Biochemical Studies on the Protective Effects of *Picrorhiza kurroa* in Experimentally Induced Hepatitis in Rats

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Summary The effect of pre-treatment with an alcoholic extract of *Picrorhiza kurroa*, a herbal drug, on D-galactosamine-induced hepatitis in rats was investigated with respect to the activities of serum marker enzymes, glycolytic enzymes, gluconeogenic enzymes, glucose-6-phosphate dehydrogenase, and membrane-bound ATPases and the levels of lipid peroxides and hepatic thiols. Prior oral treatment with *P. kurroa* extract significantly prevented the D-galactosamine-induced increases in the activities of serum marker enzymes, glycolytic enzymes, glycogen phosphorylase, and glucose-6-phosphate dehydrogenase and the decreases in the activities of key gluconeogenic enzymes and membrane-bound ATPases in the liver. The extract also exerted an antioxidant effect against D-galactosamine-induced hepatitis by blocking the induction of lipid peroxidation and by preventing the depletion of hepatic thiols. The present findings confirm that *P. kurroa* is hepatoprotective in experimentally induced hepatitis in rats.

Key Words: *Picrorhiza kurroa*, D-galactosamine hepatitis, glycolytic enzymes, gluconeogenic enzymes, membrane-bound ATPases

A single intraperitoneal injection of D-galactosamine (GalN), an amino sugar, produces acute reversible hepatocellular injury in rats that morphologically resembles both drug-induced hepatitis and viral hepatitis in human beings [1, 2]. The primary disturbance of the injury has been reported to involve a depletion of uridine nucleotides [3] and subsequent suppression of RNA synthesis [4, 5].
GalN hepatitis is characterized by a progressive rise in liver enzymes, total bilirubin, ammonia, and lactate and is associated with coagulopathy, hypoglycemia, coma, and brain death [6]. The effects of GalN-induced hepatitis on energy metabolism are due to a reduction in gluconeogenesis and involve a shift from aerobic metabolism to anaerobic glycolysis [7]. Also, Shanne et al. [8] have reported that the appearance of GalN injury is dependent on intracellular Ca2+ concentrations. Peroxidation of endogenous lipid is also a major factor in the cytotoxic action of GalN [9].

Medicinal plants have been used to cure human illness since time immemorial, and certain of these drugs are believed to promote positive health and to maintain organic resistance against infection by re-establishing body equilibrium and by conditioning the body tissues [10]. Picrorhiza kurroa Royle ex Benth (Scrophulariaceae) is a small perennial herb distributed in the alpine Himalayas tract and in some tropical parts of India. It is commonly called “kutuki” and has high priority and applicability as an indigenous medicine to cure viral hepatitis and various liver disorders [10-12]. The extract also possesses diaphoretic, antiperiodic, stimulating, and expectorant properties, and it is used in traditional medicine to cure heart ailments, lung diseases, abdominal pain, stomach disorders, anemia, and jaundice and for promoting bile secretion [10, 12]. The iridoid glycosides present in the roots of P. kurroa are well known for their free radical-scavenging activities[13].

We have already reported the hepatoprotective effect of P. kurroa on mitochondrial function [14-16], the tissue defense system [17], protein metabolism [18], and erythrocyte membrane function [19] in GalN-induced hepatitis in rats. We have also reported the antiulcerogenic effects of an ethanolic extract of P. kurroa on HCl/ethanol-induced ulcer in rats [20]. However, its protective effects on key enzymes of glucose metabolism, lipid peroxidation, and membrane-bound ATPases in GalN-induced hepatitis in rats have not yet been explored. Therefore, the present study was undertaken to examine the preventive effects of an ethanolic extract of P. kurroa rhizomes and roots (PK) on glucose metabolism, lipid peroxidation, and hepatic thiol status in GalN-induced liver intoxication in rats.

MATERIALS AND METHODS

GalN was obtained from the Sigma Chemical Company, St. Louis, MO, USA. All other chemicals used were of analytical grade. PK prepared (yield: 8.8%) from dried rhizomes and roots of P. kurroa, authenticated by Captain Sreenivasamurthi Drug Research Institute for Ayurveda, Arumbakkam, Chennai, India, was obtained from TTK Pharmaceuticals, Chennai, India [18].

Male Wistar rats weighing about 120–150 g were obtained from FIPPAT, Padappai, Chennai, India, and housed in polyurethane cages under hygienic conditions at normal room temperature (25–30°C). They were allowed commercial pelleted feed (M/s. Hindustan Lever Foods, Bangalore, India) and water ad libitum.
The experimental animals were divided into four groups of 6 rats each. Group I served as the control. Group II animals were intraperitoneally (i.p.) injected twice with Ga1N (500 mg/kg body wt, dissolved in physiological saline), once on one day, and once on the next day for the induction of hepatitis [21]. Group III animals were orally pre-treated with PK (50 mg/kg body wt/day, dissolved in distilled water and given daily for 10 days) and then i.p. injected with Ga1N (500 mg/kg body wt/day) as in Group II. Group IV animals were orally treated with PK alone at the above dosage for 10 days.

