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## Protective and ameliorative effects of *Picrorhiza kurroa* rhizome extract against drug-induced liver injury in rats model

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### ABSTRACT

**Introduction and aim.** The study evaluated the hepatoprotective and antioxidant properties of *Picrorhiza kurroa* rhizome extracts in rats, assessing their ability to scavenge free radicals and protect against liver damage.

**Material and methods.** Liver damage was observed in Wistar rats after seven days of oral paracetamol (PCM) and azithromycin (AZM) combination therapy, with serum biomarkers evaluated for effect.

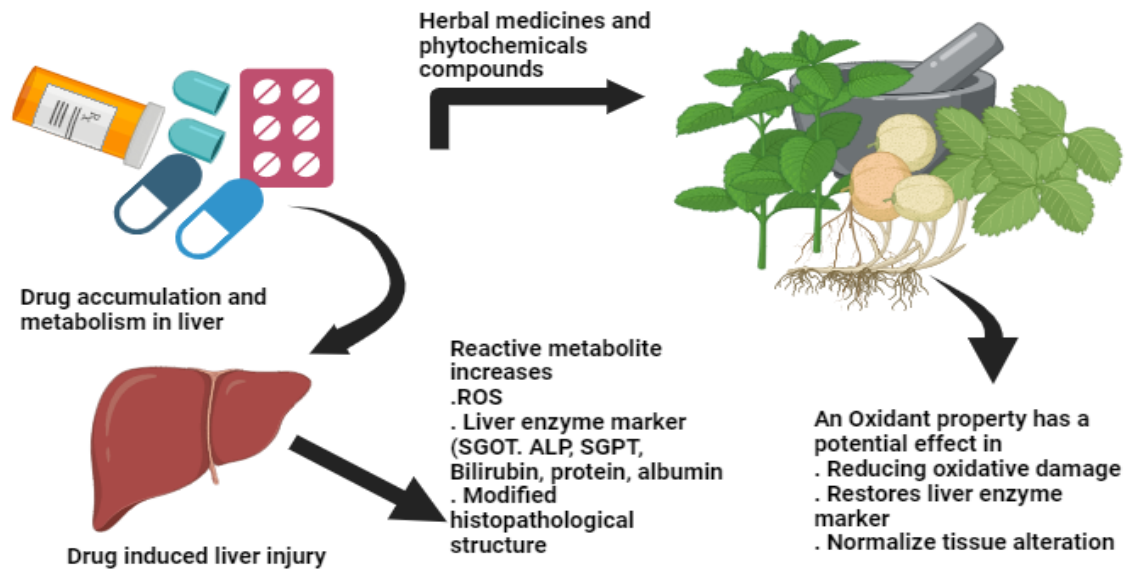
**Results.** During the DPPH experiment, the antioxidant DPPH assay on rats' livers revealed that the ethanol extract of *P. kurroa* demonstrated free radical scavenging activity. The crude ethanol extract of *P. kurroa* showed a 15.62% yield and 48.62 IC<sub>50</sub> values in an antioxidant DPPH experiment. Long-term treatment reduces liver toxicity by balancing biochemical factors. When compared to the group that received only PCM and AZM, the rats treated with *P. kurroa* crude extract showed a significant decrease in alkaline phosphatase, aspartate aminotransferase, glutamate pyruvic transaminase, and bilirubin (p<0.001) while showing an increase in protein and albumin at all doses (p<0.05). In addition, it was reprovved by *in vivo* antioxidant parameters such as superoxide dismutase, lactate dehydrogenase, catalase, and glutathione, which were also examined to verify its strong hepatoprotective effect.

**Conclusion.** The study found that the ethanolic extract of *P. kurroa* rhizome has the potential to protect against liver damage caused by PCM and AZM due to its complementary anti-oxidant properties.

