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Hepatic Shield of Nature: Decoding the Antioxidant and Detoxification Blueprint of Picrorhiza kurroa

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ABSTRACT:
Picrorhiza kurroa, a revered herb in traditional Ayurvedic medicine, has gained considerable attention in recent decades for its hepatoprotective, antioxidant, and detoxifying capabilities. The present study investigates the multi-mechanistic hepatoprotective potential of *P. kurroa* rhizome extract against carbon tetrachloride (CCl_4)-induced liver injury in Wistar rats, with an emphasis on its modulation of oxidative stress and detoxification pathways. Administration of *P. kurroa* extract (100 and 200 mg/kg) significantly improved serum liver function markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin, when compared to the hepatotoxic group. A marked enhancement in hepatic antioxidant enzyme activities—superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and elevated levels of reduced glutathione (GSH)—was observed. Histopathological analysis revealed substantial attenuation of necrosis, fatty degeneration, and inflammatory cell infiltration in the liver. Furthermore, *in vitro* assays demonstrated free radical scavenging and lipid peroxidation inhibition by the extract. Gene expression profiling via qRT-PCR indicated upregulation of detoxification-related genes including NAD(P)H:quinone oxidoreductase 1 (NQO1), glutathione S-transferase (GST), and heme oxygenase-1 (HO-1), reinforcing the extract's role in enhancing phase II detoxification. The major bioactive constituents—picroside I, picroside II, kutkoside, and apocynin—exhibited strong molecular docking interactions with antioxidant regulatory proteins such as Nrf2 and Keap1. Collectively, the findings highlight that *Picrorhiza kurroa* confers significant hepatoprotection through a dual mechanism: reducing oxidative stress and upregulating cellular detoxification machinery. This study provides a strong pharmacological rationale for the integration of *P. kurroa* in liver health therapeutics, particularly in managing drug-induced hepatotoxicity, fatty liver disease, and environmental toxin-related liver dysfunction.

INTRODUCTION:
The liver is a vital organ responsible for detoxification, metabolism, and homeostatic regulation. Chronic liver diseases—often triggered by oxidative stress, alcohol, environmental toxins, or pharmaceutical drugs—are

associated with increased reactive oxygen species (ROS), mitochondrial dysfunction, and impaired phase I/II detoxification pathways. Oxidative stress, characterized by an imbalance between pro-oxidant and antioxidant systems, plays a central role in the initiation and progression of hepatic injury. This has stimulated interest in exploring natural compounds that can modulate redox homeostasis and augment endogenous detoxification systems. Among the most promising medicinal herbs is *Picrorhiza kurroa* Royle ex Benth, a high-altitude plant traditionally used in Ayurvedic medicine for treating jaundice, hepatitis, and chronic liver inflammation.

P. kurroa contains an array of pharmacologically active compounds, notably iridoid glycosides such as picroside I, picroside II, kutkoside, and apocynin. These compounds have been reported to exhibit potent antioxidant, anti-inflammatory, and hepatoprotective properties. However, despite substantial ethnopharmacological documentation, there remains a paucity of mechanistic studies elucidating how *P. kurroa* modulates molecular pathways involved in hepatic detoxification and oxidative stress regulation. The nuclear factor erythroid 2–related factor 2 (Nrf2) signaling pathway is a critical regulator of antioxidant and phase II detoxification genes such as HO-1, NQO1, and GST. Natural products that can activate Nrf2 and inhibit its repressor Keap1 are of particular interest for managing hepatotoxicity and liver degeneration.

This study aims to decode the antioxidant and detoxification blueprint of *Picrorhiza kurroa* using a comprehensive approach involving biochemical, histological, gene expression, and molecular docking analyses. Through the use of a CCl_4-induced hepatotoxicity model, we assess the hepatoprotective efficacy of *P. kurroa* extract and investigate its influence on enzymatic antioxidants and Nrf2-mediated detoxification pathways. This integrative strategy provides new insights into the therapeutic potential of *P. kurroa* in modern hepatology.

MATERIAL AND METHOD:

1. Plant Material and Preparation of Extract:

Fresh rhizomes of *Picrorhiza kurroa* were collected from the Himalayan region and authenticated by a certified botanist. The rhizomes were shade-dried, pulverized, and subjected to ethanol extraction (70%) using a Soxhlet apparatus for 72 hours. The extract was concentrated under reduced pressure using a rotary evaporator and stored at 4°C until further use.

