

Study of the preventive effect of hydroalcoholic extract of *Peuraria tuberosa* against Paracetamol Induced Hepatotoxicity in rats

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Abstract

The present study investigated the preventive effect of hydroalcoholic extract of *Pueraria tuberosa* against paracetamol-induced hepatotoxicity in Wistar rats. The tubers were extracted using 80% methanol and subjected to phytochemical screening, which confirmed the presence of alkaloids, flavonoids, phenolics, saponins, glycosides, and terpenoids. Acute toxicity studies established safety up to 2000 mg/kg. Hepatotoxicity was induced by paracetamol (2 g/kg, p.o.), and the protective effect of *P. tuberosa* extract was evaluated at doses of 100 and 200 mg/kg, compared with silymarin (100 mg/kg) as standard. Biochemical parameters including SGOT, SGPT, ALP, bilirubin, cholesterol, and triglycerides were assessed, alongside histopathological examination. The extract significantly reduced elevated liver enzymes and restored biochemical markers toward normal values in a dose-dependent manner. Histopathological analysis further confirmed hepatocyte protection, with near-normal architecture observed in extract-treated groups. These findings suggest that *P. tuberosa* possesses hepatoprotective potential, likely mediated by its antioxidant phytoconstituents, and may serve as a cost-effective natural alternative for managing drug-induced liver injury.

Keywords

Pueraria tuberosa; paracetamol; hepatotoxicity; hydroalcoholic extract; hepatoprotective activity

Introduction

The liver is a vital organ responsible for metabolism, detoxification, and regulation of biochemical processes. However, it is highly susceptible to damage from xenobiotics, drugs, and environmental toxins, which can lead to hepatotoxicity and progressive liver diseases such as cirrhosis and hepatocellular carcinoma¹. Paracetamol (acetaminophen) is widely used as an analgesic and antipyretic, but overdose or prolonged use can result in severe hepatotoxicity due to the accumulation of its toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI)². This metabolite depletes glutathione reserves and induces oxidative stress, leading to hepatocellular necrosis and elevated serum biomarkers such as SGOT, SGPT, ALP, and bilirubin³.

Synthetic hepatoprotective agents like silymarin are effective but often limited by cost and side effects, which has encouraged the exploration of medicinal plants as safer alternatives⁴. *Pueraria tuberosa* (Indian Kudzu), a member of the Fabaceae family, is traditionally used in Ayurveda for its rejuvenating, antioxidant, and hepatoprotective properties⁵. Phytochemical studies have revealed that its tubers contain flavonoids, phenolics, alkaloids, and glycosides, which may contribute to hepatoprotection through free radical scavenging and membrane stabilization⁶.

Given the global burden of drug-induced liver injury and the need for affordable natural remedies, the present study was designed to evaluate the preventive effect of hydroalcoholic extract of *Pueraria tuberosa* against paracetamol-induced hepatotoxicity in rats. By assessing biochemical parameters and histopathological changes, this work aims to provide experimental validation for the traditional use of *Pueraria tuberosa* and explore its potential as a hepatoprotective agent.

Material and Methods

Preparation of extract and phytochemical screening

The tubers of *Peuraria tuberosa* have been collected from local market and authenticated by botanist at RB Science, Bhopal. The authenticated tubers were rinsed with distilled water to remove dirt and impurities, dried under shade and powdered using a blender at low speed.

The powdered tubers were used for the extraction process by hot continuous extraction method using soxhlet apparatus. 91 g of tuber powder was evenly placed in the extractor of the apparatus and 350 mL of 80% methanol was poured over it and was allowed to collect in the attached flask. Extraction was achieved by heating the solvent at 65°C for 9 h till a colorless solution was collected in the siphon tube of the apparatus. The extract was filtered through Whatman filter and concentrated using rotary vacuum evaporator. The resinous extract was collected and stored in desiccator to remove the excessive moisture. The dried extract was stored in desiccator for further processing⁷.

The extract was evaluated by qualitative phytochemical screening in order to identify the type of plant secondary metabolites present in it. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation was used as analytical responses to these tests⁸.

