Protective activity of picroliv on hepatic amoebiasis associated with carbon tetrachloride toxicity

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Background & objectives: Picroliv, isolated from the root and rhizome of Picrorhiza kurroa, is known to have significant hepatoprotective activity. Its effects against Entamoeba histolytica induced liver damage are not studied. This study aims to evaluate the hepatoprotective action of picroliv against the hepatotoxic changes induced by carbon tetrachloride (CCl₄) and E. histolytica infection in three animal models.

Methods: Mastomys, gerbils and albino Druckray rats were used in this study. A total of 30 animals were used for each model and divided into five groups of six animals each. Group I consisted of normal animals. The rest received six doses of CCl₄ intraperitoneally. Group II served as hepatotoxic control. The remaining animals were infected intraperitoneally with E. histolytica trophozoites, of which group III was the hepatotoxic plus amoeba infected control. The remaining animals were divided into two groups, one received hepatoprotective agent picroliv and the other silymarin. All animals were sacrificed seven days post amoeba infection.

Results: Increase in the enzyme levels induced by CCl₄ was further elevated after E. histolytica infection. Pinpoint abscesses were found to develop only in gerbils after E. histolytica infection. Picroliv was found to possess hepatoprotective activity against amoebic liver abscess.

Interpretation & conclusion: Significant recovery obtained in serum enzyme levels in all animal models and against amoebic liver abscess in gerbils on treatment with picroliv indicated that picroliv possesses therapeutic activity against E. histolytica induced hepatic damage.

Key words Carbon tetrachloride - Entamoeba histolytica - hepatic amoebiasis - picroliv

Human amoebiasis caused by the amoebic protozoan parasite Entamoeba histolytica is ubiquitous in its distribution with consistently high levels of infection. Primarily the infection with E. histolytica occurs in the intestine from ingestion of food and water contaminated with cysts of these amoebae. Excystation of the cysts establishes the infection in the lumen of the large bowel. Of all the cases of extraintestinal amoebiasis the incidence of hepatic amoebic abscess is the most encountered (3-9%)
infection. It is also believed that hepatomegaly associated with amoebic infection might be further enhanced as reaction to toxic substance.

Though, picroliv isolated from the root and rhizome of Picrorhiza kurroa (Scrophulariaceae) has been shown to have a marked hepato protective activity against many hepatotoxic compounds such as alcohol, aflatoxin B1, oxytetracycline, and carbon tetrachloride (CCl4), its effect against E. histolytica induced liver damage is not known. We undertook this study to evaluate the effect of hepatotoxic agent CCl4 on the development of hepatic abscess due to E. histolytica in rats, mastomys and gerbils. The hepatoprotective activity of picroliv to provide protection against the biochemical alterations produced by CCl4 and E. histolytica was also evaluated.

**Material & Methods**

**Animals:** Mastomys, gerbils and albino rats (Druckray strain), weighing 25-30 g of either sex, bred in the animal house at Central Drug Research Institute (CDRI), Lucknow were used in this study. The animals were fed *ad libitum* on standard pellet diet and had free access to water.

**Hepatoprotective agent:** Picroliv supplied by the Pharmaceutical Division of CDRI, and silymarin (Aldrich, USA) were used as hepatoprotective agents. Picroliv is isolated from the root and rhizome of Picrorhiza kurroa (Scrophulariaceae), traditionally known as kutki. P. kurroa is a small perennial herb that grows in hilly parts of India particular in Himalayas between 3000 and 5000 meters.

**Amoebae:** E. histolytica, isolated from the stool sample of a patient suffering from acute symptoms of dysentery and containing haematophagus trophozoites, maintained in Robinson’s medium, was used in this study.

**Amoebic inoculum:** Healthy, motile trophozoites from 48 h old cultures were pooled by low speed centrifugation (14g). The sediment containing the trophozoites were suspended in fresh overlay ensuring viability of the amoebae. The amoebae were then counted in a haemocytometer and the number adjusted to approximately 10 x 10⁴ trophozoites in 0.02 - 0.03 ml. The inoculum was incubated at 37°C till further use.

