Hepatoprotective and Antioxidant effect of *Polycarpaea corymbosa* against CCl₄ induced hepatotoxicity in rats

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**Abstract**

Carbon tetrachloride (CCl₄) intoxicated rats showed significant elevation in serum enzymes, bilirubin and lipid peroxidation of the liver tissues and reduction in serum total protein, superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase activity. Treatment with ethanol extract of *Polycarpaea corymbosa* whole plant altered the above parameters to the levels of near normal. All the above results were comparable with the standard drug silymarin (100mg/kg) treated group. Thus the present study ascertains that the ethanol extract of *Polycarpaea corymbosa* whole plant possesses significant hepatoprotective activity.

**Keywords** *Polycarpaea corymbosa*, Hepatoprotective, ALP, Bilirubin and MAD.

**Introduction**

Liver is a versatile organ of the body that regulates internal chemical environment. Liver injury induced by various hepatotoxins has been recognized as a major toxicological problem for years. Because of its unique metabolic functions and relationship to the gastrointestinal tract, liver is an important target of toxicity to xenobiotics, oxidative stress, ethanol and toxic chemicals (Patel and Shah, 2009).

Inspite of tremendous advances in modern medicine no effective drugs are available, which stimulate liver functions and often protection to the liver from the damage or help to regenerate hepatic cells (Chathopadhyay, 2003). In absence of reliable liver protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite after claimed to after significant relief (Chatterjee, 2000). There are numerous plants and polyherbal formulations claimed to have hepatoprotective activity. Nearly 150 phyto-constituents from 101 plants have been claimed to possess liver protecting activity (Subramanium and Pushpangadan, 1999).

*Polycarpaea corymbosa* (L.) Lam. belongs to “Caryophyllaceae” is commonly known as “Pallipoondu” in Paliyapahal tribals of Sirumalai hills, Western Ghats Tamil Nadu. Paste prepared from the whole plant is taken once in a day for period of 2-3 weeks to treat jaundice by the paliyars (Maruthupandian et al., 2011). However, inspite of traditional use, pharmacology of its whole plant has not yet been explored scientifically. Literature reviews indicated that the hepatoprotective activity of whole plant of *Polycarpaea corymbosa* has not been scientifically evaluated so far. An active and safe drug is...
is needed for the treatment of jaundice. In view of this, the present study was aimed at evaluating the hepatoprotective activity of whole plant of *Polycarpaea corymbosa* against CCl₄ induced hepatotoxicity in rats.

**Materials and methods**

**Plant material**

The *Polycarpaea corymbosa* (L.) Lam were collected from the Agasthiarimalai Biosphere Reserve, Western Ghats, Tamil Nadu.

**Preparation of plant extract for phytochemical screening and hepatoprotective studies**

The whole plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder, which was then subjected to successive extraction extraction in a Soxhlet apparatus using ethanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures (Brinda *et al.*, 1981, Anonymous, 1990 and Lala, 1993). The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for hepatoprotective studies.

**Animals**

Normal healthy male Wistar albino rats (180-240g) were used for the present investigation. Animals were housed under standard environmental conditions at room temperature (25±2°C) and light and dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

**Acute toxicity studies**

Acute oral toxicity study was performed as per OECD - 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD, 2002). The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 and 2000 mg/kg body weight.

**Experimental design**

In the investigation, a total of 25 rats (CCl₄ hepatic toxicity induced rats and 5 normal rats) were taken and divided into five groups of 5 rats each.

Group - I: Rats received normal saline was served as a normal control.

Group - II: CCl₄ hepatic toxicity induced control: Rats received 2.5ml/kg body weight of CCl₄ for 14 days.

Group - III: Liver injured rats received ethanol extract of whole plant of *Polycarpaea corymbosa* at the dose of 250mg/kg body weight for 14 days.

Group - IV: Liver injured rats received ethanol extract of whole plant of *Polycarpaea corymbosa* at the dose of 500mg/kg body weight for 14 days.

Group - V: Liver injured rats received standard drug silymarin at the dose of 100mg/kg body weight for 14 days.

**Biochemical analysis**

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes. Serum protein (Lowry *et al.*, 1951) and serum albumins was determined quantitatively by colorimetric method using bromocresol green. The total protein minus the albumin gives the globulin. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by using the method of Reitman and Frankel (1957). Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong (1934).
Total bilirubin and conjugated bilirubin were determined as described by Balistrei and Shaw (1987). The unconjugated bilirubin concentrations were calculated as the difference between total and conjugated bilirubin concentrations. Gamma-glutamyl transferase (GGT) was estimated by the method of Szasz (1969). Liver homogenates (10% W/V) were prepared in ice cold 10mM tris buffer (pH7.4). Quantitative estimation of MDA formation was done by determining the concentration of thiobarbituric acid reactive substances (TBARS) in 10% liver homogenates by the method of Okhawa et al., (1979). Enzymatic antioxidants, superoxide dismutase SOD (Mishra and Fridowich, 1972) catalase (CAT) (Aebi, 1974 and Colowick, 1984) and non enzymatic antioxidant glutathione peroxidase (GPx) (Pagila and Valentine, 1967) and glutathione reductase (GRD) (Goldberg and Spooner, 1983) were also assayed in liver homogenates.