Total Phenolic Content

In order to determine the total phenolic content 0.5 g dried powder was mixed with 5 mL of methanol and kept overnight. The suspension was filtered through a qualitative cellulose filter paper and the filtrate was diluted to 10 mL with methanol. The solution was stored at 4°C in amber bottles and served as the stock solution for subsequent analyses. For total phenolic content determination, 200 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After 3 min, 300 µL of 20% aqueous Na₂CO₃ solution was added to it and the mixture was allowed to settle for 2 h³³. The absorbance was measured at 760 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (20-100 ppm) were treated similarly to obtain the calibration curve. The control solution contained 200 µL of water and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample⁹.

Total Flavonoid content

The total flavonoid content will be determined using Aluminium chloride method using quercetin as the standard flavonoid for reporting the results. 0.1 g of dried extract was extracted with 10 ml methanol, filtered, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoid. Extract was mixed with 1 mL of 10% Sodium nitrite solution and 1 ml of 2% AlCl₃ methanolic solution was added to it and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm¹⁰.

Pharmacological Study

Animal Used

The male rat weighing between 180-230 g were used procured from approved suppliers from Bhopal. The rodents were allowed free access pellet diet (Lipton India Ltd, Mumbai, Ind.) and water *ad libitum*. All the laboratory conditions and animals were maintained as per CPCSEA guidelines throughout the experiments.

Acute Toxicity study

The short- and long-term toxic effects of both drugs and their extracts were performed within prescribed guideline set by OECD guideline no. 423. The albino rats of 150-200 g were employed for study and kept in 12 h day-night cycle with *ad libitum* access to water. The extract was suspended in 1% Tween 80, prepared in distilled water. Animals were fasted for 12 h with and extract was administered orally with increasing doses to 2000 mg/kg considering as maximum weight. The observation made in first 4 h for any bizarre behavior like change in skin and fur, eye, hyperactivity, grooming, convulsions, sedation, hypothermia, salivation, tremor, coma, lethargy, body weight, and mortality¹¹.

Paracetamol induced hepatotoxicity and hepatoprotective action

Paracetamol tablets and silymarin capsules (Limarin-140) was purchased from Pooja medical store, Bhopal. All other chemicals used for this study were of analytical grade.

Preparation of Paracetamol and Silymarin

Paracetamol was prepared in 0.5% sodium CMC solution and used for oral administration. Previous experiments for dose finding indicate Paracetamol 2g/kg was selected as the toxicant dose in the present study. Silymarin was dissolved in normal saline and 100mg/kg p.o. dose was selected as a standard.

Experimental Design

In the experiment, a total of 30 rats were used. The rats were divided into 5 groups comprising of 6 animals in each group as follows:

Group I: Normal control rats received 1ml/100gm of 0.5% sodium chloride using an intragastric tube for 7 days.

Group II: Negative control rats received paracetamol 2g/kg, p.o. for inducing hepatotoxicity

Group III: Rats received Silymarin (100mg/kg, p.o.) for 7 days and paracetamol 2g/kg, p.o. on 6th day.

Group IV Rats received 80% methanolic extract of *Peuraria tuberosa* 100mg/kg once daily for 7 days and paracetamol 2g/kg, p.o. on 6th day.

Group V Rats received 80% methanolic extract of *Peuraria tuberosa* 200mg/kg once daily for 7 days and paracetamol 2g/kg, p.o. on 6th day.

Sample collection

At the end of the experiment on 7th day, Rats were sacrificed by cervical dislocation. Blood was collected by orbital puncture and allowed to clot for 30 minutes at room temperature. The serum was separated by centrifugation at 3000 rpm at 30°C for 15 minutes.

Biochemical estimation¹²

The serum samples were subjected to biochemical parameters examination like ALT, SGOT, SGPT, Bilirubin, Triglyceride and Cholesterol levels were estimated by using standard kits (Span diagnostics Ltd).

SGOT (Serum Glutamic Oxaloacetic Transaminase)

In different tubes, 1 ml the buffered substrate was added to 0.1 ml of serum and incubated at 37°C for 30 min. Then 1 ml of DNPH reagent was added to arrest the reaction. To the blank tubes, 0.1 ml of enzyme was added only after the addition of DNPH reagent. The tubes were kept aside for 15 min and then 10 ml of 0.4 N sodium hydroxide was added and read at 505 nm in a UV spectrophotometer. The enzyme activity is expressed as UL⁻¹ of serum.