**Experiment design:**

(i) Production of hepatic amoebiasis: The mode of infection and grading of hepatic abscess were done according to the method of Dutta. In brief, a small incision was made on the skin below the xiphisternum towards the left from the midline. Connective tissue underlying the skin was gently removed to expose the muscular layer of the abdomen. The inoculum was inoculated into the peritoneal cavity through a 26-gauge needle near the left lobe of the liver. The incision was carefully sutured and the wound dabbed with 0.2 per cent gentian violet solution and boric acid powder. The whole procedure was carried out in properly anaesthetized animals.

(ii) Hepatotoxic agents: Animals were given intraperitoneal injections of CCl4 (0.3 ml/kg body weight) diluted ten fold with mineral oil, every alternate day for two weeks (6 doses). A total of 30 animals were used for experiments with each model. Group I consisted of six normal animals, the remaining 24 animals received treatment with CCl4. After CCl4 treatment was completed, six animals were isolated for group II which served as hepatotoxic treated control. The remaining 18 animals were inoculation with E. histolytica trophozoites, of which six animals were isolated for group III (hepatotoxic and amoebae treated control). The remaining twelve animals were used for testing hepatoprotective agents picroliv (Group IVa) and silymarin (Group IVb), six animals in each group.

(iii) Hepatoprotective agents: Both the hepatoprotective agents picroliv and silymarin, were given at daily doses of 12 mg/kg body weight orally with the help of a feeding needle. The treatment was started three days after inoculation with amoebae and continued for three consecutive days. All animals were sacrificed seven days after E. histolytica treatment.

(iv) Collection of blood samples: Blood was collected from the retro-orbital plexus before sacrificing the animals and serum stored for enzyme assays.
Enzyme assays: The activities of glutamic oxaloacetate transaminase (GOT) and glutamic pyruvate transaminase (GPT) were assayed by the method of Reitman and Frankel. Serum albumin was determined using bromocresol green. Serum levels of cholesterol, triglyceride and alkaline phosphatase were determined by standard procedures and serum protein estimated by the Lowry’s method. The study protocol was approved by the institutional ethics committee.

Statistical analysis: Data were analysed using Student’s t test.

Results

Rats: CCl₄ treatment induced liver damage and showed significantly elevated levels of albumin and triglycerides accompanied with 1.3, 1.7 and 1.8 fold increase in the GPT, GOT and alkaline phosphatase specific activities respectively (Table I). However, the level of protein and cholesterol remained unaffected after CCl₄ treatment. In CCl₄ treated and E. histolytica infected animals the levels of cholesterol, triglycerides and specific activities of GPT, GOT and alkaline phosphatase showed a further rise whereas the level of albumin remained unchanged. All these elevated levels of liver markers were significantly lowered in animals treated with hepatoprotective agents. Animals receiving picroliv (12 mg/kg) showed protection of 30.8 per cent in albumin, 68 per cent in cholesterol, 74 per cent in triglycerides, 61.37 per cent in alkaline phosphatase, 60.7 per cent in GOT and 87 per cent in GPT. Silymarin at the same dose had a similar pattern of protection but the effect was comparatively less prominent than that of picroliv.

![Table I. Protective effect of picroliv on liver enzymes in hepatic damage induced by CCl₄ and E. histolytica in rats](image-url)
Mastomys: The levels of serum protein, cholesterol and triglycerides were elevated significantly after CCl₄ treatment (Table II). The specific activities of serum enzymes viz., GPT, GOT and alkaline phosphatase showed 1.8, 2.6 and 1.9 fold increase respectively. No significant change in level of albumin was found. The CCl₄ treated rats when infected with *E. histolytica* showed a further significant rise in the level of serum triglycerides.

The increased levels of hepatospecific markers were significantly reversed after picroliv treatment.

Silymarin also showed marked hepatoprotective effect but in a less effective manner as compared to picroliv.