At the end of 24 h after the last injection of Ga1N, the animals were killed by decapitation. Blood was collected without any anticoagulant, and the separated serum was used for the assay of marker enzymes such as aspartate aminotransferase (AST) [22], alanine aminotransferase (ALT) [22], acid phosphatase (ACP) [23], alkaline phosphatase (ALP) [23], and lactate dehydrogenase (LDH) [24]. The liver was dissected out immediately, washed with chilled isotonic saline, and homogenized in ice-cold 0.1 M Tris-HCl buffer, pH 7.2, in a Potter-Elvehjem homogenizer for the estimation of hexokinase [25], aldolase [26], LDH [24], glucose-6-phosphatase [27], fructose-1,6-diphosphatase [28], glycogen phosphorylase [29], glucose-6-phosphate dehydrogenase [30], lipid peroxides [31], Na+K+-ATPase [32], Mg2+-ATPase [33], Ca2+-ATPase [34], and thiols (total, protein-bound, and non-protein) [35]. The blood sugar was estimated by the method of Sasaki et al. [36]; proteins, by the method of Lowry et al. [37], and hepatic glycogen, by the method of Morales et al. [38].

Results were expressed as the mean ± SD, and Student’s t-test was used to assess statistical significance.

RESULTS

The elevation of activities of serum ALT, AST, ACP, ALP, and LDH caused by Ga1N intoxication was significantly (p<0.001) prevented in P. kurroa extract pre-treated rats (Table 1). Increased activities of glycolytic enzymes, glycogen phosphorylase and glucose-6-phosphate dehydrogenase and decreased activities of key gluconeogenic enzymes and membrane-bound ATPases and lowered levels of blood glucose and hepatic glycogen content observed in Ga1N toxic rats were also significantly avoided by the PK pre-treatment in Group III rats (Tables 2 and 3).

Intraperitoneal administration of Ga1N also caused a significant increase in lipid peroxidation in liver with a significant decrease in hepatic thiol content as compared with the level in normal control rats (Table 3). The oral pre-treatment with PK significantly prevented all these adverse effects and maintained the levels of the evaluated parameters nearly at normal values. The normal rats receiving PK alone did not show any significant changes when compared with the control animals, indicating that PK does not have any adverse effect.
DISCUSSION

Increased activities of AST, ALT, ACP, ALP, and LDH in serum are well known diagnostic indicators of GalN-induced hepatitis [3, 39, 40]. In cases of liver damage with hepatocellular lesions and parenchymal cell necrosis, these marker enzymes are released from damaged tissues into the bloodstream [41]. Prior oral administration of PK resulted in significant reduction in the activities of these marker enzymes towards near-normalcy, as compared with the levels in the

Group II GalN-intoxicated rats, indicating the cytoprotective effect of this extract.

The observed GalN-induced hypoglycemia may be due to an activated pancreatic β-cell function and to the decreased activities of the key gluconeogenic enzymes and/or increased activities of glucose-utilizing enzymes [39, 42]. Injection of GalN induces depletion of glycogen in the liver rather than new synthesis of glycogen [43]. Increased activities of glycolytic enzymes (hexokinase, aldolase, and lactate dehydrogenase) in Group II GalN-toxic rats, which is in line with previous reports [7, 39, 43] indicate that GalN-induced depletion of ATP synthesis in the impaired mitochondria may induce an activation of glycolysis, disappearances of glycogen granules and accumulation of hepatocellular fat.

Activities of the principal gluconeogenic enzymes (glucose-6-phosphatase and fructose-1,6-diphosphatase) have been previously reported to be decreased in GalN hepatitis along with an increase in the ratio of key gluconeogenic to glycolytic enzymes, thus leading to GalN-induced hypoglycemia [6, 7, 44]. The significantly increased glycogen phosphorylase activity observed in Group II GalN-intoxicated rats indicates increased glycogenolysis. The damage to liver cell integrity caused by fatty degeneration after GalN administration might be the cause of both decreased glycogenesis and increased glycogenolysis. The rats pre-treated with PK showed nearly normal levels of blood glucose, hepatic glycogen content, and the activities of glycolytic enzymes, gluconeogenic enzymes and glycogen phosphorylase as compared with Group II GalN-intoxicated rats. It probably did so by inhibiting the glycogen depletion or by increasing gluconeogenesis [14].

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Table 3. Levels of lipid peroxides and thiols and the activities of membrane-bound ATPases in livers from normal and experimental groups of rats (mean±SD for 6 animals in each group).

