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# Investigating the Safety and Hepatoprotective Efficacy of Alkaline Extract of *Tephrosia purpurea* in a Wistar Rat Model

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**ABSTRACT**
Liver damage has become a widespread issue and is a significant metabolic disorder that leads to high rates of mortality and illness worldwide. Finding effective treatments has become a crucial topic in current research since numerous medications can cause toxic effects that harm the liver, thereby increasing interest in the healing properties of medicinal plants with hepatoprotective capabilities to alleviate or heal liver diseases. Various herbal plants have been studied for their ability to protect the liver from damage during the treatment of different liver conditions, with *Sharpunkha* (*Tephrosia Purpurea*) being one notable example. This study focuses on the hepatoprotective activity of Sharpunkha Kshar against liver toxicity which was induced by paracetamol in albino rats. Two distinct experiments were carried out: one for acute toxicity testing to confirm safety and another utilizing a paracetamol induced liver toxicity model to assess therapeutic effects. The evaluation of acute toxicity was conducted according to the OECD guideline 423. The findings demonstrate that Sharpunkha Kshar is safe even at a high dose of 2000 mg/kg and shows hepatoprotective effects on par with Liv 52 at a dosage of 200 mg/kg, which includes reducing liver cell death and normalizing biochemical markers.

**Keywords:** *Sharpaksha lavana, yakritpliha roga*, alkaline ash, paracetamol-induced hepatotoxicity, albino rats

## Introduction

Numerous herbal plants have been studied for their potential to protect the liver and treat various liver conditions, *Sharpunkha (Tephrosia purpurea)* being one of them. Sharpunkha was first introduced by Acharya Shusrut as he categorized medicinal plants into groups.(1) It was included in the *Sursadigana* (A group of herbal drugs). In Bhavprakash Samhita, Sharpunkha is referenced for the treatment of "*yakrit-pleeha roga*" (liver and spleen disorders).(2) Whole plant powder and Water-soluble Alkaline extract of *Tephrosia purpurea* is often applied to manage conditions like liver and spleen-related disorders and other digestive problems.(3) Hepatotoxicity continues to be a major health issue, frequently triggered by medications such as paracetamol.(4)Sharpunkha Kshar (alkaline extract of *Tephrosia purpurea* Linn.), which is traditionally used in Ayurveda for liver ailment and was used in this research to assess its safety and effectiveness in albino rats.

## Drugs and chemicals

Tephrosia purpurea (whole plant) was procured from the Dr. SRRAU campus in Jodhpur, and authenticated using microscopic, physicochemical, and phytochemical analysis. Lupeol, Rutin, quercetin, rotenone, and beta-sitosterol were identified as biomarkers in the Tephrosia purpurea through HPTLC fingerprinting. For the experimental study, Sharpunkha Kshar was utilized. The preparation of Sharpunkha Kshar was done in accordance with the guidelines of the Ayurvedic Formulary of India. The Kshar thus obtained was utilized for the experimental study.

## Animals

Wistar albino rats of both sexes, each weighing approximately 200±20 gm, were obtained from the Animal house associated with Bilwal Medchem and Research Laboratory, Jaipur. The animals were kept under standard husbandry conditions, including a temperature range of 22±3ºC, a relative humidity of 50–60%, and a 12-hour light and dark cycle. The chosen animals underwent a 7-day acclimatization period prior to the experiments. They were provided with standard rat pellet feed and drinking water. The experiments proceeded with the approval of the Institutional Animal Ethics Committee (BMRL/AD/CPCSEA/IAEC/2023/02/9) and in compliance with the guidelines set by CPCSEA, New Delhi.

## Acute Toxicity Assessment

The acute toxicity of Sharpunkha Kshar was assessed in two sets of albino rats in accordance with OECD guideline 423(5):
Group 1: Administered 300 mg/kg orally (n=3).
Group 2: Administered 2000 mg/kg orally (n=3).

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**Table1- Weight and Dose determination in group 1**

| **Group 1** | | | |
|---|---|---|---|
| **Marking** | **Weight (gm)** | **Dose in mg** | **Dose in ml (20% w/v Solution)** |
| **H** | 155 | 46.5 | **0.23** |
| **B** | 164 | 49.2 | **0.25** |
| **T** | 171 | 51.3 | **0.26** |

**Table2- Weight and Dose determination in group 2**

| **Group 2** | | | |
|---|---|---|---|
| **Marking** | **Weight (gm)** | **Dose in mg** | **Dose in ml (20% w/v Solution)** |
| **H** | 184 | 368 | **1.84** |
| **B** | 176 | 352 | **1.76** |
| **T** | 159 | 318 | **1.59** |

Observations are monitored over 14 days such as behavioral alterations, condition of the skin and fur, status of mucous membranes, and physiological symptoms such as salivation, lethargy, and convulsions. Additionally, food and water consumption, weight variations, and mortality rates were recorded.