Gerbils: In gerbils CCl₄ treatment significantly elevated the levels of cholesterol and triglycerides. However, protein and albumin remained unchanged. The serum activities of hepatospecific enzymes, GPT (1.8 fold), GOT (2.5 fold) and alkaline phosphatase (1.8 fold) were significantly increased. Further increase in albumin, cholesterol, alkaline phosphatase and GPT was observed after infecting the CCl₄ treated animals with *E. histolytica*. Picroliv treatment exhibited high rate of protection by reversing the altered levels of markers while silymarin exhibited protective effect to a lesser extent (Table III).

It was also observed that these animals developed pinpoint amoebic abscesses, which showed motile amoebae in the smears prepared from the infected portion. Positive cultures were obtained when part of the infected liver was inoculated into Robinson’s medium along with sterile rice starch.

### Table II. Protective effect of picroliv on liver enzymes in hepatic damage induced by CCl₄ and *E. histolytica* in mastomys

<table>
<thead>
<tr>
<th>Serum parameter</th>
<th>Group I (control)</th>
<th>Group II (CCl₄ treated)</th>
<th>Group III (CCl₄ + amoeba)</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IVa Picroliv (12mg/kg) + CCl₄ + amoeba</td>
<td>IVb Silymarin (12mg/kg) + CCl₄ + amoeba</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.38±0.26</td>
<td>4.78±0.4</td>
<td>5.08±0.20</td>
<td>4.1±0.05† (76.56)</td>
</tr>
<tr>
<td>Protein (g/dl)</td>
<td>5.11±0.106</td>
<td>8.93±0.20***</td>
<td>9.2±0.10</td>
<td>6.96±0.22*** (56.09)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>175.11±3.94</td>
<td>394.1±6.54***</td>
<td>396.06±6.52</td>
<td>218.18±5.95*** (80.9)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>162.45±3.45</td>
<td>393.9±8.09***</td>
<td>428±56.5.95**</td>
<td>280.18±6.35*** (71.44)</td>
</tr>
<tr>
<td>Alkaline phosphate* (U/l)</td>
<td>191.45±3.45</td>
<td>354.56±12.62***</td>
<td>358.53±13.73</td>
<td>211.4±6.9*** (88)</td>
</tr>
<tr>
<td>GOT b (U/l)</td>
<td>24.26±1.88</td>
<td>65.5±4.63***</td>
<td>73.0±6.10</td>
<td>43.16±1.99*** (61.22)</td>
</tr>
<tr>
<td>GPT b (U/l)</td>
<td>49.6±2.00</td>
<td>95.83±5.09***</td>
<td>99.33±4.89</td>
<td>66.83±2.58*** (65.33)</td>
</tr>
</tbody>
</table>

Data are mean ± SE of 6 animals

*P*<0.01, **P**<0.001 compared to group I

*P*<0.001 compared to group II

**P**<0.05, *P*<0.01 compared to group III

Enzyme unit expressed

a, µ moles of p-nitrophenol released/min/l serum

b, µ moles of pyruvate formed/min/l serum

Figures in parenthesis indicate per cent hepatoprotective effect.
The toxic effect of CCl₄ is due to free radical generation (CCl₃⁻), which may cause lipid peroxidation thus altering the permeability of the liver cell membranes. In the present study, further increase in the serum enzyme levels was seen after E. histolytica infection. This suggests disturbance in the transport function of hepatocytes resulting in leakage of enzyme from cells due to altered permeability of membranes.

In our study only gerbils were found to be susceptible to E. histolytica infection and developed pinpoint amoebic abscess in the liver. The finding is in confirmation with earlier reports. The toxic effect exerted by CCl₄ might have created an atmosphere for the survival and colonization of the trophozites of E. histolytica in the liver tissue.