Statistical analysis
The data were expressed as the mean ± S.E.M. The difference among the means has been analyzed by one-way ANOVA. p<0.05 and p<0.01 were considered as statistical significance using SPSS Software.

Results
The ethanol extract of whole plant of *Polycarpaea corymbosa* subjected for phytochemical study showed the presence of alkaloids, coumarin, glycosides, flavonoids, saponins, steroids, phenols, tannins and xanthoproteins. The ethanol extract did not show any sign and symptoms of toxicity and mortality upto 2000mg/kg dose. Table - 1 shows the body weight of the normal, liver damaged and drug treated rats. The effect of ethanol extract of *Polycarpaea corymbosa* serum total protein, albumin, globulin, A/G ratio, serum transaminases, alkaline phosphatases in CCl₄ intoxicated rats are summarized in Table - 2.

There was a significant (p<0.01) increase in serum GOT, GPT and ALP levels in CCl₄ intoxicated group (Group - II) compared to the normal control group (Group - I). The total protein and albumin levels were significantly (p< 0.01) decreased to 7.11 g/dl and 4.34 g/dl in CCl₄ intoxicated rats from the levels of 8.14g/dl and 4.56 g/dl respectively in normal group. Ethanol extract of *Polycarpaea corymbosa* at the dose of 250 and 500 mg/Kg orally significantly decreased the altered serum marker enzymes and reversed the altered total protein and albumin to almost normal level.

The effect of ethanol extract of *Polycarpaea corymbosa* on total, conjugated, unconjugated bilirubin and gamma-glutamyltransferase is shown in Table - 3. A significant elevation of total, conjugated, unconjugated bilirubin and gamma-glutamyltransferase in the serum of CCl₄ intoxicated group (Group - II) when compared to normal control (Group - I). The ethanol extract of *Polycarpaea corymbosa* at the dose 250 and 500mg/Kg reduced the levels of total, conjugated and unconjugated bilirubin (Group - III and Group - IV). The decreases in the concentration of total bilirubin, conjugated bilirubin, unconjugated bilirubin and gamma-glutamyltransferase were found to be greater in standard silymarin (Group - V) followed by Group - IV and Group III (Table - 3).

The effects of ethanol extract of *Polycarpaea corymbosa* on lipid peroxidation (LPO), Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Catalase (CAT) activity is shown in Table - 4. Lipid peroxidation level was significantly (p< 0.01) increased and glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase activity were significantly (p< 0.01) decreased in CCl₄ intoxicated rats when compared with those of the animals in normal control group. Rats treated with ethanol extract of *Polycarpaea corymbosa* at the doses of 250 and 500 mg/kg significantly decreased the elevated lipid peroxidation levels and restored the altered glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase levels towards the normal levels in a dose
Table - 1. Effect of *Polycarpaea corymbosa* whole plant extract on the body weight of the rats before and after treatment in the normal, liver damaged and drug treated rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Initial Body weight (Gm)</th>
<th>Final Body weight (Gm)</th>
<th>Mean weight Gain (G↑) / loss(L↓) (Gm)</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.9% Saline</td>
<td>198.45±6.34</td>
<td>211.65±6.31</td>
<td>13.2↑</td>
<td>6.65</td>
</tr>
<tr>
<td>Liver damaged Control</td>
<td>0.9% Saline</td>
<td>208.14±9.37</td>
<td>189.21±4.37</td>
<td>18.93↓**</td>
<td>9.09</td>
</tr>
<tr>
<td>Liver Damaged Animal + POW Extract</td>
<td>100(mg/Kg)</td>
<td>193.83±7.19</td>
<td>204.11±5.13</td>
<td>10.28↑</td>
<td>5.30</td>
</tr>
<tr>
<td>Standard Drug (Silymarin)</td>
<td>100(mg/Kg)</td>
<td>201.44±8.15</td>
<td>214.98±7.08</td>
<td>13.54↑**</td>
<td>6.72</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 5 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. * p< 0.05; ** p< 0.01 as compared with Normal Control to liver damaged control: a p< 0.05; aa, p< 0.01 as compared with liver damaged control to drug treated animal.