**Hepatoprotective Investigation**

A model of PCM-induced liver toxicity was utilized:

- Subjects: Eighteen healthy albino Wistar rats were separated into three groups (n=6/group).
- Induction: 1000 mg/kg of PCM was administered orally for a duration of seven days.

**Group design**

- Group 1 (Control): this group was treated with distilled water (5 mL/kg/day).
- Group 2 (Standard): treated with Liv 52 (800 mg/kg/day).
- Group 3 (Test): treated Sharpunkha Kshar (200 mg/kg/day).

**Dosing Chart**

**Table 3- Dose administration of Paracetamol to induce the hepatotoxicity in Group-1**

| **Group 1** | | | | |
|---|---|---|---|---|
| **Marking** | **Weight (gm)** | **Dose of PCM** | | **Dose in ml (Distilled Water)** |
| | | **Dose in mg** | **Dose in ml** | |
| **H** | 153 | 153 | **1.53** | **0.765** |
| **B** | 147 | 147 | **1.47** | **0.735** |
| **T** | 169 | 169 | **1.69** | **0.845** |
| **HB** | 168 | 168 | **1.68** | **0.84** |
| **BT** | 175 | 175 | **1.75** | **0.875** |
| **HT** | 195 | 195 | **1.95** | **0.975** |

**Table 4- Dose administration of Paracetamol to induce the hepatotoxicity in Group-2**

| **Group 2** | | | | | |
|---|---|---|---|---|---|
| | | **Dose of PCM** | | **Dose of Liv 52** | |
| **Marking** | **Weight (gm)** | **Dose in mg** | **Dose in ml** | **Dose in mg** | **Dose in ml** |
| **H** | 175 | 175 | **1.75** | 140 | **1.4** |
| **B** | 169 | 169 | **1.69** | 135.2 | **1.352** |
| **T** | 158 | 158 | **1.58** | 126.4 | **1.264** |
| **HB** | 176 | 176 | **1.76** | 140.8 | **1.408** |
| **BT** | 178 | 178 | **1.78** | 142.4 | **1.424** |
| **HT** | 169 | 169 | **1.69** | 135.2 | **1.352** |

**Table 5- Dose administration of Paracetamol to induce the hepatotoxicity in Group-3**

| **Group 3** | | | | | |
|---|---|---|---|---|---|
| | | **Dose of PCM** | | **Sharpunkha Kshar** | |
| **Marking** | **Weight(gm)** | **Dose(mg)** | **Dose(ml)** | **Dose(mg)** | **Dose(ml)** |
| **H** | 185 | 185 | **1.85** | 37 | **0.37** |
| **B** | 195 | 195 | **1.95** | 39 | **0.39** |
| **T** | 174 | 174 | **1.74** | 34.8 | **0.348** |

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| | | | | | |
|---|---|---|---|---|---|
| **HB** | 165 | 165 | **1.65** | 33 | **0.33** |
| **BT** | 157 | 157 | **1.57** | 31.4 | **0.314** |
| **HT** | 169 | 169 | **1.69** | 33.8 | **0.338** |

**Evaluated Parameters:**

- Biochemical indicators: SGOT, SGPT, Serum ALP, and Serum Bilirubin levels.
- Histopathological analysis of liver tissue for signs of necrosis, degeneration, and edema.

**Statistical test**

Statistical evaluation using Student's t-test and ANOVA followed by Dunnett's post hoc test.

**Tissue Processing and Staining:**

Liver tissues were preserved using 10% buffered formalin. Dehydration involved the use of increasing concentrations of alcohol. Sections measuring 5 µm in thickness were sliced and stained with hematoxylin and eosin. The prepared slides were analyzed under a light microscope for histopathological assessment. Prepared slides were examined under a light microscope for histopathological evaluation.

**Results & observation:**

**Acute toxicity**

During the 14-day observation period, no signs of toxicity were observed in either group. Behavioral parameters, food and water consumption, and body weight remained within normal ranges. Both the 300 mg/kg and 2000 mg/kg doses were well tolerated, with no recorded mortality.

