RESEARCH PAPER

Evaluation of Hepatoprotective Activity of Coix lacryma-jobi L. Seed Extract for Isoniazid-Induced Hepatotoxicity in Experimental Animal

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ABSTRACT

Background: Drug-induced liver injury (DILI) is a major cause of hepatic morbidity. Isoniazid (INH), a first-line antitubercular drug, is frequently associated with hepatotoxicity due to the formation of reactive metabolites that deplete hepatic antioxidant defenses. Plant-derived antioxidants may offer protection against such injury.

Objective: To evaluate the hepatoprotective potential of the ethanolic seed extract of *Coix lacryma-jobi* L. (CLJSE) against isoniazid-induced hepatotoxicity using in vitro and in vivo models.

Methods: CLJSE was prepared by Soxhlet extraction using 70% ethanol and standardized through pharmacognostic, physicochemical, phytochemical, and FTIR analyses. Antioxidant activity was assessed by the DPPH assay, while hepatoprotective activity was evaluated in HepG2 cells against H_2O_2-induced cytotoxicity using the MTT assay. Acute oral toxicity was conducted according to OECD Guideline 423. For the in vivo study, Wistar rats were divided into normal control, toxic control (INH, 50 mg/kg i.p., twice weekly for 28 days), standard (INH + silymarin 100 mg/kg p.o.), and extract-treated groups (INH + CLJSE 200 or 400 mg/kg p.o.). Serum liver markers, hepatic antioxidant parameters, liver weight, and histopathology were evaluated.

Results: CLJSE showed concentration-dependent DPPH radical scavenging activity (IC_{50} = 74.56 µg/mL) and protected HepG2 cells against oxidative stress, producing 94.46% cell viability at 100 µg/mL. The extract was non-toxic up to 2000 mg/kg. INH significantly increased serum AST, ALT, ALP, total bilirubin, liver weight, and MDA levels, while reducing GSH, SOD, and CAT levels ($p < 0.01$). CLJSE treatment significantly and dose-dependently reversed these changes, with the 400 mg/kg dose showing effects comparable to silymarin. Histopathological findings confirmed marked protection of hepatic architecture.

Conclusion: CLJSE exhibits significant dose-dependent hepatoprotective activity against isoniazid-induced liver injury, primarily through antioxidant, anti-lipid-peroxidative, and membrane-stabilizing mechanisms, supporting its potential as an adjuvant phytotherapeutic agent during antitubercular therapy.

Keywords: Coix lacryma-jobi; hepatoprotective activity; isoniazid; drug-induced liver injury; oxidative stress; DPPH; HepG2; Wistar rats

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1. INTRODUCTION

The liver occupies a central position in metabolic homeostasis, performing essential functions in carbohydrate, lipid, and protein metabolism, plasma protein synthesis, bile secretion, and biotransformation of endogenous and exogenous compounds [1,2]. Because of its continuous exposure to xenobiotics, drugs, and environmental toxins during these processes, the liver is uniquely susceptible to chemical injury, and drug-induced liver injury (DILI) remains a major cause of hepatic morbidity, treatment discontinuation, and acute liver failure worldwide [3-5].

Isoniazid (INH), a cornerstone first-line agent in antitubercular chemotherapy, is among the most

extensively documented hepatotoxic drugs [6,7]. During hepatic biotransformation, INH is sequentially metabolized to acetylhydrazine and hydrazine; further oxidation of these intermediates via the cytochrome P450 system generates reactive free radicals that initiate lipid peroxidation, mitochondrial dysfunction, and hepatocellular necrosis [8-10]. Given the prolonged duration of antitubercular therapy and the high global burden of tuberculosis, INH-induced hepatotoxicity represents a substantial clinical concern affecting treatment adherence and outcomes [6].

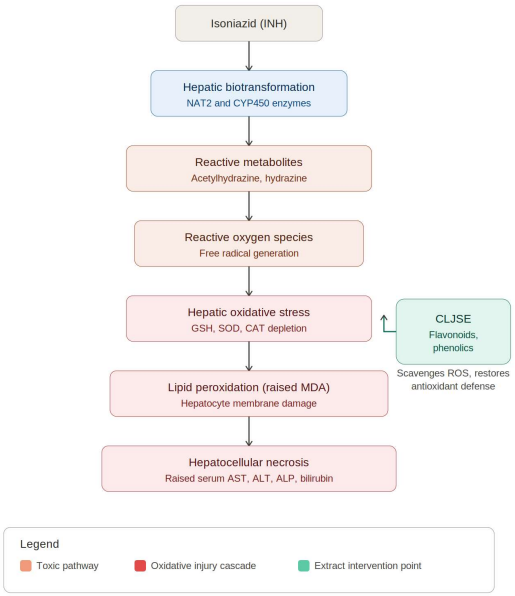


Figure 1. Schematic representation of the proposed mechanism of isoniazid-induced hepatotoxicity, illustrating sequential bioactivation via NAT2/CYP450 to reactive intermediates that deplete antioxidant reserves and culminate in hepatocellular injury