Table – 2. Effect of *Polycarpaea corymbosa* whole plant extracts on the protein, albumin, globulin concentration and enzyme activity of serum GOT, GPT, and ALP in the normal, liver damaged and drug treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>T.Protein (mg/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>A/G Ratio</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td>8.14±0.16</td>
<td>4.56±0.12</td>
<td>3.58±0.11</td>
<td>1.27:1</td>
<td>11.26±0.31</td>
<td>13.92±0.86</td>
<td>134.61±4.26</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td>7.11±0.15*</td>
<td>4.34±0.21</td>
<td>2.77±0.09*</td>
<td>1.56:1</td>
<td>58.91±2.56*</td>
<td>54.86±1.93**</td>
<td>211.63±7.36*</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td>6.93±0.16</td>
<td>4.21±0.18</td>
<td>2.72±0.11*</td>
<td>1.54:1</td>
<td>24.68±2.93*</td>
<td>20.33±1.08**</td>
<td>163.05±5.11*</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td>7.84±0.26</td>
<td>4.89±0.22</td>
<td>2.96±0.13</td>
<td>1.65:1</td>
<td>14.33±2.36**</td>
<td>13.68±1.68**</td>
<td>131.26±3.91**</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>8.07±0.17</td>
<td>4.55±0.18</td>
<td>3.51±0.74</td>
<td>1.29:1</td>
<td>12.88±0.98**</td>
<td>10.23±0.85**</td>
<td>142.81±4.16*</td>
</tr>
</tbody>
</table>

Each Value is SEM ± 5 individual observations* p< 0.05; ** p< 0.01 Compared normal control vs liver injured rats a: p< 0.05; aa p<0.01 Compared liver injured rats vs drug treated.

Table - 3. Effect of *Polycarpaea corymbosa* whole plant extracts on the serum Total, conjugated and unconjugated bilirubin and GGTP levels in the normal control, liver injured and drug treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Total Bilirubin (µmol/L)</th>
<th>Conjugated (µmol/L)</th>
<th>Unconjugated (µmol/L)</th>
<th>GGTP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td>0.61±0.04</td>
<td>0.21±0.06</td>
<td>0.40±0.04</td>
<td>10.93±0.21</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td>3.05±0.31**</td>
<td>1.29±0.07**</td>
<td>1.76±0.14**</td>
<td>26.23±0.72**</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td>1.94±0.11*</td>
<td>0.75±0.03*</td>
<td>1.29±0.34ns</td>
<td>12.37±0.81*</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td>0.91±0.07**</td>
<td>0.24±0.03**</td>
<td>0.67±0.24</td>
<td>10.44±0.73*</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>0.76±0.06*</td>
<td>0.24±0.07**</td>
<td>0.62±0.05*</td>
<td>8.93±0.13**</td>
</tr>
</tbody>
</table>

Each Value is SEM ± 5 individual observations* p< 0.05; ** p< 0.01 Compared normal control vs liver injured rats a: p< 0.05; aa p<0.01 Compared liver injured rats vs drug treated, ns- Not significant.
The results are well comparable with silymarin (standard drug) treated group.

**Discussion**

Carbon tetrachloride is one of the most commonly used hepatotoxin. It is well documented that carbon tetrachloride is biotransformed under the action of cytochrome P<sub>450</sub> in the microsomal compartment of liver to trichloromethyl radical which readily reacts with molecular oxygen to form trichloromethyl peroxy radical (Raucy et al., 1993). Both the radicals can bind covalently to the macromolecules and induce peroxidase degradation of the membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids (Recknagel et al., 1989). This leads to the formation of lipid peroxides of protein synthesis, elevated levels of serum marker enzymes such as SGPT, SGOT and ALP (Faroon et al., 1994). SGPT is thought to be one of the indices of the degree of cell membrane damage while SGOT is an indicator for mitochondrial damage since mitochondria contains 80% of the enzyme (Dabba and Abdel Rahman, 1998). The increased activity of the liver marker enzymes such as SGPT, SGOT and ALP in the serum of CCl<sub>4</sub> induced rats. Both *Polycarpaea corymbosa* and silymarin treated rats possess significantly lower SGPT, SGOT and ALP levels as compared to CCl<sub>4</sub> treated animals. So in the present study can speculate that the protective effect is on both mitochondria and hepatocytes the normalized levels of the enzymes SGPT, SGOT and ALP after the treatment with *Polycarpaea corymbosa* in CCl<sub>4</sub> intoxicated rats demonstrated its hepatoprotective action.

Increase in serum bilirubin levels may be found in hepatocellular damage, hemolytic jaundice or hepatitis. CCl<sub>4</sub> injury causes significant degeneration of hepatocytes and blockade of the bile ducts which results into significant increase in the serum total bilirubin and direct bilirubin levels (Saraswat et al., 1993). As increased in the levels by CCl<sub>4</sub> intoxication. Pretreatment with ethanol extract of *Polycarpaea corymbosa* whole plant normalized the elevated total bilirubin and direct bilirubin levels.