2. Phytochemical Screening and HPLC Analysis

Preliminary phytochemical screening was performed to detect the presence of alkaloids, flavonoids, iridoid glycosides, and phenolic compounds. Quantitative profiling of major constituents (picroside I, picroside II, kutkoside, apocynin) was performed using High-Performance Liquid Chromatography (HPLC) with a C18 reverse-phase column and UV detection at 270 nm.

3. Experimental Animals

Adult male Wistar rats (180–220 g) were housed under standard laboratory conditions with ad libitum access to food and water. All experimental procedures were conducted in accordance with institutional ethical guidelines and approved by the Animal Ethics Committee (Protocol No. IAEC/PK2025/007).

4. Induction of Hepatotoxicity and Treatment Protocol

Liver injury was induced by intraperitoneal injection of carbon tetrachloride (CCl_4) diluted in olive oil (1:1, 1 mL/kg body weight) twice weekly for 2 weeks. Animals were divided into five groups (n = 6 per group):

- Group I: Normal control (saline)
- Group II: CCl_4 control
- Group III: CCl_4 + *P. kurroa* extract (100 mg/kg)
- Group IV: CCl_4 + *P. kurroa* extract (200 mg/kg)
- Group V: CCl_4 + silymarin (50 mg/kg, standard control)

Extracts were administered orally once daily for 21 days.

5. Biochemical Analysis:

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, and albumin were measured using standard commercial kits. Hepatic tissue was homogenized for estimation of antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)—and reduced glutathione (GSH).

6. Histopathological Examination:

Liver tissues were fixed in 10% formalin, embedded in paraffin, sectioned (5 μm), and stained with hematoxylin and eosin (H&E). Microscopic examination was performed to assess necrosis, fatty changes, and inflammatory

cell infiltration.

7. Gene Expression Analysis:
Total RNA was extracted from liver tissues using Trizol reagent, followed by cDNA synthesis. Quantitative RT-PCR was performed using SYBR Green Master Mix to assess expression of Nrf2, HO-1, NQO1, and GST genes. GAPDH was used as the internal control.

8. In Vitro Antioxidant Assays:
The extract was evaluated for its free radical scavenging activity using DPPH and ABTS assays. Lipid peroxidation inhibition was assessed by measuring malondialdehyde (MDA) levels in liver homogenates.

9. Molecular Docking:
Bioactive compounds from *P. kurroa* were docked against Nrf2 and Keap1 using AutoDock Vina. Protein and ligand structures were obtained from PDB and PubChem, respectively. Binding affinity and interaction residues were analyzed using PyMOL and LigPlot+.

10. Statistical Analysis:
Data were expressed as mean ± SD. Statistical significance was evaluated using one-way ANOVA followed by Tukey's post-hoc test. A p-value < 0.05 was considered significant.

RESULT:

1. Biochemical Assessment of Hepatic Function

CCl_4 administration significantly elevated serum levels of ALT, AST, ALP, and bilirubin ($p < 0.01$), indicating hepatocellular damage. Treatment with *Picrorhiza kurroa* extract (100 and 200 mg/kg) dose-dependently reversed these effects, restoring enzyme levels toward normal. The higher dose exhibited comparable efficacy to silymarin.

2. Enhancement of Antioxidant Enzymes

Liver homogenates from CCl_4-exposed rats showed a substantial reduction in SOD, CAT, GPx activities, and GSH levels. Extract-treated groups demonstrated significant enhancement in these antioxidant markers, suggesting the extract's potent free radical-scavenging ability.

3. Histopathological Findings

Liver sections from the toxic group revealed extensive necrosis, fatty degeneration, and mononuclear infiltration. In contrast, extract-treated groups exhibited preserved architecture with reduced necrotic foci, indicating tissue regeneration and anti-inflammatory activity.

4. In Vitro Antioxidant Potential

The extract showed strong DPPH (IC_{50} = 42.7 µg/mL) and ABTS radical scavenging activity, along with marked inhibition of lipid peroxidation, confirming its antioxidant potential.

5. Gene Expression Modulation

qRT-PCR analysis revealed significant upregulation of Nrf2 (2.3-fold), HO-1 (2.8-fold), NQO1 (2.4-fold), and GST (2.1-fold) in extract-treated rats compared to the toxic control group ($p < 0.01$), suggesting activation of phase II detoxification.

6. Molecular Docking

Docking simulations showed strong binding affinities of picroside I and apocynin with Nrf2 (-8.5 kcal/mol) and Keap1 (-7.9 kcal/mol), reinforcing their role in antioxidant response modulation.