SGPT (Serum Glutamic Pyruvic Transaminase)

In different tubes, 1 ml the buffered substrate was added to 0.1 ml of serum and incubated at 37°C for 1 h. Then 1 ml of DNPH reagent was added to arrest the reaction. To the blank tubes, 0.1 ml of enzyme was added only after the addition of DNPH reagent. The tubes were kept aside for 15 min and then 10 ml of 0.4 N sodium hydroxide was added and read at 505 nm in a UV spectrophotometer. The enzyme activity is expressed as UL⁻¹ of serum.

Alkaline Phosphatase (ALP)

The incubation mixture contained the following compounds in a final volume of 3 ml of carbonate-bicarbonate buffer: 1 ml of substrate and 0.1 ml magnesium chloride and necessary amount of the enzyme source (0.2 ml of serum). The reaction mixture was incubated at 37°C for 15 min. The reaction was terminated by the addition of 1 ml Folin's phenol reagent. If turbidity appeared, the tubes were centrifuged. Controls without enzyme source were also incubated and the enzyme source was added after the addition of Folin's phenol reagent. Then 1 ml of 15% sodium carbonate solution was added and incubated further for 10 min at 37°C. The blue color developed was read at 510 nm against blank. The standard was also reared similarly. The activity is expressed as UL⁻¹ of serum.

Total bilirubin

Fresh serum 2 ml was pipetted in each of the two test tubes. Two of one tube 0.5 ml diazo reagent was added (test) and to the tube 0.5 ml diazo blank was added (blank). Then kept for 15 min and after those tubes are mixed with 5.7 ml of distilled water and 70% alcohol and kept for 10 min. The reading was taken at 540 nm. The level of total bilirubin was expressed as mg dL⁻¹ of serum.

Total cholesterol

Add 0.1 ml of serum to 10 ml of the ferric chloride acetic acid reagent in a glass stoppered centrifuge tube, mix well and stand for 10-15 min. for the proteins to flocculate and transfer 5 ml of the clear supernatant fluid to a another glass stoppered centrifuge tube. For the standard mix 0.1 ml of physiological saline and 10 ml of the cholesterol for the use and transfer 5 ml to a second stoppered centrifuge tube. As blank take 5 ml of the ferric acetic acid reagent in the third tube. Add 3 ml of sulphuric acid from a burette to all the three tubes stopper tightly and mix by repeated inversion. Remove the stopper carefully and stand for 20-30 min. Read unknown and standard against the blank at 505 nm.

Histopathological studies

The livers were excised quickly and fixed in 10% formalin and stained with haemotoxylin and eosin and then observed under microscope for degeneration, fatty changes, necrotic changes and evidence of hepatotoxicity if any.

Statistical analysis

All the values are expressed as mean $\pm$ standard error of mean (SEM) and analyzed for ANOVA and post hoc Dunnet's *t*-test by employing statistical software, GraphPad InStat 3. Differences between groups were considered significant at $p < 0.05$.

Results and Discussion

The extraction of the tuber was carried out using 80% methanol as the solvent. The extract was green in color with sticky appearance and yield of 16.37%w/w of crude drug. The 80% methanolic extract of *P. tuberosa* contained alkaloids, phenolics, flavonoids, saponins, glycosides, terpenoids and carbohydrates. The 80% methanolic extract of *Peuraria tuberosa* was found to contain 71.94 ± 1.224 GAE mg/g of phenolics and 88.99 ± 0.801 QE mg/g of flavonoids.

Acute toxicity of extract

The acute toxicity test was performed by using the dried extract at concentration of 2000 mg/kg to the test animal, administered orally. No animal died and hence the dose of upto 2000 mg/Kg was considered to be safe. As none of the animals died, the LD₅₀ was considered to be more than 2000 mg/Kg. From the observations and study, 1/10th and 1/20th of the lethal dose was used as therapeutic dose & cut-off values were chosen as 100 & 200 mg/kg to evaluate dose dependent action for the assessment of hepatoprotective activity.

