

Protective effect of *Picrorhiza kurroa* on mitochondrial glutathione antioxidant system in D-galactosamine-induced hepatitis in rats

Indian ayurvedic medicine claims a lasting cure for human viral hepatitis through oral administration of an extract of *Picrorhiza kurroa* (a member of the family Scrophulariaceae). This medicinal plant occurs in alpine Himalayas from Kashmir to Sikkim at altitudes of 2700–4500 m. In traditional medicine, it has also been used to cure heart ailments, lung diseases, abdominal pain, stomach disorders, anaemia and jaundice and to promote secretion of bile^{1–3}. Yet there is little documentary evidence regarding its nature, chemistry or action of the therapeutic principle.

Hepatitis induced by D-galactosamine (GalN) shows many metabolic and morphological aberrations in the livers of experimental animals similar to that seen in human viral hepatitis⁴. GalN hepatitis is induced by a multiple step mechanism⁵. Peroxidation of endogenous lipid is a major factor in the cytotoxic action of GalN⁶. A growing body of evidence is emerging which suggests that reactive oxygen-derived radicals play a crucial role in the pathogenesis of GalN hepatitis⁷.

Glutathione antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species⁸. The cellular tripeptide GSH (L-glutamyl-cysteinyl glycine) exerts protective antioxidant functions through a complex enzyme system including glutathione peroxidase (GPX) and glutathione-S-transferase (GST)⁹. Since the alcoholic extract of dried rhizomes and roots of *P. kurroa* have been reported to contain two electrophilic free radical scavenging principles called picroside I and kutkoside^{10,11}, we have now attempted to assess the protective effect of *P. kurroa* on mitochondrial glutathione antioxidant system in GalN-induced hepatitis in rats.

The lyophilized ethanolic extract (yield 8.8%) of dried rhizomes and roots of *P. kurroa* (authenticated by Captain Srinivasamurthi Drug Research Institute for Ayurveda, Arumbakkam, Chennai), was supplied by TTK Pharmaceuticals Limited, Chennai. Wistar strain male albino rats weighing 120–150 g were

maintained at constant temperature ($29 \pm 3^\circ\text{C}$) and light (12L:12D) controlled room with provision for food (Hindustan Lever Ltd., Bangalore) and water *ad libitum*. Seven days after acclimatization, the animals were divided into four groups of six each. Group I served as the controls. Group II animals received by i.p. injection, 500 mg GalN (dissolved in physiological saline)/kg body wt/day for two days^{2,12}. Group III animals were pre-treated with 50 mg of an alcoholic extract of *P. kurroa* (in distilled water)/kg body wt/day for 10 days by oral intubation and then with GalN (500 mg/kg body wt/day) by i.p. injection for 2 days. Group IV animals were treated with *P. kurroa* alone at the above dosage for 10 days to test for any side-effects.

At the end of the experimental period, the animals were sacrificed by cervical decapitation. Blood was collected without any anticoagulant and the serum separated was used for the assay of diagnostic marker enzymes such as alanine aminotransferase (ALT) [EC 2.6.1.2]¹³, aspartate aminotransferase (AST) [EC 2.6.1.1]¹³, acid phosphatase (ACP) [EC 3.1.3.2]¹⁴, alkaline phosphatase (ALP) [EC 3.1.3.1]¹⁴, and lactate dehydrogenase (LDH) [EC 1.1.1.27]¹⁵. Liver was excised immediately and washed with chilled physiological saline. Liver mitochondria were isolated by the method of Johnson and Lardy¹⁶.

Mitochondrial protein was estimated by the method of Lowry *et al.*¹⁷. Lipid peroxide content was determined by the thiobarbituric acid (TBA) reaction as described by Ohkawa *et al.*¹⁸ and the non-enzymatic lipid peroxidation in the presence of promoters such as ascorbic acid, ferrous sulphate (FeSO_4) and *t*-butyl hydroperoxide (*t*-BH), 2mM each was studied. Reduced glutathione was measured by the method of Ellman¹⁹. The method described by Habig *et al.*²⁰ was followed for the estimation of GST [EC 2.5.1.18] activity and the activity of GPX [EC 1.11.1.9] was assayed by the method of Pagila and Valentine²¹.

The results presented here are the mean \pm SD of six animals. Level of significance has been evaluated by using Student's *t*-test.

Increased activities of serum ALT, AST, ACP, ALP and LDH are well-known diagnostic indicators of GalN hepatitis. In cases like liver damage with hepatocellular lesions and parenchymal cell necrosis, these enzymes are released from damaged tissues into the blood stream²². The present results indicate a marked elevation in the activities of these marker enzymes in serum (Table 1), which is in accordance with previous reports^{23–25}. Prior oral administration of *P. kurroa* extract resulted in a significant reduction in the levels of these enzymes towards near normalcy as compared to Group II GalN toxic rats, establishing its hepatoprotective effect.

GalN has been proposed to be hepatotoxic due to its ability to destruct liver cells, possibly by a free radical mechanism⁷. Lipid peroxidation reaction, a type of oxidative degeneration of polyunsaturated fatty acids, has been linked with altered membrane structure and enzyme inactivation²⁶. The highly significant elevation in non-enzymatic lipid peroxidation reactions in GalN-induced hepatitis suggests the enhanced susceptibility of the membranes. Significant increases in the levels of mitochondrial lipid peroxides after i.p. administration of GalN have already been reported²⁷. GalN-induced hypoglycaemia-related glucose auto-oxidation^{28,29} may also be responsible for the increased generation of free radicals in GalN hepatitis. Our results also suggest that GalN-intoxicated rats may be less resistant and more susceptible to lipid peroxidation in the presence of promoters like ascorbate, FeSO_4 and *t*-BH (Table 2). Pre-treatment with *P. kurroa* extract in our study significantly prevented this alteration, because of its antioxidant nature¹⁰ against lipid peroxidation induced by GalN²⁷.