**Keywords.** antioxidant activity, hepatoprotective effects, liver damage, *Picrorhiza kurroa*

## Introduction

Although drug-induced hepatotoxicity is well understood, it remains a significant public health concern. A serious problem is the glutamate pyruvic transaminase (SGPT) issue. The two primary areas affected by the obstacles presented by this condition are the development of novel pharmaceuticals and the removal of promising pharmaceuticals from the market. Troglitazone, a potentially effective antidiabetic medication, was taken off the market in a matter of years due to severe liver damage that occurred when the drug was being administered, as per the report by Niknahad and Fisher et al.<sup>1,2</sup> Significant idiosyncratic hepatotoxicity is thought to result from its sulfate conjugate's inhibition of bile SGPT transport from hepatocytes.<sup>1,3</sup> Hepatotoxicants can be classified into two categories: those that cause direct harm to the liver (intrinsic hepatotoxicity) and those that cause idiosyncratic hepatotoxicity, which is the toxicant that mediates an immunological response (hypersensitivity). Direct or intrinsic hepatotoxicity typically has a constant onset phase, is dose-dependent, and can be replicated in several animal models. For example, the hepatotoxicity caused by acetylpara-aminophenol (APAP) varies in dose.<sup>4</sup> Lack of repeatable animal models, dose-independent and host-dependent effects on the immune system, and genetic variability contribute to idiosyncratic drug-induced hepatotoxicity.<sup>5,6</sup> Troglitazone and chlorpromazine are two examples of idiosyncratic hepatotoxins.<sup>6,7</sup> Although not entirely understood, the mechanisms of action are known. Hepatotoxicants work in various ways, some directly interfering with the function of vital cellular structures such as the nucleus, mitochondria, endoplasmic reticulum, and plasma membrane. Numerous hepatotoxicants attach themselves to enzymes and mitochondrial membranes, affecting cellular respiration and energy metabolism.<sup>8</sup> Several hepatotoxicants operate as direct inhibitors and uncouplers of the mitochondrial electron transport chain.<sup>9,10</sup> Lipid peroxidation, redox recycling, and disruption of calcium homeostasis are other well-known modes of action.<sup>10</sup> All hepatotoxins bring on numerous different clinical and histological manifestations of liver injury. Certain biochemical markers, such as albumin levels, bilirubin protein, alkaline phosphatase (ALP), glutamate oxaloacetate transaminase (SGOT), and SGPT, can be used to diagnose liver injury (Fig. 1).<sup>11-13</sup> Increases in bilirubin levels are markers of overall liver function, while elevated serum enzyme levels are considered pertinent indicators of hepatic toxicity.<sup>14-16</sup> A rise in bilirubin to more than twice the upper limit of normal, accompanied by an elevation in transaminase levels, is thought to be a warning sign of hepatotoxicity.<sup>17</sup>



**Fig. 1.** Liver injury and protective effect of herbal plant

Hepatocellular and cholestatic toxicity are the two primary categories of hepatotoxicity, each with a unique mechanism of harm.<sup>18</sup> Although cholestatic injury is typified by predominantly initial ALP level elevations that precede or are relatively more prominent than increases in serum aminotransferase, hepatocellular or cytolytic injury involves primarily initial serum aminotransferase level elevations that typically precede increases in bilirubin levels and modest increases in ALP levels. In general, injuries of a mixed type that incorporate cholestatic and hepatocellular processes happen.<sup>19</sup> Selecting the type of hepatotoxic liver injury depends partly on the SGPT to ALP ratio value. When hepatocellular damage is prevalent, the ratio is larger than or equal to five ( $\geq 5$ ), but when cholestatic liver damage is predominant, the ratio is less than or equal to two ( $\leq 2$ ). When liver damage is combined, the ratio falls between two and five. In nonclinical investigations, SGPT, SGOT, and bilirubin in combination are often advised to evaluate hepatic damage in rodents and non-rodents. Since mitochondrial membrane damage, which is intracellular to the cell/plasma membrane, is required for an elevated SGOT level, SGPT is considered a more sensitive and specific biomarker of hepatocellular injury than SGOT. However, SGOT and SGPT are only cytoplasmic enzymes readily released into the bloodstream when hepatocyte cell/plasma membranes are damaged.<sup>20,21</sup>

Herbal therapies have demonstrated effective alternatives to hepatotoxic drug side effects. Only four plants have been scientifically clarified using internationally accepted standard protocols to develop evidence-based SGPT alternative herbal hepatoprotective drugs,<sup>22,23</sup> out of the many herbs and herbal medicines reported to have hepatoprotective effects.<sup>24-26</sup> A potent herbal hepatoprotective agent, silymarin, a flavonolignan derived from *Silybum marianum* (milk thistle), protects the liver from damage through antioxidative, antilipid peroxidative, anti-inflammatory, membrane-stabilizing, immunomodulatory, and liver-regenerating

mechanisms.<sup>27,28</sup> Typically, oral Silymarin is prescribed at 600 mg/day, or 200 mg thrice daily, for medical therapeutic purposes. This study will employ an oral daily dose of 50 mg/kg of silymarin for treatment, since the 200 mg milk thistle plant extract typically includes 140 mg of silymarin or 70% silymarin concentration. In light of the ongoing search for more potent hepatoprotective agents, we plan to assess the hepatoprotective effects of the crude ethanolic rhizome extract of *Picrorhiza kurroa*, also known as the mango plant, in a mouse model by following the internationally recognized standard scientific protocol.<sup>29</sup> Kutki, also known as *P. kurroa* Royle ex Benth, is a member of the Scrophulariaceae family. North Burma, West China, South-ESGOT Tibet, and the Himalayan region (Garhwal to Bhutan) are home to the perennial herb *P. kurroa*. It grows spontaneously in organic soils and on rock fissures in alpine areas. Though considered an important medicinal herb, it is mainly used in traditional medicine to treat snake bites, fever, jaundice, liver problems, and malaria. *P. kurroa* has anti-microbial, anti-oxidant, anti-bacterial, anti-mutagenic, cardio-protective, hepato-protective, anti-inflammatory, anti-diabetic, anti-malarial, anti-ulcer, anti-cancer, and nephroprotective pharmacological qualities.<sup>30,31</sup> For herbal/botanical products sold commercially, ensuring that the raw material used is real *P. kurroa*. Overusing *P. kurroa* for medicinal purposes has jeopardized the plant's conservation status in several places. The plant population is severely impacted by the widespread use of this remedy by locals to treat a wide range of ailments.<sup>32-35</sup> Additionally, the purpose of this work is to examine the hepatoprotective, ameliorative and antioxidant properties of the crude ethanolic rhizome extract against azithromycin and paracetamol-induced hepatotoxicity in a mouse model.