Evaluation of Hepatoprotective action

The hepatitis associated with liver cirrhosis even hepatocellular carcinoma, which has become one of the most prevalent diseases in the world, can be induced by viruses, alcohol or other toxic chemicals. Increasing transaminase activities and jaundice were significantly observed in most hepatitis sufferers. Liver damage in animal models can be induced by Paracetamol from transaminases and ALP leakage in the blood can be detected, which is often associated with hepatonecrosis. In this study, the protective effect of 80% methanolic extract of tuber of *Peuraria tuberosa* on paracetamol induced hepatotoxicity was evaluated through various biochemical parameters.

Effect of extract on serum glutamate oxalacetate transminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) levels

The levels of SGOT and SGPT in normal control group were 87.03 ± 3.57 and 63.27 ± 2.84 respectively, while the levels of SGOT and SGPT were elevated in paracetamol treated rats and found to be 178.34 ± 7.81 and 158.46 ± 1.25 , respectively. The 80% methanolic extract of *Peuraria tuberosa* (100/200 mg/kg) decreased the levels of SGOT and SGPT, when compared with toxic group.

Effect of extract on alkaline phosphatase (ALP) level

The level of ALP in normal control group was found to be 67.41 ± 0.62 and elevated value was found to be in paracetamol treated rats (137.53 ± 0.81). The 80% methanolic extract *Peuraria tuberosa* decreased the level of ALP, when compared with toxic group at both the tested doses.

Effect of extract on total bilirubin (TB) level

The level of TB in normal control group was found to be 1.4 ± 0.32 and paracetamol treated rats was found to be 4.19 ± 0.68 . The 80% methanolic extract of *Peuraria tuberosa* decreased the level of TB, when compared with toxic group. The TB value of 100 and 200 mg/kg of extract were 3.13 ± 0.46 and 2.63 ± 0.32 respectively.

Effect of extract on total cholesterol (TC) level

The level of TC in normal control group was 74.84 ± 2.34 and elevated level was found to be paracetamol treated rats (88.38 ± 3.15). The 80% methanolic extract of *Peuraria tuberosa* lowered the levels of TC, almost to the normal levels.

Effect of extracts on triglyceride (TG) level

The level of triglyceride was found to be increased in the paracetamol treated rats (176.31 ± 9.94), where the level of TG in normal rat group was found to be 126.93 ± 5.36 . The 80% methanolic extract of *Peuraria tuberosa* (100 and 200mg/kg) significantly restored the level of TG, when compared with toxic rats. The TG level of 100 and 200 mg/kg of 80% methanolic extract was found to be 163.35 ± 5.68 and 154.08 ± 6.83 , respectively.

Table 1 Effect of 80% methanolic extract of *Peuraria tuberosa* on serum biochemical parameters in PCM induced hepatic injury in rats.

Group	ALP (IU/L)	SGOT (IU/L)	SGPT (IU/L)	Total Bilirubi n (mg/dl)	Triglyceri des (mg/dl)	Cholester ol (mg/dl)
Group I (Normal Control)	67.41 ± 0.62	87.03 ± 3.57	63.24 ± 2.84	1.4 ± 0.32	126.93 ± 5.36	74.84 ± 2.34
Group II (-ve Control PCM)	137.53 ± 0.81	178.34 ± 7.81	158.46 ± 1.25	4.19 ± 0.68	176.31 ± 9.94	88.38 ± 3.15
Group III (Std. Silymari n)	$71.56 \pm 0.37^{**}$	$90.72 \pm 4.79^{**}$	$79.56 \pm 4.97^{**}$	1.77 ± 0.76	138.04 ± 2.63	76.35 ± 0.49
Group IV (PTE, 100mg/k g)	$115.53 \pm 0.89^{**}$	$123.71 \pm 5.35^{**}$	$121.23 \pm 5.97^{**}$	3.13 ± 0.46	163.35 ± 5.68	79.28 ± 0.68
Group V (PTE, 200mg/k g)	$93.32 \pm 0.52^{**}$	$104.46 \pm 2.45^{**}$	$98.24 \pm 4.96^{**}$	2.63 ± 0.32	154.08 ± 6.83	78.27 ± 2.69

The data obtained were analyzed by one-way ANOVA followed posthoc Dunnet's *t*-test. Each value represents the mean $\pm$ SEM., n= 6. ****P < 0.05**, compared with negative control group

Histopathology

Histopathological study was performed to provide direct evidence of the hepatotoxicity of paracetamol and the effect of the 80% methanolic extract of *Peuraria tuberosa*. Prominent disturbances of the structure of hepatocytes were noted in liver tissue of rats treated with paracetamol (Figure 1).

