Hepatoprotective and antioxidant properties of aqueous rhizome extracts of *Picrorhiza kurroa* on *CCl₄* induced liver toxicity in *albino* rats

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ABSTRACT

The aqueous rhizome extract of *Picrorhiza kurroa* (Scrophulariaceae) was investigated for its hepatoprotective and antioxidant effects in wistar albino rats. Different group of animals were administered with 30% carbon tetrachloride (*CCl₄*) in olive oil (1.0 ml/kg body wt., i.p) every 72 hrs for 3 doses. After the 3 doses the aqueous rhizome extract at a dose of 250mg/kg body wt., was administered orally by intragastric tube for 10 days. Animal was sacrificed and liver tissue were collected for the biochemical estimation. Biochemical marker enzymes such as Aspartate aminotransaminase (AST), Alkaline aminotransaminase (ALT), Alkaline phosphatase (ALP) and bilirubin were measured. Further, the antioxidant defense enzymes like Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and Glutathione-S-transferase (GST) were also estimated. The rhizome extract showed significant (*P*<0.05) hepatoprotective effect by decreased the biochemical marker enzymes level in serum and the antioxidant enzymes were significantly (*P*<0.05) increased when compared to the *CCl₄* induced control groups.

Keywords: *Picrorhiza kurroa*, biochemical marker enzymes, antioxidant defense enzymes, carbon tetrachloride (*CCl₄*)

INTRODUCTION

Liver is an important organ actively involved in metabolic functions, production and secretion of bile, prothrombin and fibrinogen. The liver is also responsible for detoxifying poisonous substances in the body by transforming and removing toxins, waste, and pollutant xenobiots ([1]). The liver is expected not only perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. Carbon tetrachloride (*CCl₄*) is one of the oldest and most widely used toxins for experimental induction of liver fibrosis in laboratory animals ([2]). It is a well known for its hepatic and renal toxic actions. Metabolism of *CCl₄* in to trichloromethyl (*Cl*) and peroxy trichloromethyl (OCCl₃) free radicals has been reported to cause acute liver damages like Cirrhosis, steatosis and necrosis ([3]). It has been established that trichloromethyl (*CCl₃*) radical and Cl are formed as a result of the metabolic conversion of *CCl₄* by cytochrome P-450 ([4]). The principle causes of *CCl₄* is induced hepatic damage in lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals ([5]).

India is the largest producer of medicinal herbs and appropriately called the botanical garden of the world ([6]). Plants have been used in traditional medicine for several thousand years Conventional synthetic drugs used in the treatment of liver diseases are inadequate and can have serious adverse effects. Hence there is a world wide trend to go back to traditional medicinal plants.

*Picrorhiza kurroa* (Scrophulariaceae) is a small perennial herb that grows in North West India on the slopes. It is an important herb in the traditional ayurvedic system of medicine and has been used to treat liver troubles and bronchial problems. Other traditional uses include dyspepsia, bilious fever, chronic dysentery and scorpion sting. *P.kurroa* has immunomodulating properties which may be another mechanism by which it is beneficial for allergies ([7]). *P.kurroa* has hepatoprotective and antioxidant effect against amanita poisoning ([8]). Bioactivity studies of *Picrorhiza kurroa* like anti-inflammatory ([9]), antibacterial activity ([10]) were recorded earlier. The present study the aqueous rhizome extract of *P.kurroa* have been selected for hepatoprotective and antioxidant studies in *CCl₄* induced liver toxicity in *albino* rats.

MATERIALS AND METHODS

Plant Material

The rhizome of *Picrorhiza kurroa* (Family: Scrophulariaceae) were collected from Sikkim State, India. The plant materials were cleaned with distilled water and shade dried at room temperature. The plant materials were authenticated by the Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore District, Tamilnadu, and voucher specimens (No.CAHC-05-2008), were kept at the Department of Botany, C.Abdul Hakeem College, Melvisharam, Vellore Dt., Tamilnadu. The shade dried plant materials were powdered by using electric blender.

Preparation of plant extracts

The powdered plant materials were extracted separately to exhaustion in a soxhlet apparatus using distilled water. The extract was filtered by using what man filter paper (No.1). The extract was concentrated by using a rotary evaporator at low temperature (40-50°C) and reduced pressure to get solid yield aqueous fraction. The extract was preserved in airtight container and kept at 4°C until further use. The aqueous crude extract was taken for hepatoprotective and antioxidant studies.

