly prevented (Plate-21,22). with the same effect of Silymarin drug in group-V (Plate 23). So the present study revealed that *E.maculatus* has the ability to protect the liver from diseases. In the traditional Chinese medicine liver disease is thought to be caused by the stagnation of pathogenic damp-heat and liver stasis or invasion of the stomach and spleen by hepatic Qi (sub-state-characterized by accumulation of cells in G1 phase, with lowered cellular RNA content). Patients with liver disease might manifest different syndromes in different phase (Tsai et al, 1997). Therefore, one disease can be treated in different ways. In conclusion, this study has demonstrated that the extracts of *E.macleatus* can be used as a crude hepatoprotective drug.
Plate - 19. Normal control rats: Section of liver shows normal hepatic cells (40X)

Plate: 20 CCL4 treated rats: Section of liver shows fatty degeneration, cloudy appearance, necrosis (NC), increase of Kupffer cells (kf) (40X)
Plate – 20a CCl4 treated rats: Section of liver shows fatty degeneration, increase of kupffer cells (kf) and necrosis (nc) in hepatic cells (40X)

Plate – 21 E.maculatus extract 50mg/kg treated rats: Section of liver shows regaining of normalcy in hepatic cells after 15 days (40x)
Plate 22. *E. maculatus* extract 100 mg/kg treated rats: Section of liver shows regaining of normalcy in hepatic cells after 15 days (40x)

Plate 23. Silymarin treated rats: Section of livers shows normalcy in hepatic cells after 15 days (40x)