## **Aim**

This study aimed to evaluate the protective and ameliorative effects of *P. kurroa* rhizome extract against drug-induced liver injury in a rat model.

## **Material and methods**

### ***Sampling and authentication of plant extracts***

From July to September 2023, fresh *P. kurroa* rhizomes were harvested from hill crops at the Bimla Logistics Om complex in Raja Rani Vihar, Haldwani (UK), India. Botanists verified the taxonomic identification. Mr. R S. Jayasomu, the Chief Scientist Head of RHMD, and Dr. Sunita Garg, the Former Chief Scientist Head of RHMD, CSIR-National Institute of Science Communication and Information Resources (NISCAIR), verified the plants. The ethical number for animal experiments is added in the form of CPCSEA (1204/PO/Re/S/08/CPCSEA) Animal Welfare, Swami Vivekanand Subharti University Meerut.

### ***Physico-chemical characteristics***

#### ***Plant extraction method***

To remove any earthy elements, the *P. kurroa* rhizome was thoroughly cleansed with water. After letting it air dry in the shade at room temperature, it was carefully weighed and machine-ground into a fine powder using a mixer grinder. Using a Soxhlet device, the powdered material was subjected to solvent extraction with ethanol (99.9%) at 50–60°C for 48 hours at room temperature. To dry the extract, an oven preheated to 40°C was utilized. The crude extract was kept dry and sealed in an airtight container for the next experiment.

#### *Physical-chemical property evaluation*

Using the Unani Pharmacopoeia of India, different physicochemical properties were determined.<sup>36</sup> The following chemical and physical attributes have been evaluated: drying loss, total amount of ash, sulfate ash, water-soluble ash, and acid-insoluble ash.<sup>37</sup>

#### *Preliminary phytochemical analysis*

A preliminary phytochemical examination was performed using recognized techniques to determine the presence or absence of specific phytoconstituents.<sup>37</sup>

#### *Assay to determine antioxidant activity (DPPH)*

Using the standard protocol described by Braca A. et al. the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to assess the free radical scavenging activity of the ethanol extract of the *P. kurroa* rhizome *in vitro*.<sup>38</sup> The proportion of radical scavenging activity (RSA) was calculated using the following formula:  $RSA = [(A_0 - A_1)/A_0] \times 100$  where  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance of the samples after thirty minutes. The  $IC_{50}$  value indicated the plant extracts' ability to scavenge free radicals. The  $IC_{50}$  value is the concentration of a sample (in  $\mu\text{g/mL}$ ) that inhibits 50% of the DPPH radical.<sup>39</sup>

#### *Pharmacological studies*

##### *Experimental animals*

We chose all sexes of Wistar albino rats weighing 180–220 g. The experiments were carried out according to the CPCSEA criteria for the use and care of experimental animals and the moral principles approved by the Institutional Animal Ethics Committee (IACE) guidelines for animal care. The ethical number for animal experiments is added as CPCSEA (1204/PO/Re/S/08/CPCSEA) Animal Welfare, Swami Vivekanand Subharti University Meerut.

##### *Assessment of acute toxicity*

The acute oral toxicity was evaluated at a test limit test dose of 2000 mg/kg following the parameters described in the Organization for Economic Cooperation and Development (OECD)-425.

### *Evaluation of hepatoprotective activity*

The PCM and AZM-induced liver damage method was modified and utilized to evaluate the hepatoprotective effect *in vivo*. This method has been reported in several studies.

### *Experiments and procedures*

There were six groups (n=6) of the animals. Wistar albino rats (150–250 g body weight) of both sexes (male and female) were used. Work carried out on accredited CPCSEA (1204/PO/Re/S/08/CPCSEA) Animal Welfare, Swami Vivekanand Subharti University Meerut.<sup>40</sup> Oral administration of Silymarin (50 mg/kg) and *P. kurroa* (200 and 400 mg/kg) was performed for 14 days. To cause liver damage, PCM (250 mg/kg, p.o.) and AZM (200 mg/kg, p.o.) were administered continuously for 7 days, from the 7th to the 14th day, 2 hours after the test, in addition to the usual drug administration.<sup>41-44</sup>

The following is a summary of the experimental groups:

Group 1: Normal control (seven days of 0.5 percent CMC administration to rats),

Group 2: Toxicant control (Rats were administered 0.5% CMC for seven days in conjunction with 250 mg/kg of PCM and 200 mg/kg of AZM),

Group 3: Silymarin (Rats received 50 mg/kg of Silymarin suspension (p.o.) for 14 days along with toxicants (7 days' worth of 250 mg/kg PCM and 200 mg/kg AZM),

The rats in groups 4 and 5 were given a 14-day course of treatment consisting of 200 and 400 mg/kg p.o. extract of *P. kurroa*, along with a combination of toxicants PCM (250 mg/kg) and AZM (200 mg/kg) for 7 days.