Animals

Adult male *albino* rats of Wistar strain weighing around 180 to 200gms were procured from Tamilnadu Veterinary and Animal Sciences University, Chennai. Animals were housed in clean polypropylene cages with 12±1 hr light/dark schedule and fed with rat chow (Hindustan Lever Ltd., Bangalore, India) and free access to water. The experiments were designed and conducted in accordance with the institutional guidelines.

Experimental design

The rats were randomly divided into 4 groups of 6 rats each.
Group I: Animals served as normal control and received distilled water orally for 10 days.

Group II: Animals received olive oil 1ml/kg body wt i.p every 72 hrs for 3 doses

Group III: Animals were induced hepatotoxic by injection of 30% CCl4 in Olive oil (1 ml/kg body wt., i.p) every 72 hrs for 3 doses.

Group IV: CCl4 treated groups administered with Picrorhiza kurroa aqueous rhizome extracts orally, 250 mg/kg body wt for 10 days.

Estimation of biochemical parameter

At the end of the experimental period, animals were sacrificed by cervical decapitation. Blood were collected and serum was separated for biochemical analysis. Such as Aspartate amino transaminase (AST), Alanine amino transaminase (ALT) were estimated by method of Reitman and Frankel (11), Alkaline phosphatase (ALP) in serum were estimated by King and Armstrong (12) and bilirubin was estimated by Malloy and Evelyn method (13).

Preparation of tissue homogenate

Known amount of hepatic tissue was homogenized in suitable buffer and centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant was collected and stored at -80°C for assay of the marker enzymes and antioxidant studies.

Measurement of antioxidant activity

From all the experimental groups, the portion of the liver was collected and rinsed with 0.025M Tris–HCl (pH 7.5). 10% liver homogenate was prepared in 0.25M M Tris –HCl buffer and processed for the estimation of lipid peroxidation in the from of Malonialdelyde (MDA) in liver for measuring the thiothibarbituric acid reactive substance (TBARS) by the method of Nichans and Samue110. The lipid hydroperoxide in the liver was determined by Jiang method (14). The rest of the homogenate was centrifuged at 12,000 rpm for 20mns at 4°C. The Supernatant thus obtained was used for the estimation of Super oxide dismutase (SOD) (15), Catalase (CAT) (18), Glutathione peroxidase (GPx) (19), Glutathione-S-transferase (GST) (19). The results were expressed in Mean ± S.E.M. Statistical analysis was carried out by using one-way ANOVA as in standard statistical software package of social science (SPSS).

RESULTS

The serum cellular marker enzymes like Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and bilirubin play a vital role in hepatic damage. Leakage of cellular enzymes into the plasma is the hall mark sign of hepatic injury or damage. In addition, the extent and type of liver injury or damage can be assessed based on the presence or absence of specific marker enzymes in the bloodstream.

The cellular marker enzymes like AST, ALT, ALP and bilirubin of group II olive oil induced liver damaged animals were found to have been elevated was not significant. But the ALP levels of these animals were found to have been significantly elevated P<0.001. When compared to the levels in normal animals. The levels of cellular enzymes in CCl4 induced (Group III) liver damaged animals were found to have significantly elevated by increasing P<0.001 respectively. When compared to the levels in normal animals. In group IV animals treated with Pkurror plant extract the elevated levels of these cellular enzymes were found to have decreased significantly (Table:1) when compared to the levels in CCl4 induced groups.

Liver toxicity was assessed by measuring the levels of thiothibarbituric acid reactive substances (TBARS), lipid hydro peroxides (Table:2) and antioxidant defense enzymes viz., Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and Glutathione–S-transferase (GST) (Table:3) in CCl4 induced liver damaged as well as plant extract treated groups. The levels of antioxidant enzymes such as SOD, CAT, GPx and GST were observed in normal, olive oil, CCl4 as well as plant treated groups. In group II animals in which liver damage was induced by olive oil, the levels of antioxidant enzymes like SOD and CAT were found to have decreased P<0.001 which was not significant, but GPx and GST were found to have significantly decreased respectively P<0.001 (Table:3) when compared to the levels in normal animals. The activity levels of antioxidant enzymes in group III rats (CCl4, liver damaged) were found to have decreased P<0.001 significantly (Table:3) when compared to the levels in normal animals. The CCl4 induced hepatotoxic animals group IV were treated with Pkurror rhizome extract. The decreased levels of antioxidant defense enzymes were found to have increased significantly P<0.001 when compared to the levels in CCl4 administered rats (Table:3).