Currently available hepatoprotective agents, such as silymarin, show variable clinical efficacy, and long-term pharmacological management is constrained by cost and tolerability concerns [11,12]. This has intensified interest in plant-derived hepatoprotective agents, whose multi-targeted antioxidant, anti-inflammatory, and membrane-stabilizing actions offer a comparatively favourable safety margin [13,14].

Coix lacryma-jobi L. (Poaceae), commonly known as Job's tears, is a cereal-medicinal plant widely distributed across Asia and traditionally used for liver disorders, inflammation, and diabetes [15,16]. Its seeds are reported to contain flavonoids, phenolic acids, polysaccharides, sterols, and bioactive lactones such as coixenolide and coixol, several of which possess documented antioxidant and anti-inflammatory properties [16-18]. Despite this ethnomedicinal background and preliminary pharmacological evidence, systematic experimental validation of the hepatoprotective efficacy of *C. lacryma-jobi* seed extract specifically against isoniazid-induced hepatotoxicity has not been comprehensively reported [19].

(A) Mature plant in habitat

(B) Seed (pseudocarp) clusters

Figure 2. Coix lacryma-jobi L. (Job's tears) in its natural growth habitat. (A) Mature plant showing characteristic grass-like foliage; (B) Pseudocarp (seed) clusters at maturity, the plant part used for extraction in this study (photographs taken by the authors during plant collection).

The present study was therefore designed to evaluate the hepatoprotective potential of the ethanolic seed extract of *C. lacryma-jobi* (CLJSE) using an integrated experimental approach encompassing pharmacognostic standardization, in vitro antioxidant and cytoprotective assays, acute toxicity assessment, and an in vivo isoniazid-induced hepatotoxicity model in Wistar rats, supported by biochemical, antioxidant, and histopathological endpoints. The findings are intended to provide mechanistic and translational evidence supporting the traditional hepatoprotective use of this plant.

2. MATERIALS AND METHODS

2.1. Plant Material, Chemicals, and Drugs

Seeds of *Coix lacryma-jobi* L. were procured and authenticated by Dr. N. V. Malpure, In-charge Professor, Shri Sadguru Gangageer Maharaj Science, Gautam Arts and Sanjivani Commerce College, Kopargaon, Maharashtra, India, with reference to the standard taxonomic flora of the region (Authentication Certificate Ref. No. 1782/2025-26, dated 06/02/2026). A voucher specimen was deposited for future reference. Isoniazid was procured from Research Lab Fine Chem Industries; silymarin from Vaishno Herbal. Carboxymethyl cellulose (1% CMC) was used as the vehicle for extract suspension, and 10% neutral buffered formalin was used for tissue fixation. All

other chemicals and reagents used were of analytical grade.

2.2. Preparation of Seed Extracts

Air-dried, coarsely powdered seeds (200 g) were subjected to continuous hot extraction in a Soxhlet apparatus, separately using 70% ethanol and distilled water (1000 mL each) at 70-80 °C for 3-4 days. The resulting extracts were filtered and concentrated under reduced pressure using a rotary vacuum evaporator (40-45 °C) to obtain semisolid crude extracts, which were stored in airtight containers at 4 °C until further use. Percentage yield was calculated as (practical yield/theoretical yield) × 100.

2.3. Pharmacognostic and Physicochemical Standardization

The crude drug and extract were characterized by organoleptic evaluation (colour, odour, texture, taste), macroscopic and microscopic examination (transverse seed sections and powder microscopy), and standard physicochemical parameters including loss on drying, total ash, acid-insoluble ash, water-soluble ash, and water-/alcohol-soluble extractive values, performed according to standard pharmacopoeial procedures [20].

2.4. Preliminary Phytochemical Screening

The ethanolic extract was screened qualitatively for alkaloids (Dragendorff's test), flavonoids (Shinoda test), tannins (ferric chloride test), saponins (foam test), glycosides (Keller-Killiani test), carbohydrates (Molisch's test), proteins (Biuret test), phenolic compounds (ferric chloride test), and steroids (Salkowski test), following standard protocols [20].