**Behavioral Observation**

**Table 5- Behavioral observations of Group-1 in Oral Acute toxicity**

| **Group 1** | | | | | | |
|---|---|---|---|---|---|---|
| **Observation** | **30min.** | **4hr.** | **24hr.** | **48hr.** | **1w** | **2w** |
| **Skin and Fur** | N | N | N | N | N | N |
| **Eyes** | N | N | N | N | N | N |
| **Mucous Membrane** | N | N | N | N | N | N |
| **Salivation** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Lethargy** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Sleep** | N | N | N | N | N | N |
| **Coma** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Convulsions** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Tremors** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Diarrhea** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Morbidity** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Mortality** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Weight changes (%)** | 0 | 0 | 1.324 | 2.574 | 6.241 | 13.854 |
| **Food consumption (gm)/rat** | 2.5 | 7.65 | 12.58 | 25.84 | 85.74 | 170.65 |
| **Water consumption (ml)/rat** | 4 | 5 | 40 | 75 | 255 | 520 |

**Table 6- Behavioral observations of Group-2 in Oral Acute toxicity**

| **Group 2** | | | | | | |
|---|---|---|---|---|---|---|
| **Observation** | **30min.** | **4hr.** | **24hr.** | **48hr.** | **1w** | **2w** |
| **Skin and Fur** | N | N | N | N | N | N |
| **Eyes** | N | N | N | N | N | N |
| **Mucous Membrane** | N | N | N | N | N | N |
| **Salivation** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Lethargy** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Sleep** | N | N | N | N | N | N |
| **Coma** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Convulsions** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Tremors** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Diarrhea** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Morbidity** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Mortality** | Ab | Ab | Ab | Ab | Ab | Ab |
| **Weight changes (%)** | 0 | 0 | 2.651 | 3.147 | 7.617 | 14.954 |
| **Food consumption (gm)/rat** | 3.45 | 8.74 | 13.54 | 21.36 | 90.45 | 181.36 |
| **Water consumption (ml)/rat** | 2.5 | 4 | 35 | 80 | 265 | 545 |

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http://www.veterinaria.org
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**Hematology Observation**

**Table 7- Hematological observations of Group-1 in Oral Acute toxicity**

| **Group 1** | | | |
|---|---|---|---|
| **Tests** | **H** | **B** | **T** |
| **RBC (x10 /mm³)** | 7.65 | 8.18 | 7.96 |
| **PCV (%)** | 41.36 | 45.74 | 39.68 |
| **Hb (g/dl)** | 14.85 | 13.65 | 15.74 |
| **WBC (X10³/mm³)** | 9.32 | 12.74 | 9.91 |
| **Neutrophils (%)** | 24.32 | 21.36 | 25.74 |
| **Lymphocytes (%)** | 74.19 | 76.35 | 74.35 |
| **Eosinophils (%)** | 1.35 | 1.85 | 2.47 |
| **Monocytes (%)** | 2.45 | 1.95 | 2.12 |
| **Basophils (%)** | 0.0 | 0.0 | 0.0 |
| **Platelets (X10³/mm³)** | 685 | 745 | 673 |

**Table 8- Hematological observations of Group-2 in Oral Acute toxicity**

| **Group 2** | | | |
|---|---|---|---|
| **Tests** | **H** | **B** | **T** |
| **RBC x10 /mm³)** | 8.95 | 8.15 | 9.15 |
| **PCV (%)** | 41.32 | 38.87 | 44.58 |
| **Hb(g/dl)** | 15.85 | 14.73 | 14.81 |
| **WBC(X10³/mm³)** | 14.36 | 13.74 | 15.71 |
| **Neutrophils (%)** | 31.25 | 29.74 | 24.69 |
| **Lymphocytes(%)** | 67.85 | 69.32 | 71.65 |
| **Eosinophils(%)** | 3.54 | 2.41 | 1.75 |
| **Monocytes(%)** | 2.47 | 2.37 | 2.17 |
| **Basophils(%)** | 0.00 | 0.00 | 0.00 |
| **Platelets(X10³/mm³)** | 758 | 691 | 789 |