Table.1. Effects of plant extract Picrorhiza kurroa (PK) treatment on CCl4 induced hepatotoxicity: Activity levels of ALT, AST, ALP and bilirubin.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/mm/mg protein)</th>
<th>AST (U/mm/mg protein)</th>
<th>ALP (U/mm/mg protein)</th>
<th>Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I Normal</td>
<td>57.16±2.22</td>
<td>147±6.8</td>
<td>232.5±4.96</td>
<td>0.40±0.02</td>
</tr>
<tr>
<td>Group-II oil</td>
<td>59.16±1.47</td>
<td>150.5±6.7</td>
<td>250±3±3.77</td>
<td>0.41±0.02</td>
</tr>
<tr>
<td>Control</td>
<td>4.39</td>
<td>2.38</td>
<td>7.65</td>
<td>2.5</td>
</tr>
<tr>
<td>Group-III CCl4</td>
<td>116.8±2.04</td>
<td>210.3±6.02</td>
<td>456.1±4.44</td>
<td>0.60±0.02</td>
</tr>
<tr>
<td>Control</td>
<td>104.3</td>
<td>43.06</td>
<td>96.17</td>
<td>50</td>
</tr>
<tr>
<td>Group-IV Pk fed</td>
<td>62.6±2.50</td>
<td>155.8±2.85</td>
<td>252.8±4.87</td>
<td>0.48±0.01</td>
</tr>
<tr>
<td>% of changes</td>
<td>-46.9</td>
<td>-29.5</td>
<td>-44.5</td>
<td>-20</td>
</tr>
<tr>
<td>(Normal Vs CCl4)</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>(Normal Vs olive oil)</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean of six individual observations in each group ± S.D. ‘P’ denotes statistical significance, ‘+’ and ‘-’ indicate % of changes over the CCl4 induced liver damaged groups.

Table.2. Effects of plant extract Picrorhiza kurroa (Pk) treatment on CCl4 induced hepatotoxicity: Levels of thiothibarbituric acid reactive substances (TBARS) and lipid hydroperoxide in liver tissue

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nm/100 g tissue)</th>
<th>Lipid peroxide (nm/100 g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I Normal</td>
<td>0.67±0.02</td>
<td>69.6±1.63</td>
</tr>
<tr>
<td>Group-II Olive oil</td>
<td>0.67±0.34</td>
<td>72.16±1.16</td>
</tr>
<tr>
<td>Control</td>
<td>+1.5</td>
<td>+3.5</td>
</tr>
<tr>
<td>Group-III CCl4</td>
<td>1.42±0.05</td>
<td>111.16±2.13</td>
</tr>
<tr>
<td>Control</td>
<td>+11.5</td>
<td>+59.57</td>
</tr>
<tr>
<td>Group-IV Pk fed</td>
<td>0.84±0.36</td>
<td>76.16±1.32</td>
</tr>
<tr>
<td>% of changes</td>
<td>-40.8</td>
<td>-31.48</td>
</tr>
<tr>
<td>(CCl4 Vs Pk fed)</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean of six individual observations in each group ± S.D. ‘P’ denotes statistical significance, ‘+’ and ‘-’ indicate % of changes over the CCl4 induced liver damaged groups.

Table.3. Effects of plant extract Picrorhiza kurroa (Pk) treatment on CCl4 induced hepatotoxicity: Levels of antioxidants defense viz., superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) in the liver tissue