2.5. Spectral Characterization

UV-visible spectroscopic analysis (Shimadzu UV-1900, double-beam) was performed at 280 nm and 345 nm to estimate total phenolic and flavonoid content across a concentration series (20-100 ppm). Fourier-transform infrared (FTIR) spectroscopy was carried out over the range 4000-400 cm^{-1} to identify characteristic functional groups corresponding to major phytoconstituents.

2.6. In Vitro Antioxidant Activity (DPPH Assay)

Free radical scavenging activity was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. One millilitre of test extract or ascorbic acid (standard) at concentrations of 20, 40, 60, 80, and 100 µg/mL was mixed with 1.5 mL of 0.1% methanolic DPPH solution and incubated in the dark for 30 min. Absorbance was recorded at 510 nm, and percentage radical scavenging activity was calculated as: [(Absorbance of control - Absorbance of test)/Absorbance of control] × 100. The half-maximal inhibitory concentration (IC50) was derived from the concentration-response curve.

2.7. In Vitro Hepatoprotective Activity (HepG2/MTT Assay)

HepG2 (human hepatocellular carcinoma) cells were seeded at 1 × 10^4 cells/well in 96-well plates and incubated for 24 h (37 °C, 5% CO2). Cytotoxic injury was induced with H2O2, and cells were co-treated with CLJSE or silymarin (20-100 µg/mL) in triplicate, with DMSO (0.2% in PBS)-treated cells serving as control. Following 24 h incubation, MTT reagent (5 mg/mL) was added, and after 4 h formazan crystals were dissolved in DMSO. Absorbance was measured at 550 nm using a microplate reader, and percentage viability and inhibition were calculated relative to control.

2.8. Ex Vivo Pharmacological Screening on Isolated Chicken Ileum

Pharmacological response of CLJSE (ethanolic and aqueous extracts) was additionally assessed on isolated chicken ileum tissue mounted in an organ bath containing physiological salt solution at 37 °C with continuous aeration, using isoniazid as the toxic agent and silymarin as the reference standard. Contractile responses were recorded using a kymograph following standard tissue-bath pharmacology procedures.

2.9. Animals

Healthy Wistar rats of either sex were used for the acute toxicity (120-150 g) and sub-acute/hepatoprotective (150-250 g) studies. Animals were housed under standard laboratory conditions (23 ± 2 °C, 55 ± 5% relative humidity, 12 h light/dark cycle) with free access to standard pellet diet and water ad libitum, and acclimatized for 7 days prior to experimentation. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), RJS College of Pharmacy (Approval No. IAEC-I/RJSCOP/2025-2026/05), and conducted in accordance with OECD Guidelines 423 and 407.

2.10. Acute Oral Toxicity Study

Acute oral toxicity of CLJSE was evaluated as per OECD Guideline 423. Overnight-fasted rats were divided into two groups (n = 6): Group I received normal saline (5 mL/kg, p.o.), and Group II received a single oral dose of CLJSE (2000 mg/kg, p.o.). Animals were observed continuously for the first 4 h and

periodically thereafter for 14 days for mortality, behavioural changes, and signs of toxicity (CNS, CVS, and autonomic effects), and body weight was recorded daily.

2.11. Isoniazid-Induced Hepatotoxicity Model

Hepatotoxicity was induced by intraperitoneal administration of isoniazid (50 mg/kg) twice weekly for 28 days [8-10]. Thirty rats were randomly allocated into five groups (n = 6 each):

Group I (Normal control): 1% CMC, 1 mL/kg, p.o., once daily for 28 days.

Group II (Toxic control): Isoniazid 50 mg/kg, i.p., twice weekly for 28 days.

Group III (Standard): Isoniazid (as above) + silymarin 100 mg/kg, p.o., daily for 28 days.

Group IV (Test, low dose): Isoniazid (as above) + CLJSE 200 mg/kg, p.o., daily for 28 days.

Group V (Test, high dose): Isoniazid (as above) + CLJSE 400 mg/kg, p.o., daily for 28 days.

Following the 28-day treatment period, animals were rested for 3 days, fasted overnight, and then anaesthetized with ether for blood collection via the retro-orbital plexus. Serum was separated by centrifugation (3000 rpm, 10 min, 4 °C) for biochemical estimation. Animals were then sacrificed, and the liver was excised, rinsed in ice-cold saline, weighed, and divided for antioxidant assay (10% w/v homogenate) and histopathological fixation (10% neutral formalin).