Glutathione has a direct antioxidant function. It functions by reaction with superoxide radicals, peroxy radicals and

Table 1. Activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in normal and experimental groups of rats (mean \pm SD for six animals in each group)

Group	Group I	Group II	Group III	Group IV
ALT	110.8 \pm 9.8	374.3 \pm 35.9***	133.4 \pm 11.6***	112.9 \pm 10.3
AST	85.7 \pm 6.2	395.8 \pm 36.4***	118.6 \pm 9.7***	89.3 \pm 6.6
ACP	12.6 \pm 1.0	39.7 \pm 2.1***	19.2 \pm 1.2***	11.8 \pm 0.8
ALP	108.7 \pm 8.9	263.8 \pm 14***	126.2 \pm 10.5***	109.2 \pm 11.2
LDH	215.6 \pm 19.8	398.5 \pm 26.4***	228.6 \pm 19.6***	212.3 \pm 17.4

Group I, Normal controls; Group II, D-galactosamine-intoxicated rats; Group III, *P. kurroa* pre-treated and D-galactosamine-intoxicated rats; Group IV, *P. kurroa* treated normal rats.

Values expressed: ALT, AST, and LDH, μ moles of pyruvate liberated/h/l; ACP and ALP, μ moles of phenol liberated/h/l. As compared with respective controls, i.e. Group II vs Group I, Group III vs Group II: ****P* < 0.001.

Table 2. Protective effects of *P. kurroa* on mitochondrial glutathione antioxidant system in D-galactosamine-induced hepatitis in rats (mean \pm SD of six animals in each group)

Parameter	Group I	Group II	Group III	Group IV
Lipid peroxidation				
Basal	158.14 \pm 14.7	269.44 \pm 24.3***	182.61 \pm 16.5***	163.83 \pm 15.2
Ascorbate	324.35 \pm 23.8	514.81 \pm 40.7***	398.73 \pm 29.5***	316.78 \pm 25.7
FeSO ₄	452.83 \pm 39.5	686.54 \pm 48.9***	524.17 \pm 40.9***	445.35 \pm 41.4
t-BH	593.46 \pm 47.3	864.62 \pm 67.2***	678.71 \pm 53.4***	602.73 \pm 51.7
GSH	38.14 \pm 2.78	22.76 \pm 1.83***	33.58 \pm 2.61***	39.71 \pm 2.83
GPX	22.63 \pm 2.31	9.84 \pm 0.76***	17.58 \pm 1.42***	23.21 \pm 1.98
GST	2636.33 \pm 158.9	1471.24 \pm 104.3***	2265.32 \pm 129.8***	2594.42 \pm 164.3

Values expressed: Lipid peroxides, nmol MDA/mg protein; Glutathione, nmol/g tissue; GPX, nmol of glutathione oxidized/min/mg protein; GST, nmol of CDNB units/min/mg protein. As compared with respective control values, i.e. Group II vs Group I; Group III vs Group II: ****P* < 0.001.

singlet oxygen, followed by the formation of oxidized glutathione and other disulphides³⁰. Depletion of GSH results in enhanced lipid peroxidation³¹, and excessive lipid peroxidation can cause increased GSH consumption²⁶, as observed in the present study (Table 2). Tappel³² has reported that GSH protects the mitochondrial membrane from the damaging action of lipid peroxide. The oral pre-treatment of *P. kurroa* extract resulted in the elevation of GSH level, which protects against oxidative damage by regulating the redox status of proteins in the cell membrane³³.

GPX, an antioxidant enzyme, offers protection to the mitochondrial membrane from peroxidative damage³⁴. A decrease in the activity of GPX makes mitochondria susceptible to GalN-induced damage, which leads to a change in mitochondrial composition and function. Our studies also show decreased GPX activity in Group II GalN toxic rats (Table 2), which is in line with the report by Neihorster *et al.*³⁵. GPX and the cellular NADPH-

generating mechanism together form a system for removing hydroperoxides from the cell. Prior oral treatment of *P. kurroa* maintained the GPX activity significantly at near normal, as observed in Group III animals.

GST, another scavenging enzyme, binds to many different lipophilic compounds³⁶; so it would be expected to bind GalN and act as an enzyme for GSH conjugation reactions. The significant decrease in its activity noted in this study (Table 2) might have been due to the decreased availability of GSH. This is consistent with a reported study³⁷, which showed a reduction of GST activity in liver. These findings led to the conclusion that GSH and GSH-dependent enzyme systems may be directly related to the pathogenic mechanism of GalN hepatitis.

Prior oral administration with the alcoholic extract of *P. kurroa* in our study significantly prevented the alterations in the GST activity. It probably did so by counteracting the free radicals due to the presence of two electrophilic sub-

stances, picroside I and kutkoside, present in the roots of *P. kurroa*^{10,11}. The unpaired electron present in the hydroxyl free radical might have been trapped by these two substances.

Our observations indicate that the pre-treatment with *P. kurroa* prevents GalN-induced hepatitis in rats. This present study suggests a hepatoprotective effect of *P. kurroa* in experimental animals.

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R. ANANDAN
R. DEEPA REKHA
T. DEVAKI

Department of Biochemistry and
Molecular Biology,
University of Madras,
Guindy Campus,
Chennai 600 025, India

Ayan Guha