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
<th>GST (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I Normal</td>
<td>6.25±0.18</td>
<td>106.3±2.58</td>
<td>11.18±2.01</td>
<td>6.11±0.14</td>
</tr>
<tr>
<td>Group-II Olive oil</td>
<td>6.05±0.10</td>
<td>161.6±2.73</td>
<td>10.56±0.38</td>
<td>5.71±0.11</td>
</tr>
<tr>
<td>Control</td>
<td>-3.2</td>
<td>-4.16</td>
<td>-5.5</td>
<td>-6.54</td>
</tr>
<tr>
<td>Group-III CCl4</td>
<td>1.30±0.14</td>
<td>38.81±5.94</td>
<td>9.58±0.18</td>
<td>2.78±0.11</td>
</tr>
<tr>
<td>Control</td>
<td>0.01±0.01</td>
<td>0.01±0.01</td>
<td>0.01±0.01</td>
<td>0.01±0.01</td>
</tr>
<tr>
<td>Group-IV Pk fed</td>
<td>5.93±0.12</td>
<td>155.3±5.53</td>
<td>9.95±0.24</td>
<td>5.5±0.17</td>
</tr>
<tr>
<td>% of changes</td>
<td>+104.8</td>
<td>+74.8</td>
<td>+67.05</td>
<td>+97.84</td>
</tr>
<tr>
<td>(CCl4 Vs Pk fed)</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean of six individual observations in each group ± S.D. ‘P’ denotes statistical significance, ‘+’ and ‘-’ indicate % of changes over the CCl4 induced liver damaged groups.
SOD – U - One unit of activity was taken as the enzymes reaction which gives 50% inhibition of NBT reduction in one minute.
CAT – U- µmoles of hydrogen peroxide consumed per minute.
GPx – U - µg of glutathione consumed per minute.
GST – U - µmoles of CDNB – GSH conjugate formed per minute

DISCUSSION

The levels of the cellular marker enzymes viz., ALT, AST, ALP and bilirubin in serum reflect the physiological state of the liver. The levels of these enzymes change according to distortion of the liver, resulting from cellular injury of the organ caused by toxic metabolites and diseases. The increased levels of AST, ALT, ALP and serum bilirubin are conventional indicators of liver injury [20,21]. The efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effects or in maintaining the normal hepatic physiological mechanisms which have been altered by a hepatotoxin [22].

In our experiments when CCl4 was given to the experimental animals, the levels of cellular marker enzymes were found to have been significantly elevated in them indicating increased cellular permeability, damage and necrosis of hepatocytes which might be due to the leakage of the cellular enzymes into the serum. The significant decrease of cellular enzymes in P.kurroa plant extract treated groups might be due to decreased leakage from the hepatic cells. This suggests that the P.kurroa plant extract could repair the hepatic injury and or restore the cellular permeability, thus reducing the toxic effect of CCl4 damaged liver tissue.

The levels of TBARS and lipid peroxides were found to have been significantly elevated in CCl4 induced liver damaged animals. This might be due to increased lipid peroxidation and increase of free radicals in hepatic cells. The significant depletion of levels of TBARS and lipid peroxides in the liver tissue of the plant extract administered animal groups might be due to reduced lipid peroxidation and or elevation of activity levels of tissue antioxidant defense enzymes indicating that the plant extract could reduce the generation of free radicals and increase free radicals scavenging mechanism.

In CCl4 induced liver damaged animals were depleted hepatic GSH levels, inhibited the activities of SOD, GPx and CAT, and promoted hepatic lipid peroxidation, indicating an impaired anti-oxidative capacity and sustained oxidative stress. Serum activities of superoxide dismutase (SOD) and Catalase (CAT) are the most sensitive enzymatic indices of liver injury caused by ROS and oxidative stress; SOD is one of the most abundant intracellular antioxidant enzymes present in all aerobic cells and it has an antioxidative effect against ROS [23]. CAT is a heatonoprotein; it protects the cells from the accumulation of H2O2 by dismutating it to form H2O and O2 [24]. Therefore reduction in the activities of these enzymes may indicate the toxic effects of ROS produced by toxictants.

In our study, in CCl4 administered groups; the levels of antioxidant enzymes were found to have decreased significantly. The decreased levels of these enzymes promote lipid peroxidation and increase the free radicals in the hepatic tissues. The significant increase in the activity levels of antioxidant defense enzymes can be corroborated with the increased free radicals scavenging mechanism. Similar reduction in lipid peroxidation increased antioxidant enzyme activity levels during plant extract supplementation were recorded [23,26,27,28]. The plant extract treated animals revealed higher protection from ROS that may lessen the oxidative damage to the hepatoctye, lipid peroxidation and elevated antioxidant defense enzyme activity levels thus protecting the liver from CCl4 induced damage.

In conclusion these results suggest that, the P.kurroa plant extract has a more potent and synergetic hepatoprotective effect and more therapeutic value to combat the hepatotoxic condition in rats.

ACKNOWLEDGEMENT

Authors are thankful to C.Abdul Hakeem College, Melvisharam, Tamilnadu for providing laboratory facilities to carry out this study. The authors thank to Dr.V.Chelladurai, Botanist, Thirunelveli district for his help in collecting and identifying the plant material.

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Source of support: Nil, Conflict of interest: None Declared