**Biochemical tests**

**Table 9- Biochemical analysis of Group 1&2 in Oral Acute toxicity**

| **Name of Test** | **Group 1** | | | **Group 2** | | |
|---|---|---|---|---|---|---|
| | **H** | **B** | **T** | **H** | **B** | **T** |
| **Protein (g/dl)** | 6.41 | 6.18 | 5.96 | 5.96 | 6.67 | 6.15 |
| **Albumin (g/dl)** | 3.47 | 2.94 | 3.65 | 3.74 | 3.87 | 3.91 |
| **Globulin (g/dl)** | 2.75 | 2.96 | 2.95 | 2.98 | 3.15 | 2.85 |
| **Glucose (mg/dl)** | 95 | 96 | 75 | 77 | 96 | 83 |
| **Urea nitrogen(mg/dl)** | 18.36 | 17.69 | 19.74 | 16.58 | 20.65 | 19.28 |
| **Creatinine (mg/dl)** | 0.27 | 0.65 | 0.48 | 0.37 | 0.57 | 0.69 |
| **Bilirubin (mg/dl)** | 0.35 | 0.41 | 0.39 | 0.39 | 0.51 | 0.41 |
| **Cholesterol (mg/dl)** | 75 | 84 | 91 | 85 | 95 | 82 |

**Table 10- Biochemical mean comparison of Group 1&2 in Oral Acute toxicity**

| **Name of Test** | **Group 1 Mean±SEM** | **Group 2 Mean±SEM** | **Reference Value** |
|---|---|---|---|
| **Protein (g/dl)** | 6.18±0.130 | 6.26±0.212 | 5.6 - 7.6 |
| **Albumin (g/dl)** | 3.35±0.213 | 3.84±0.051 | 2.8 - 4.8 |
| **Globulin (g/dl)** | 2.89±0.068 | 2.99±0.087 | 1.8 – 3.2 |
| **Glucose (mg/dl)** | 88.67±6.839 | 85.33±5.608 | 50 – 135 |
| **Urea nitrogen(mg/dl)** | 18.60±0.603 | 18.84±1.196 | 15 – 21 |
| **Creatinine (mg/dl)** | 0.47±0.110 | 0.54±0.093 | 0.2 - 0.8 |
| **Bilirubin (mg/dl)** | 0.38±0.018 | 0.44±0.037 | 0.2 - 0.55 |
| **Cholesterol (mg/dl)** | 83.33±4.631 | 87.33±3.930 | 40 – 130 |

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**Hepatoprotective study**

**Table 11- SGOT analysis of Group 1,2 & 3**

| SGOT (IU/L) | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| H | 236 | 94 | 156 |
| B | 245 | 96 | 126 |
| T | 215 | 89 | 142 |
| HB | 236 | 114 | 135 |
| BT | 195 | 96 | 120 |
| HT | 219 | 84 | 169 |

**Table 12- SGPT analysis of Group 1,2 & 3**

| SGPT(IU/L) | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| H | 195 | 86 | 110 |
| B | 186 | 75 | 123 |
| T | 174 | 69 | 142 |
| HB | 169 | 58 | 142 |
| BT | 175 | 75 | 126 |
| HT | 165 | 69 | 105 |

**Table 13- Serum alkaline phosphates analysis of Group 1,2 & 3**

| Serum Alkaline Phosphates (IU/L) | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| H | 354 | 156 | 215 |
| B | 296 | 198 | 269 |
| T | 315 | 145 | 159 |
| HB | 275 | 169 | 163 |
| BT | 296 | 175 | 148 |
| HT | 245 | 169 | 169 |

**Table 14- Total serum bilirubin analysis of Group 1,2 & 3**

| Total Serum Bilirubin (g/dl) | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| H | 0.23 | 0.19 | 0.24 |
| B | 0.31 | 0.21 | 0.31 |
| T | 0.29 | 0.24 | 0.25 |
| HB | 0.31 | 0.18 | 0.24 |
| BT | 0.25 | 0.21 | 0.23 |
| HT | 0.29 | 0.25 | 0.31 |

**Biochemical Parameters (Hepatoprotective Study)**

**Table 15- Mean comparison of Biochemical parameters of Group 1,2 & 3**

| Parameter | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| SGOT (IU/L) | 224.33 ± 7.47 | 95.50 ± 4.16 | 141.33 ± 7.55 |
| SGPT (IU/L) | 177.33 ± 4.57 | 72.00 ± 3.78 | 168.67 ± 7.35 |
| ALP (IU/L) | 296.83 ± 15.01 | 168.67 ± 7.35 | 187.17 ± 18.90 |
| Bilirubin (mg/dL) | 0.280 ± 0.013 | 0.213 ± 0.011 | 0.263 ± 0.015 |

**Statistical Analysis**

Dunnett's multiple comparison test revealed significant reductions ($p < 0.0001$) in SGOT, SGPT, and ALP levels in Groups 2 and 3 compared to Group 1, with Group 3 showing comparable efficacy to Liv 52.