Picroliv at a dose of 12 mg/kg body wt provided significant protection of the altered serum enzyme levels due to CCl₄ followed by E. histolytica administration. Pilot experiments were conducted with 3, 6, 12 and 20 mg/kg doses of the hepatoprotective agents. It was observed that 12 mg/kg dose offered better protection than 3 and 6 mg/kg while 20 mg/kg did not give a significantly higher degree of protection, which is in confirmation with earlier findings. Hence, in the present study, a dose of 12 mg/kg of the hepatoprotective agents was used. Concentration-dependent restorative effect was observed when hepatocytes damaged by exposure to galactosamine, thioacetamide, and CCl₄ were incubated with Picrorhiza constituents.

Biochemical changes in rats induced by aflatoxin B1 poisoning were significantly prevented by oral administration of Picrorhiza extract. Picroliv, when given at a dose of 3-12 mg/kg

### Table III. Protective effect of picroliv on liver enzymes in hepatic damage induced by CCl₄ and E. histolytica in gerbils

<table>
<thead>
<tr>
<th>Serum parameter</th>
<th>Group I (control)</th>
<th>Group II (CCl₄ treated)</th>
<th>Group III (CCl₄ + amoeba)</th>
<th>Group IV</th>
<th>Group IVb Silymarin (12mg/kg) + CCl₄ + amoeba</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IVa Picroliv (12mg/kg) + CCl₄ + amoeba</td>
<td>IVb Silymarin (12mg/kg) + CCl₄ + amoeba</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.73±0.11</td>
<td>3.71±0.04</td>
<td>3.95±0.07**</td>
<td>3.63±0.04** (54)</td>
<td>3.85±0.03 (45.45)</td>
</tr>
<tr>
<td>Protein (g/dl)</td>
<td>6.85±0.24</td>
<td>6.96±0.37</td>
<td>7.05±0.08</td>
<td>6.8±0.03* (75)</td>
<td>6.8±0.04 (75)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>117.86±14.88</td>
<td>287.93±8.15**</td>
<td>327.23±4.48**</td>
<td>210.15±7.84*** (78)</td>
<td>253.03±6.33*** (49.66)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>163.8±5.16</td>
<td>301.48±9.88***</td>
<td>313.93±5.86</td>
<td>195.15±5.4*** (79%)</td>
<td>226.5±4.44*** (58.25)</td>
</tr>
<tr>
<td>Alkaline phosphate* (U/l)</td>
<td>203±6.23</td>
<td>373.75±5.30***</td>
<td>400.11±4.47**</td>
<td>295.95±7.36*** (52.8)</td>
<td>324.53±4.67*** (36)</td>
</tr>
<tr>
<td>GOT b (U/l)</td>
<td>29.05±2.26</td>
<td>73.78±1.25***</td>
<td>79.10±2.37</td>
<td>59.68±1.16*** (38.80)</td>
<td>63.23±1.31*** (31.7)</td>
</tr>
<tr>
<td>GPT b (U/l)</td>
<td>44.40±1.22</td>
<td>83.61±0.81***</td>
<td>87.9±1.59*</td>
<td>65.78±1.27*** (51)</td>
<td>74.98±1.31*** (29.70%)</td>
</tr>
</tbody>
</table>

Data are mean ± SE of 6 animals
P <0.01, **P<0.001 compared to group I
***P<0.01compared to group II
P<0.05, **P<0.01 ***P<0.001compared to group III
Enzyme unit expressed
a, µ moles of p-nitrophenol released/min/l serum
b, µ moles of pyruvate formed/min/l serum
Figures in parenthesis indicate per cent hepatoprotective effect
orally for 45 days, was also shown to be effective in reversing ethanol-induced liver damage in rats. Picroliv given orally to rats at 12 mg/kg daily for 7 days, prior to induced ischaemia, demonstrated improved hepatocyte glycogen preservation and reduced apoptosis, compared to control animals. Recovery induced by Picroliv might be due to restoration of plasma membrane permeability including repair of injured hepatic cells and increasing protein and nucleic acid synthesis.

Picroliv showed significant and more potent hepatoprotective activity than silymarin in all the test animals. The results of the present study indicated the usefulness of picroliv in hepatic disorders due to *E. histolytica*.

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**References**


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