On the fourteenth day, the animals were anesthetized with diethyl ether. The blood was then extracted and coagulated and serum was separated to determine enzyme activity. A section of liver tissue was immediately placed in 10% formalin for histological examination.

### *Estimation of serum biochemical parameters*

Biochemical indicators include serum levels of SGOT. As directed by the manufacturer, we measured serum levels of total bilirubin (TB), alkaline phosphatase (ALP) and SGPT using commercial enzyme biochemical diagnostic kits (Sigma-Aldrich (Merck KGaA), Darmstadt, Germany).

### *Antioxidant activity (in vivo)*

To assess *in vivo* antioxidant activity, the levels of SOD, CAT, LDH, and GSH were measured in blood plasma and liver tissue.

1. Superoxide dismutase (SOD) activity: The nitro blue tetrazolium (NBT) reduction method, as outlined by Marklund and Marklund, was used to measure the SOD activity. NBT, xanthine oxidase, and tris-HCl buffer were all present in the reaction mixture. The NBT decrease was tracked at 560 nm.<sup>45</sup>

2. Catalase (CAT) activity: By detecting the breakdown of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), as explained by Aebi, CAT activity was evaluated. At 240 nm, a reduction in absorbance was noted.<sup>46</sup>
3. Lactate dehydrogenase (LDH) activity: LDH activity was assessed with a standard commercial enzymatic test kit in accordance with the manufacturer's instructions. The transformation of pyruvate to lactate was observed at 340 nm.<sup>47</sup>
4. Reduced glutathione (GSH) level: Ellman's reagent method was used to measure GSH levels. At 412 nm, the yellow-colored complex's development was measured.<sup>48</sup>

Absorbance measurements were conducted utilizing a Thermo Fisher Scientific Multiskan GO microplate reader (Waltham, MA, USA), which is outfitted with a UV-visible spectrophotometer.

#### *Histopathological studies*

Liver tissue was embedded in paraffin, fixed for 24 hours in 10% formalin, and then sectioned into 5-mm-thick pieces using a rotary microtome. The sections were stained with hematoxylin-eosin dye under a microscope to examine the liver's histology.

#### *Statistical analysis*

Data were expressed as means±standard errors of mean (SEM). The analysis was performed using the software application GraphPad InStat 3 (GraphPad Software, La Jolla, California, USA). Each experiment was completed, and all data are shown as mean±SD. A one-way ANOVA with Post Hoc Tukey's test assesses the significance of mean differences: a=extremely significant, b=significant, and ns=not significant.

## **Results**

### *The percentage yield of crude extract*

Table 1 presents the crude ethanol extract yield percentage was found.

**Table 1.** The crude ethanol extract's yield percentage was found

Fraction	Color of extract	Yield of extract (g)	Yield of extract (%)
Ethanol	Brown	24.6	15.62

### *Physicochemical property analysis*

As indicated in (Table 2), the inorganic components of the ash are essential reference points for determining the validity and purity of the drug. The consistency, kind, color, and yield of each plant material extract were also examined.



**Table 2.** Physicochemical analysis of *P. kurroa*

Physicochemical parameters	% w/w
Ash value	
Total ash	13.6
Acid insoluble ash	1.57
Water soluble ash	7.25
Extractive value	
Ethanol 95%	2.544
Loss on drying	8.345

**Preliminary phytochemical research**

Alkaloids, glycosides, flavonoids, tannins, steroids, saponins, and quercitin are some phytochemicals that can be found in plant materials. Table 3 shows that the ethanolic extract of *P. kurroa* contained all the phenolic and flavonoid content.

**Table 3.** A preliminary phytochemical screening was performed on the ethanolic extract of the *P. kurroa* rhizome to assess its effectiveness\*

Constituents	Test	Observation	Inference of the ethanolic extract of the <i>P. kurroa</i> (rhizome)
Saponins	Frothing	Frothing persists for 15 min.	+
Alkaloids	Mayer's	White-cream ppt	+
	Draggondorf's	Orange ppt	+
	Wagner's	Reddish-brown ppt	+
Flavonoids	FeCl <sub>3</sub>	Green or violet ppt	-
	Shinoda	Orange-red ppt	+
Tannins	Lead subacetate	Cream ppt	+
Steroids and terpenes	Lieberman-Buchard	Blue-green color at interphase	-
	Salkowski	Reddish color	+
Carbohydrates	Molish's	Reddish ring	+
	Fehling's	Red	+
Phenols	FeCl <sub>2</sub>	Bluish black color Pin blood	+
Glycosides	Legal's	Red ppt	+