**Table 15- Statistical comparison of Biochemical parameters of Group 1,2 & 3**

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| **SGOT** | | | | | |
| **Group 1 vs. Group 2** | 128.8 | 106.1 to 151.6 | Yes | **** | <0.0001 |
| **Group 1 vs. Group 3** | 83.00 | 60.27 to 105.7 | Yes | **** | <0.0001 |
| **SGPT** | | | | | |
| **Group 1 vs. Group 2** | 105.3 | 88.04 to 122.6 | Yes | **** | <0.0001 |

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http://www.veterinaria.org
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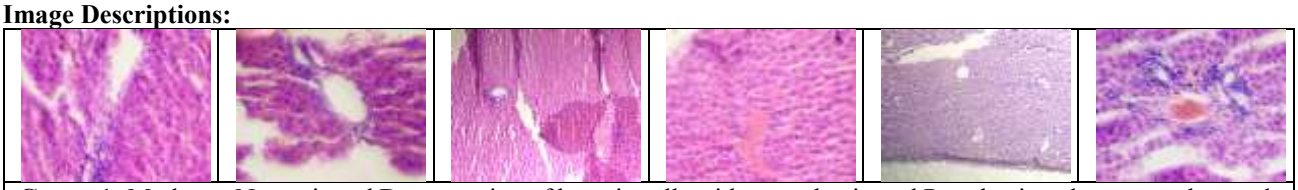
| | | | | | |
|---|---|---|---|---|---|
| **Group 1 vs. Group 3** | 52.67 | 35.38 to 69.96 | Yes | **** | <0.0001 |
| **Serum ALP** | | | | | |
| **Group 1 vs. Group 2** | 128.2 | 77.92 to 178.4 | Yes | **** | <0.0001 |
| **Group 1 vs. Group 3** | 109.7 | 59.42 to 159.9 | Yes | *** | 0.0002 |
| **Serum Bilirubin** | | | | | |
| **Group 1 vs. Group 2** | 0.06667 | 0.02086 to 0.1125 | Yes | ** | 0.0055 |
| **Group 1 vs. Group 3** | 0.01667 | 0.02914 to 0.06247 | No | ns | 0.5890 |

After Dunnett's multiple comparisons test between Group 1 vs. Group 2 and Group 1 vs. 3, the mean difference, at a 95% Confidential interval of difference, and P Values to determine the significance were calculated.
The mean difference for SGOT between Group 1 vs. Group 2 is 128.8 and 83.0 between Group 1 and 3, and the Mean difference for SGPT between Group 1 vs. Group 2 is 105.3 and 52.67 between Group 1 and 3, the Mean difference for Serum ALP between Group 1 vs. Group 2 is 128.2 and 109.7 between Group 1 and 3, the Mean difference for Serum bilirubin between Group 1 vs. Group 2 is 0.06667 and 0.01667 between Group 1 and 3.

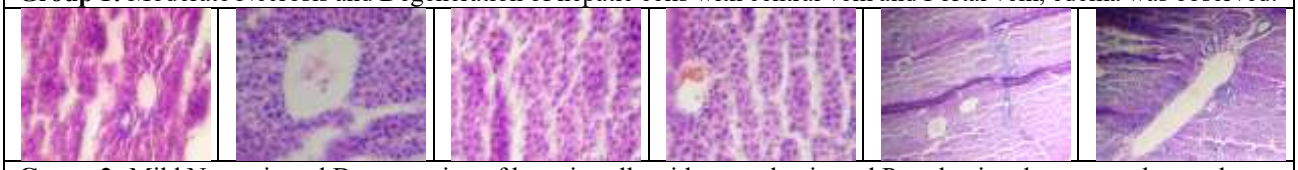
**P value significance**
P -value in group-1 vs group-2 is less than 0.0001. it is suggested that SGOT is significantly decreased in group-2 as compared to group-1.
p- value in group-1 vs group-3 is also less than 0.0001. it is suggested that SGOT is significantly decreased in group-3 as compare to group -1.
p- value in group-1 vs group-2 is also less than 0.0001. it is suggested that SGPT is significantly decreased in group-2 as compare to group -1.