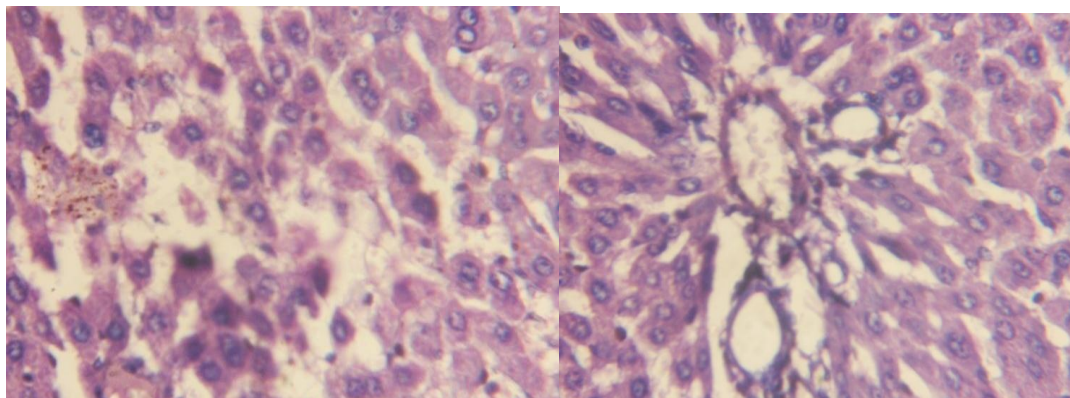


Figure 1 Liver section of (A) normal (B) toxic groups

The cells were able to be restored completely in the Silymarin treated group and were almost restored in the extract treated group (Figure 2).

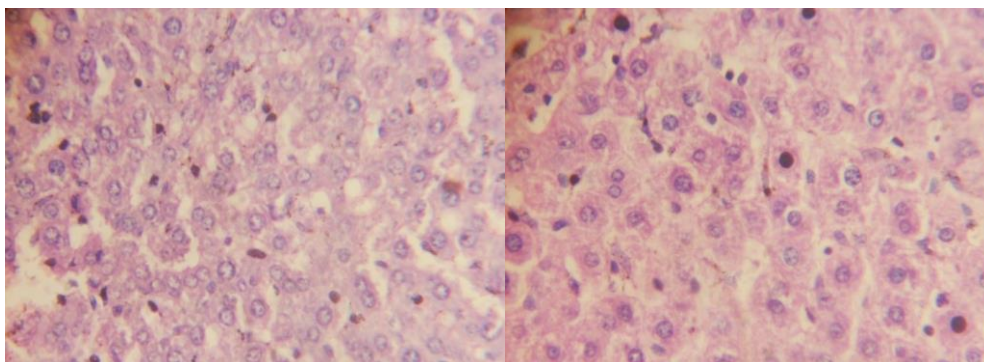


Figure 2 Liver section of (A) Silymarin treated (B) Extract treated groups

Conclusion

The hydroalcoholic extract of *Pueraria tuberosa* demonstrated significant hepatoprotective activity against paracetamol-induced liver damage in rats. Treatment with the extract at 100 and 200 mg/kg effectively reduced elevated serum levels of SGOT, SGPT, ALP, bilirubin, cholesterol, and triglycerides, indicating restoration of liver function. Histopathological studies supported these findings, showing marked improvement in hepatic architecture compared to the toxic control group. The hepatoprotective effect was dose-dependent, with the higher dose approaching the efficacy of silymarin. The safety profile established in acute toxicity studies further supports its potential therapeutic use. Overall, the study validates the traditional use of *Pueraria tuberosa* and highlights its promise as a natural, affordable hepatoprotective agent. Future research should focus on isolating active compounds, elucidating molecular mechanisms, and conducting clinical evaluations to confirm its efficacy in humans.

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