Fehling's	Red ppt	+
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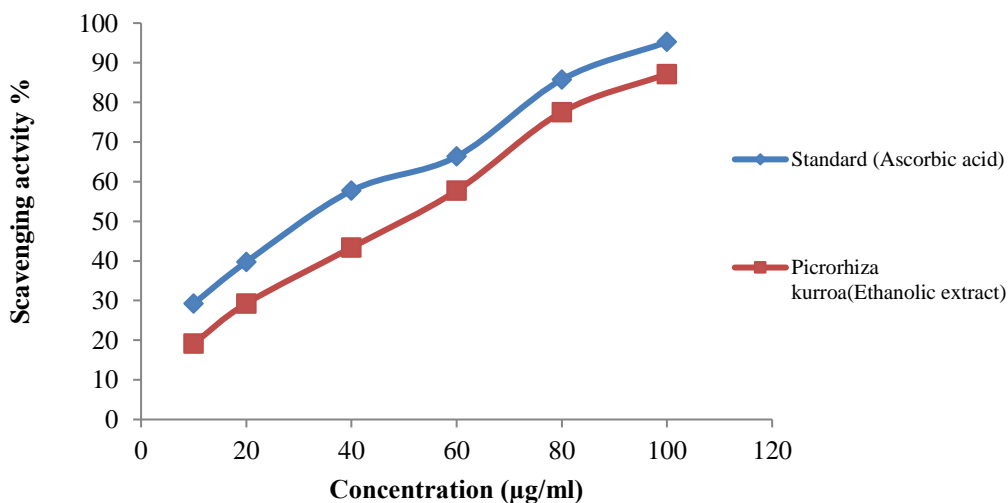
\* (+) – present, (-) – absent

### *In vitro* antioxidant activity assays

Plants with antioxidant qualities have medicinal uses. Due to this, the well-known DPPH technique was used to detect free radical scavenging activity in the ethanolic extract of *P. kurroa*, as Table 4. 87.15±0.12 percent of DPPH radical scavenging activity is found in the ethanolic extract of the rhizome of *P. kurroa*, depending on the dose. Whereas the standard ascorbic acid had an IC<sub>50</sub> of 35.20 g/mL, the extract was 48.62 g/mL (Fig. 2).

**Tables 4.** Free radical scavenging activity of 1,1-diphenyl-2 picrylhydrazyl (DPPH) for ethanolic extract of *P. kurroa*

Concentration (µg/mL)	DPPH scavenging activity (% inhibition)	
	Standard (ascorbic acid)	Ethanol extract of <i>P. kurroa</i>
10	29.22±0.32	19.12±0.30
20	39.73±0.07	29.25±0.19
40	57.71±0.81	43.36± 0.19
60	66.34±0.94	57.71±0.53
80	85.75±0.56	77.54±0.25
100	95.23±0.17	87.15±0.12
	<b>IC<sub>50</sub>= 35.20</b>	<b>IC<sub>50</sub>= 48.62</b>



**Fig. 4.** Comparison of DPPH Scavenging activity between *P. kurroa* and standard (ascorbic acid)

*Acute oral toxicity study of the ethanol extract of P. kurroa (rhizome) in rats*

**Table 5.** Acute oral toxicity study of the ethanol extract of *P. kurroa* in rats

S.N.	Response	Animals	
		Before treatment	After treatment
1	Alertness	Normal	Normal
2	Grooming	Absent	Absent
3	Restlessness	Absent	Absent
4	Touch response	Absent	Absent
5	Torch response	Normal	Normal
6	Pain response	Normal	Normal
7	Tremors	Absent	Absent
8	Convulsion	Absent	Absent
9	Gripping strength	Normal	Normal
10	Corneal reflex	Present	Present
11	Writing	Absent	Absent
12	Pupils	Normal	Normal
13	Salivation	Normal	Normal
14	Skin colour	Normal	Normal

*Hepatoprotective parameter estimation*

**Table 6.** Impact of *P. kurroa* on liver enzymes against PCM and AZM-induced liver toxicity in rats\*

Group	SGPT (IU/l)	SGOT (IU/l)	ALP (IU/l)	Bilirubin (mg/dl)	Protein (Gm/dl)	Albumin (gm/dl)
Normal (control)	31.5±1.6	33±1.3	144.25±1.48	0.8±0.10	7.521±0.09	4.986±0.2
PCM (250 mg/kg) ±AZM (200 mg/kg)	107.76±6.5 <sup>a</sup>	196±8.6 <sup>a</sup>	427.6±70.1 <sup>a</sup>	1.0245±0.02 <sup>a</sup>	4.96±0.4 <sup>a</sup>	4.726±0.3 <sup>a</sup>
PCM (250 mg/kg) ±AZM (200 mg/kg)±Silymarin (50 mg/kg)	44.4 ±4.6 <sup>ns</sup>	58.24±4.6 <sup>ns</sup>	146.85±2.3 <sup>ns</sup>	0.751±0.07 <sup>ns</sup>	7.852±0.3 <sup>b</sup>	4.94±0.3 <sup>ns</sup>
PCM (250 mg/kg ±AZM	62.24±3.2 <sup>b</sup>	143.24±11.2 <sup>b</sup>	154.4±4.5 <sup>b</sup>	0.8132±0.02 <sup>b</sup>	6.675±0.3 <sup>b</sup>	4.876±0.4 <sup>b</sup>