**Histopathology**
Group 1: Moderate necrosis and edema.
Group 2: Mild necrosis and edema.
Group 3: Mild necrosis, reduced edema, and sinusoidal dilation.

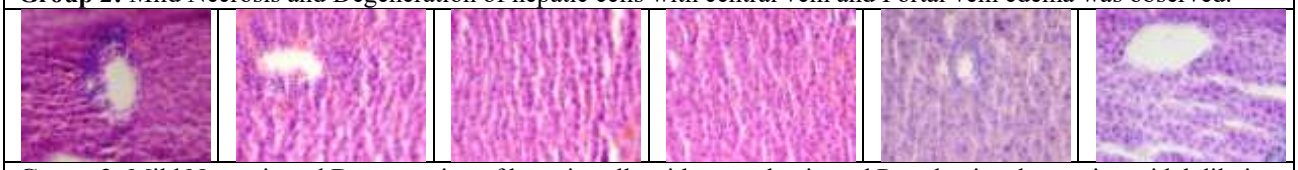
**Image Descriptions:**



**Group 1:** Moderate Necrosis and Degeneration of hepatic cells with central vein and Portal vein, edema was observed.



**Group 2:** Mild Necrosis and Degeneration of hepatic cells with central vein and Portal vein edema was observed.



**Group 3:** Mild Necrosis and Degeneration of hepatic cells with central vein and Portal vein edema, sinusoidal dilation was observed.

**Discussion**
The present study investigated the acute toxicity and hepatoprotective activity of *Sharpunkha Kshar* in albino Wistar rats using a paracetamol-induced hepatotoxicity model. The findings demonstrate that *Sharpunkha Kshar* is safe at doses up to 2000 mg/kg and exhibits significant hepatoprotective activity comparable to the standard hepatoprotective agent, Liv-52.
The acute toxicity study revealed no mortality or behavioral abnormalities in rats treated with *Sharpunkha Kshar* at doses of 300 mg/kg and 2000 mg/kg. Parameters such as food and water intake, body weight gain, and hematological and biochemical indices remained within normal ranges.
In the present study, control group exhibited marked elevation in serum SGOT, SGPT, ALP, and bilirubin levels. Treatment with *Sharpunkha Kshar* (200 mg/kg) significantly reduced the serum levels of SGOT, SGPT, and ALP compared to the toxic control group ($p < 0.0001$), indicating effective restoration of liver function. Although the reduction in bilirubin

levels was not statistically significant, the trend towards normalization suggests partial correction of impaired bilirubin metabolism. Microscopic examination of liver tissues further corroborated the biochemical observations. The control group showed moderate necrosis, edema, and degeneration of hepatocytes, while the Liv-52-treated group exhibited only mild pathological changes. Similarly, *Sharpunkha Kshar*-treated rats displayed mild necrosis and reduced edema with sinusoidal dilation, indicating substantial hepatocellular protection.

Earlier research showed that oxidative stress in the liver is marked by reduced levels of glutathione (GSH) as well as lower activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transfe*Rasa*e (GST), along with a rise in malondialdehyde (MDA) production. Glutathione (GSH), recognized as a key non-enzymatic antioxidant, is vital for safeguarding cells and its abundance significantly influences how vulnerable tissues are to oxidative injury.(6)

The hepatoprotective effect of *Sharpunkha Kshar* can be attributed to its phytoconstituents such as lupeol, rutin, quercetin, and beta-sitosterol, which are known for their antioxidant, anti-inflammatory, and membrane-stabilizing properties. These bioactive compounds likely mitigate oxidative stress and enhance hepatic cell regeneration.

Although Liv-52 showed greater efficacy in reducing biochemical markers than *Sharpunkha Kshar*, the latter demonstrated a significant protective effect and was well tolerated.

Its effectiveness is linked to its antioxidant characteristics, which help decrease oxidative stress and enhance liver function. These results are consistent with traditional beliefs concerning Tephrosia purpurea's potential for liver protection.

**Conclusion**

Alkaline extract of Tephrosia purpurea is considered safe at doses up to 2000 mg/kg and shows protective effects on the liver. Administered at 200 mg/kg, it significantly mitigates liver damage caused by PCM-induced hepatotoxicity, suggesting it could serve as a potential replacement for Liv 52 in liver protection.

**Future Scope**

Future research with larger sample sizes and clinical trials are advised to validate these results.

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