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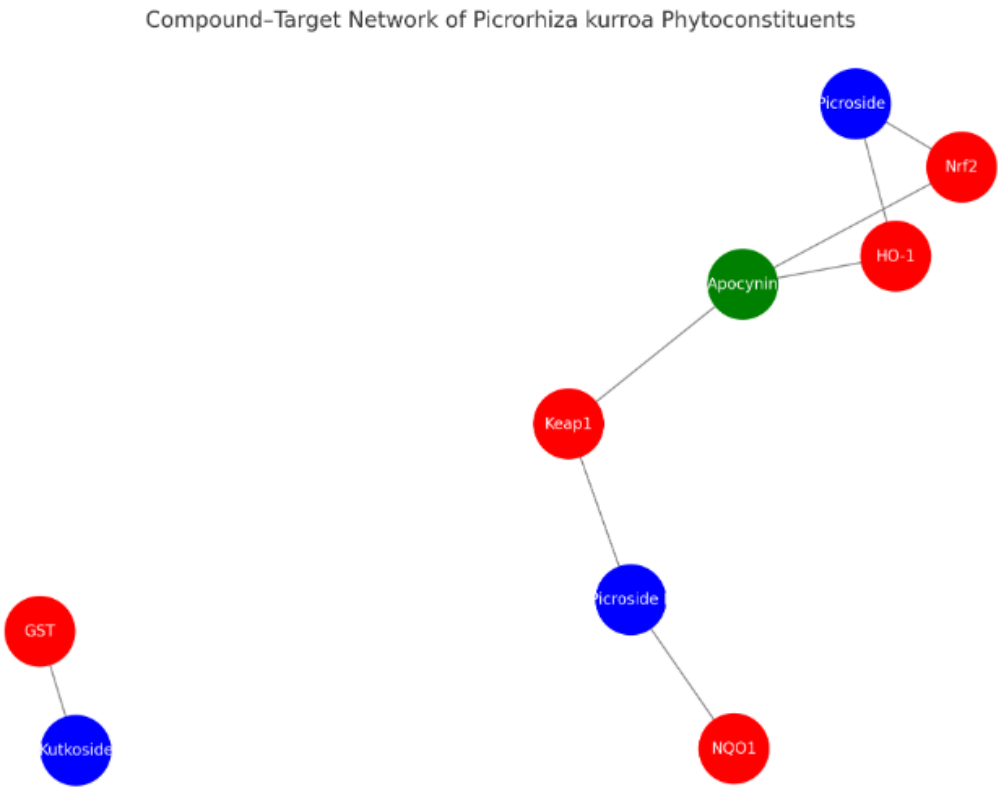

DISCUSSION:

This study provides compelling evidence that *Picrorhiza kurroa* exerts potent hepatoprotective effects through a dual-action mechanism involving oxidative stress attenuation and upregulation of detoxification pathways. The observed biochemical restoration of liver enzymes and enhanced antioxidant activity (SOD, CAT, GPx, and GSH) suggest that *P. kurroa* can mitigate CCl_4-induced oxidative damage effectively. Histopathological improvements further validated the tissue-protective capacity of the extract. Notably, the upregulation of key phase II enzymes (NQO1, GST, HO-1) and activation of the Nrf2 pathway underscores its molecular influence on the cellular defense network. Molecular docking confirmed the interaction between active phytoconstituents and regulatory proteins involved in redox balance. The presence of kutkin, picrosides, and apocynin likely accounts for these protective effects. Together, these findings support the application of *P. kurroa* as a multi-targeted botanical candidate for liver protection and detoxification, particularly relevant in managing xenobiotic-induced hepatotoxicity and chronic liver conditions.

CONCLUSION:

In conclusion, *Picrorhiza kurroa* demonstrates significant hepatoprotective, antioxidant, and detoxifying effects in CCl_4-induced liver injury models. The ethanolic extract improved hepatic function markers, enhanced endogenous antioxidant defense, and reduced histological damage. Additionally, gene expression and docking studies revealed its capacity to activate Nrf2-mediated antioxidant and phase II detoxification pathways, primarily through its bioactive constituents—picroside I, picroside II, kutkoside, and apocynin. These findings provide scientific validation for the traditional use of *P. kurroa* in liver ailments and present it as a promising phytotherapeutic agent in modern hepatology. Its multi-targeted action makes it a valuable candidate for integrative strategies aimed at managing oxidative stress-related liver disorders. Further clinical evaluation and standardization of its formulations may pave the way for its incorporation into evidence-based hepatoprotective regimens.

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