(200 mg/kg)± <i>P. kurroa</i>						
(200 mg/kg)						
PCM (250 mg/kg ±AZM	59.6±5.2 <sup>ns</sup>	71.74±4.9 <sup>ns</sup>	145.24±1.7 <sup>b</sup>	0.55±0.05 <sup>b</sup>	7.7±0.3 <sup>ns</sup>	5.467±0.09 <sup>b</sup>
(200 mg/kg)± <i>P. kurroa</i>						
(400 mg/kg)						

\* all data are presented as mean±SD, the significance of mean differences is determined using a one-way ANOVA with Post Hoc Tukey's test, a – highly significant, b – significant, ns – not significant

**Table 7.** Protective effects of *P. kurroa* on hepatic antioxidant enzyme activities against PCM and AZM-induced hepatotoxicity in rats\*

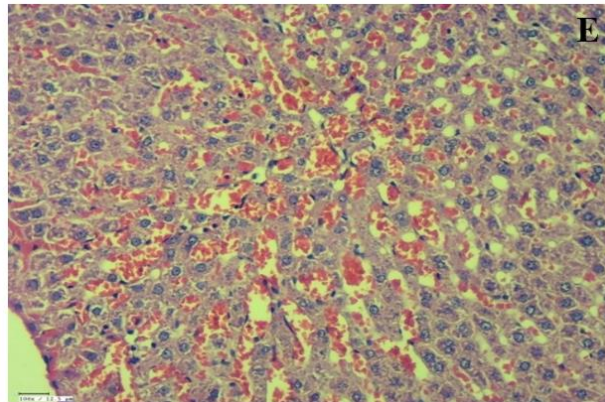
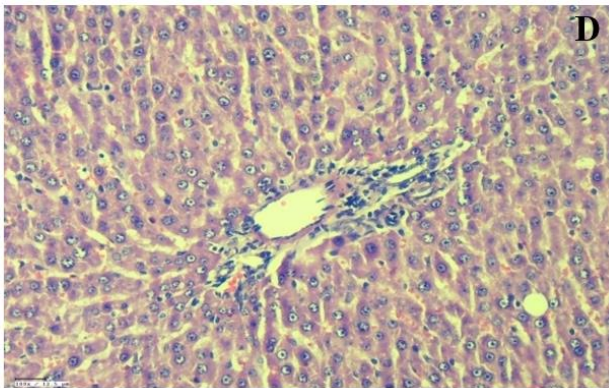
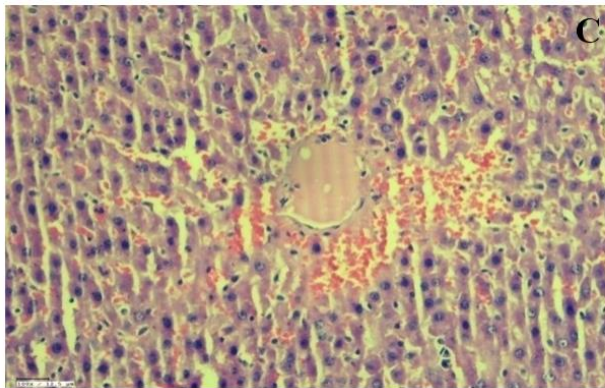
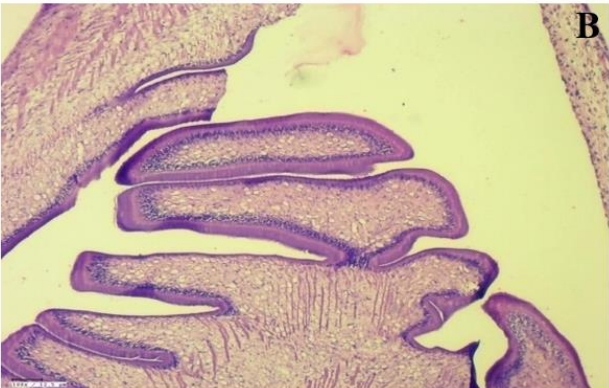
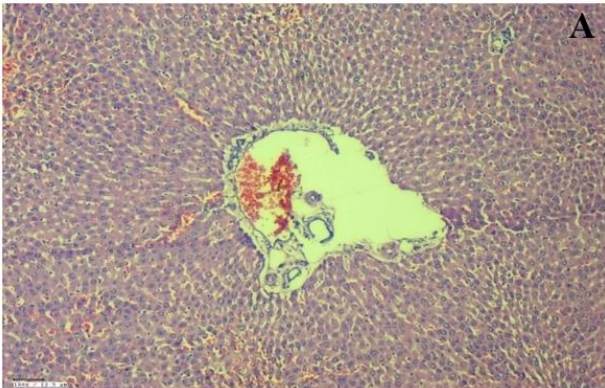
Group	SOD (U/mg of protein)	CAT (µM/min/mg of protein)	LDH (U/L)	GSH (µ/mg of protein)
Normal (control)	46.42±5.5	196.44±15.95	155.89±39.17	20.32±1.13
PCM (250 mg/kg)±AZM (200 mg/kg)	9.97±2.09 <sup>a</sup>	32.97±9.85 <sup>a</sup>	703.94±28.87 <sup>a</sup>	6.87±1.65 <sup>a</sup>
PCM (250 mg/kg) ±AZM (200 mg/kg)±Silymarin (50 mg/kg)	43.94±3.49 <sup>ns</sup>	177.43±38.33 <sup>ns</sup>	171.845± 33.26 <sup>b</sup>	18.34±3.34 <sup>b</sup>
PCM (250±AZM (200 mg/kg)± <i>P. kurroa</i> (200 mg/kg)	35.69±7.83 <sup>a</sup>	127.52±7.98 <sup>b</sup>	234.13±36.22 <sup>b</sup>	11.17±3.30 <sup>a</sup>
PCM (250±AZM (200 mg/kg)± <i>P. kurroa</i> (400 mg/kg)	41.02±7.69 <sup>ns</sup>	160.45±5.70 <sup>b</sup>	180.65±28.56 <sup>b</sup>	15.48±2.01 <sup>ns</sup>