Since the results obtained for the serum total protein and albumin concentrations followed the same trend, it thus implicated the same mechanism by which the ethanol extracts of *Polycarpaea corymbosa* exerts its effect on these parameters. The administration of CCl<sub>4</sub> alone may adversely interfere with protein metabolism probably by inhibiting the synthesis of proteins. Administration of ethanol extract of *Polycarpaea corymbosa* whole plant reversed these changes may be by increasing

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Total Bilirubin (µmol/L)</th>
<th>Conjugated (µmol/L)</th>
<th>Unconjugated (µmol/L)</th>
<th>GGTP (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
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</tr>
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<td>III</td>
<td></td>
<td>1.94±0.11*</td>
<td>0.75±0.03a</td>
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<td>12.37±0.81a</td>
</tr>
<tr>
<td>IV</td>
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<td>0.24±0.03aa</td>
<td>0.67±0.24a</td>
<td>10.44±0.73a</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>0.76±0.06a</td>
<td>0.24±0.07aa</td>
<td>0.62±0.05a</td>
<td>8.93±0.13 a</td>
</tr>
</tbody>
</table>

Each Value is SEM ± 5 individual observations *p< 0.05; **p< 0.01 Compared normal control vs liver injured rats a: p< 0.05; aa p< 0.01 Compared liver injured rats vs drug treated, ns- Not significant
protein synthesis. This indicates the hepatoprotective activity of Polycarpaea corymbosa whole plant against damage by CCl₄. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates regeneration of cells (Awang, 1993).

γ-glutamyl transferase (GGT) is a microsomal enzyme, which is widely distributed in tissue including liver. The activity of serum γ-glutamyl transferase is generally elevated as a result of liver disease, since γ-glutamyl transferase is a hepatic microsomal enzyme. Serum γ-glutamyl transferase is most useful in the diagnosis of liver diseases. Changes in γ-glutamyl transferase are parallel to those of amino transferases. The acute damage caused by CCl₄ increased the γ-glutamyl transferase level but the same attains the normal after Polycarpaea corymbosatreatment due to its antioxidant activity.

Lipid peroxidation has been postulated to the destructive process of liver injury due to CCl₄ administration. In the present study the elevations in the levels of end products of lipid peroxidation in the liver of rat treated with CCl₄ were observed. The increase in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with ethanol extract of Polycarpaea corymbosa significantly reversed these changes. Hence it may be possible that the mechanism of hepatoprotection by Polycarpaea corymbosa extract is due to its antioxidant effect.

The enzyme antioxidant defense system is the nature protector against lipid peroxidation. SOD, CAT and GPx enzymes are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage (Scott et al., 1991). In the present study, it was observed that the ethanol extract of Polycarpaea corymbosa significantly (p< 0.01) increased the hepatic SOD activity in CCl₄ induced liver damage in rats. This show ethanol extract of Polycarpaea corymbosa can reduce reactive free radicals that might lesson oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzyme.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and in the liver. CAT decomposed hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals (Chance et al., 1952). Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of ethanol extract of Polycarpaea corymbosa increased the activities of CAT in CCl₄ induced liver damage in rats to prevent the accumulation of excessive free radicals and protected the liver from CCl₄ in toxication.

To prevent lipid peroxidation, it is very important to maintain the level of GSH, GSSG is reduced to GSH by GR, which is NADPH-dependent. It plays a role in maintaining adequate amounts of GSH. Accordingly, the reduction of GR results in decreasing GSH (Reckenge et al., 1991). In CCl₄ intoxicated rats, the activity of GR is significantly (p< 0.05) decreased. However, ethanol extract of Polycarpaea corymbosa with 250 and 500 mg/kg bodyweight brought the activity of GR towards of normalization.

In conclusion the present study has demonstrated that ethanol extract of Polycarpaea corymbosa has hepatoprotective effect against CCl₄ induced hepatotoxicity in rats. It is suggested that, saponins in Polycarpaea corymbosa whole plant play an important role an antioxidant for prevention of oxidative hepatic damage. Furthermore, the flavonoids and saponins of Polycarpaea corymbosa may able to stabilize reactive oxygen species by reacting with them and oxidizes...
subsequently to more stable and less reactive radicals. The enhanced levels of antioxidant enzymes and reduced amount of lipid peroxides are suggested to be the major mechanism of *Polycarpaea corymbosa* ethanol extract in preventing the development of liver damage induced by CCl$_4$.

**Acknowledgement**

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of chemicals/Section 4: Health Effects Test No. 423; Acute oral Toxicity- Acute Toxic Class method, OECD. Paris.


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