<table>
<thead>
<tr>
<th>Group</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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</thead>
<tbody>
<tr>
<td>I. Lipid peroxidation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.43±0.18</td>
<td>2.21±0.25***</td>
<td>1.64±0.16***</td>
<td>1.48±0.16</td>
</tr>
<tr>
<td>t-Butyl hydroperoxide</td>
<td>10.41±1.16</td>
<td>15.64±1.68***</td>
<td>12.18±1.22**</td>
<td>10.37±1.20</td>
</tr>
<tr>
<td>FeSO₄</td>
<td>14.94±1.28</td>
<td>24.16±1.45***</td>
<td>16.90±1.31***</td>
<td>15.08±1.24</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>6.89±1.04</td>
<td>12.87±1.12***</td>
<td>8.27±1.10**</td>
<td>6.82±1.06</td>
</tr>
<tr>
<td>II. Membrane-bound ATPases</td>
<td></td>
<td></td>
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<tr>
<td>Ca²⁺-ATPase</td>
<td>0.82±0.07</td>
<td>0.61±0.05***</td>
<td>0.74±0.07***</td>
<td>0.79±0.08</td>
</tr>
<tr>
<td>Na⁺, K⁺-ATPase</td>
<td>1.25±0.08</td>
<td>0.98±0.05***</td>
<td>1.09±0.06**</td>
<td>1.23±0.07</td>
</tr>
<tr>
<td>Mg²⁺-ATPase</td>
<td>1.36±0.12</td>
<td>1.15±0.18*</td>
<td>1.31±0.16*</td>
<td>1.34±0.14</td>
</tr>
<tr>
<td>III. Thiols</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>410.12±29.15</td>
<td>248.65±24.12***</td>
<td>368.44±26.63***</td>
<td>406.98±30.08</td>
</tr>
<tr>
<td>Protein-bound</td>
<td>386.83±30.12</td>
<td>228.72±24.43***</td>
<td>342.16±27.33***</td>
<td>382.27±29.82</td>
</tr>
<tr>
<td>Non-protein</td>
<td>3.23±0.63</td>
<td>5.82±0.73***</td>
<td>3.92±0.68**</td>
<td>3.18±0.64</td>
</tr>
</tbody>
</table>

Group designations are the same as in Table 1.

Values are expressed as follows: Lipid peroxidation, nmol of thiobarbituric acid-reactive substance/mg protein; membrane-bound ATPases, μmol of Pi liberated/min/mg protein; thiols, nmol/g wet liver.

As compared with respective controls, i.e., group II vs. group I, group III vs. group II: ***p<0.001; **p<0.01; *p<0.05.
The hexose monophosphate shunt pathway is one of the NADPH-generating systems. A significant increase in the activity of glucose-6-phosphate dehydrogenase in GalN hepatitis has already been reported \[7\]. The prior oral treatment with \( PK \) in our study prevented the GalN-induced increase in glucose-6-phosphate dehydrogenase activity, thus reducing the availability of NADPH for excess fatty acid synthesis and establishing its hypolipidemic effect \[11\].

A significant rise in the level of lipid peroxides following GalN intoxication in our study suggests that glucose auto-oxidation related to GalN-induced hypoglycemia \[42, 45\] might be responsible for the increased generation of free radicals in GalN hepatitis. The rats pre-treated with \( PK \) showed a significant decrease in lipid peroxidation as compared with the Group II GalN-intoxicated rats. We suspect that this antioxidant effect was due to the presence two electrophilic substances called "picroside I and kutkoside" present in the roots of \( P. kurroa \) \[11\]. The unpaired electron present in the hydroxyl free radicals generated by GalN \[46\] might have been trapped by picroside I and kutkoside.

Activities of \( \text{Na}^+, \text{K}^+-\text{ATPase}, \text{Mg}^{2+}-\text{ATPase} \) and \( \text{Ca}^{2+}-\text{ATPase} \) in the liver were significantly lower in Group II GalN toxic rats, which is in line with previous reports \[9\]. The intracellular concentration of calcium regulates the activity of the \( \text{Mg}^{2+} \) and \( \text{Na}^+, \text{K}^+-\text{ATPases} \). Bironaite and Ollinger \[47\] have reported that lipid peroxidation can influence the function of \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+}-\text{ATPases} \) and the activity of membrane \( \text{Ca}^{2+}\)-translocase. Levels of membrane protective thiols, both protein bound and non-protein bound showed a notable decrease in the rats with GalN hepatitis, which also agrees with previous reports \[48, 49\]. The marked loss in the activities of membrane-bound ATPases registered in GalN hepatitis may also be due to the loss of protein-SH, because of increased lipid peroxidative damage of cell membranes.

Prior oral treatment with \( PK \) in our study largely prevented the above-mentioned GalN-induced alterations in the \( \text{Na}^+, \text{K}^+-\text{ATPase}, \text{Mg}^{2+}-\text{ATPase} \), and \( \text{Ca}^{2+}-\text{ATPase} \) activities and in the protein and non-protein thiol levels found in the Group II GalN-induced hepatotoxic rats. It probably did so by its antioxidant nature \[13\] against lipid peroxidation induced by GalN \[9\]. This present study confirms the hepatoprotective effect of \( P. kurroa \) against GalN-induced hepatitis in rats.

REFERENCES