\* all data are presented as mean±SD, the significance of mean differences is determined using a one-way ANOVA with Post Hoc Tukey's test, a highly significant, b – significant, ns not significant

### Histopathological evaluation

The cellular architecture of liver tissue in each group of rats was examined using histopathological examination, and the results are shown in Figure 3. The photomicrograph of the liver revealed normal hepatic cell architecture, with transparent cytoplasm, slightly dilated central veins, normal kupffer cells, and normal prominent nuclei in every cell (Fig. 3A). Figure 3B illustrates the deformed architecture and a vast area of necrosis in the liver tissue of the PCM and AZM control groups. Figure 3C of the Silymarin group's -3 also revealed no necrosis and fewer inflammations in the liver cells. Following pretreatment with 200 mg/kg and 400 mg/kg of *P. kurroa* extract, respectively, animal groups 4 and 5 displayed more of the liver tissue's normal architecture with the SGOT amount of inflammation (Figures 3D and 3E). All histological observations were

performed with hematoxylin and eosin (H&E stain, 200X) techniques, with 200 magnification power, and corroborated the production of hepatotoxicity by PCM in combination with AZM and the hepatoprotective action of *P. kurroa* extract.



**Fig. 3.** Effects of *P. kurroa* on histopathological changes induced by PCM and AZM in rats, A: control group, B: animals treated with PCM (250 mg/kg) and AZM (200 mg/kg), C: animal treated with PCM (250 mg/kg) and AZM (200 mg/kg) and Silymarin (50 mg/kg), D and E: animals treated with PCM (250 mg/kg) and AZM (200mg/kg) and *P. kurroa* (200 and 400mg/kg) (H&E,200X)

## Discussion

In the current study, we demonstrated the antioxidant approach of *P. kurroa* and Silymarin for hepatotoxicity induced by PCM and AZM: a histological and biochemical study would typically interpret the findings, relate them to the existing literature and suggest implications for future research and clinical practice.<sup>49,50</sup> Here is a structured discussion based on what the article probably covers: the exposure of PCM and AZM exposure to the rats' consequence in a vital increase in serum SGOT, SGPT, ALP, bilirubin, protein and albumin for *P. kurroa* when levels were compared from the control Group in Table No. 6. In group II, IV and V, the lethal activity of PCM and AZM was steadily reversed in the rats by demonstrating the vital reduction into the serum SGOT, SGPT, ALP and Bilirubin. Important increments were found in the protein and albumin levels. Group III, administration of Silymarin (standard) demonstrated a significant decrease in marker enzymes of the liver; however, an important increment was found in protein and albumin levels.<sup>51</sup>

To further understand the hepatoprotective activity of *P. kurroa*, this investigation verified the activity and potency of antioxidant enzymes, for example, SOD, CAT, and LDH, in addition to the levels of GSH in the animal's liver. Antioxidant enzymes such as CAT and GSH were regarded as major protective systems for protection from oxidative damage. Administration of PCM and AZM caused vital alterations in SOD, CAT, LDH, and GSH when they were compared with rats of the normal group. The data obtained demonstrated the effects of PCM and AZM-induced liver toxicity on the liver's functions parameter; for example, SOD, CAT, LDH, and GSH could be efficiently balanced by the ethanolic extract of *P. kurroa* rhizome.<sup>52,53</sup> The study shows that both *P. kurroa* and Silymarin significantly ameliorate liver damage caused by PCM and AZM. The reduction in liver enzyme levels and improved histological outcomes indicate that these natural antioxidants (effectively counteract hepatotoxicity). This supports the hypothesis that *P. kurroa* and Silymarin have potent hepatoprotective properties.<sup>50</sup> The findings align with previous research highlighting the hepatoprotective effects of Silymarin, which has been extensively studied and used clinically for liver disorders. However, the study adds value by providing new information on the protective effects of *P. kurroa*, particularly in the context of drug-induced liver injury.

The combination of *P. kurroa* and Silymarin may offer enhanced protection compared to either agent alone, suggesting a potential synergistic effect. This is consistent with other studies showing that the combination of different antioxidants can result in better therapeutic outcomes.<sup>54</sup> The protective effects observed in this study can be attributed to the antioxidant properties of *P. kurroa* and Silymarin. *P. kurroa*, rich in picrosides,

likely exerts its effects by scavenging free radicals, reducing oxidative stress, and modulating inflammatory pathways. On the contrary, silymarin stabilizes hepatocyte membranes, inhibits lipid peroxidation, and enhances protein synthesis, promoting liver cell regeneration. The reduction in oxidative stress markers and the preservation of hepatic architecture in the treated groups further support the notion that both *P. kurroa* and Silymarin mitigate the damaging effects of NAPQI (from PCM metabolism) and azithromycin-induced oxidative stress.<sup>55</sup>

The histological analysis showing reduced necrosis, inflammation, and fatty changes in the liver tissue of treated groups correlates well with the biochemical findings. This concordance reinforces the conclusion that *P. kurroa* and silymarin prevent biochemical alterations and protect the structural integrity. Normalization of liver enzymes, coupled with histological improvement, suggests that *P. kurroa* and Silymarin facilitate the recovery of liver function, which is crucial for overall health of the organism. Given the widespread use of PCM and AZM, the hepatoprotective effects of *P. kurroa* and Silymarin could have significant clinical implications. These findings suggest that *P. kurroa* and Silymarin could be considered adjunct therapies in patients receiving potentially hepatotoxic drugs, especially in individuals with preexisting liver conditions or those at higher risk of drug-induced liver injury. The study also underscores the importance of exploring natural antioxidants as safer alternatives or complements to synthetic drugs in managing liver toxicity. Although the study provides strong evidence for the hepatoprotective effects of *P. kurroa* and silymarin, some limitations should be acknowledged. For instance, the study was conducted in animal models, and thus, the results may not directly translate to humans without further clinical trials. Furthermore, the exact molecular mechanisms underlying the protective effects of *P. kurroa* and Silymarin could be further elucidated through more detailed studies, such as those involving gene expression analysis or advanced imaging techniques.<sup>56</sup> Future research could explore the long-term effects of treatment with *P. kurroa* and Silymarin, potential dose-response relationships, and the efficacy of these compounds in human clinical trials. Moreover, investigating the impact of *P. kurroa* and Silymarin on other forms of drug-induced hepatotoxicity or in combination with other hepatoprotective agents could be valuable. The exploration of different natural products with similar antioxidant properties could also lead to the discovery of new therapeutic agents for liver protection.<sup>57,58</sup>

## **Conclusion**

The study concludes that *P. kurroa* and Silymarin significantly protect against PCM and AZM-induced hepatotoxicity. These findings reinforce the therapeutic potential of natural antioxidants for liver protection and suggest a promising avenue for the development of adjunctive therapies in hepatology. This discussion integrates the study findings with broader scientific knowledge, highlights the implications for clinical practice, and suggests avenues for further research. In summary, this study has shown that the rhizome extract of *P. kurroa* exhibits strong hepatoprotective properties against paracetamol and azithromycin in

rats. In addition, phenolic compounds and flavonoids have been reported in plants on the basis of phytochemical data. Their potential as antioxidants may account for their hepatoprotective properties.

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### ***Author contributions***

Conceptualization, M.K. and V.R.; Methodology, V.R. and S.K.; Software, M.K.; Validation, M.K., V.R. and A.K.M.; Formal Analysis, M.K.; Investigation, V.S.; Resources, V.R.; Data Curation, M.K.; Writing – Original Draft Preparation, M.K. and V.R.; Writing – Review & Editing, V.R.; Visualization, V.R.; Supervision, V.R.; Project Administration, V.R.; Funding Acquisition, V.R.

### ***Conflicts of interest***

The authors declare that they have no potential conflicts of interest regarding the research, authorship, and publication of this article.

### ***Data availability***

All data generated or analyzed during this study are included in the published article. Any additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

### ***Ethics approval***

The ethical number for animal experiments is added in the form of CPCSEA (1204/PO/Re/S/08/CPCSEA) Animal Welfare, Swami Vivekanand Subharti University Meerut.

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