2.12. Assessment of Hepatotoxicity

(a) Morphological parameters: Absolute liver weight was recorded as an index of hepatic inflammation/oedema.

(b) Biochemical parameters: Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin (TB) were estimated by standard enzymatic/colorimetric methods.

(c) Hepatic antioxidant status: Malondialdehyde (MDA) was estimated by the thiobarbituric acid (TBA) method of Wills (1966) at 532 nm; reduced glutathione (GSH) was estimated using DTNB at 412 nm; superoxide dismutase (SOD) activity was assessed by the nitroblue tetrazolium (NBT) inhibition method at 560 nm; and catalase (CAT) activity was determined by monitoring H2O2 decomposition at 240 nm.

(d) Histopathological examination: Formalin-fixed liver sections were processed, stained with haematoxylin and eosin, and examined under a light microscope for architectural changes, necrosis, and inflammatory infiltration.

2.13. Statistical Analysis

All data are expressed as mean ± standard error of the mean (SEM) for six animals per group. Statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Extraction Yield and Pharmacognostic Standardization

Soxhlet extraction of 200 g powdered seeds yielded 5.095 g (2.5475% w/w) of ethanolic extract and 4.985 g (2.4925% w/w) of aqueous extract, indicating marginally greater extractive efficiency of ethanol (Table 1). Organoleptic evaluation showed ovoid, smooth, white-to-creamy-white seeds with a mildly sweet taste. Microscopic examination revealed a lignified sclerenchymatous seed coat, a single aleurone layer, a starch-rich endosperm with polygonal starch granules, and a laterally positioned embryo, consistent with characteristic Poaceae seed anatomy.

Table 1. Percentage yield of Coix lacryma-jobi seed extracts

Extract type	Powder taken (g)	Practical yield (g)	Yield (% w/w)	Nature of extract
Ethanolic extract	200	5.095	2.5475	Dark brown, semisolid
Aqueous extract	200	4.985	2.4925	Brown, semisolid

3.2. Physicochemical Evaluation

Loss on drying (7% w/w), total ash (4% w/w), acid-insoluble ash (1% w/w), water-soluble ash (1.5% w/w), water-soluble extractive value (8.5% w/w), and alcohol-soluble extractive value (5% w/w) were all within standard pharmacopoeial reference ranges, confirming the purity and quality of the crude drug (Table 2).

Table 2. Physicochemical parameters of Coix lacryma-jobi seed

Parameter	Observed value (%)	Standard reference range (%)
Loss on drying	7.0	5-8
Total ash	4.0	3-5
Acid-insoluble ash	1.0	0.5-1.5
Water-soluble ash	1.5	1-2
Water-soluble extractive value	8.5	8-15
Alcohol-soluble extractive value	5.0	5-8

3.3. Preliminary Phytochemical Screening

The ethanolic extract tested positive for flavonoids, tannins, saponins, glycosides, carbohydrates, proteins, phenolic compounds, and steroids, while alkaloids were not detected (Table 3).

Table 3. Preliminary phytochemical screening of CLJSE

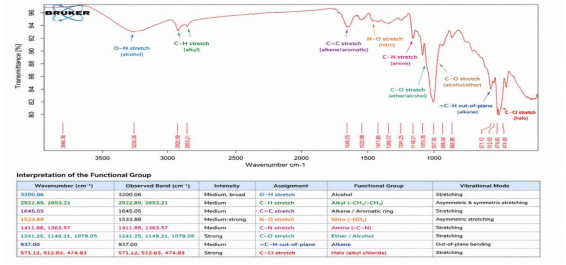
Phytoconstituent	Test performed	Result
Alkaloids	Dragendorff's test	–
Flavonoids	Shinoda test	+
Tannins	Ferric chloride test	+
Saponins	Foam test	+
Glycosides	Keller-Killiani test	+
Carbohydrates	Molisch's test	+
Proteins	Biuret test	+
Phenolic compounds	Ferric chloride test	+
Steroids	Salkowski test	+

"+" indicates presence; "–" indicates absence.

3.4. Spectral Characterization

UV-visible spectroscopy showed concentration-dependent absorbance at 280 nm (phenolics) and 345 nm (flavonoids); the test sample registered absorbance values of 0.351 and 0.039 at these wavelengths, respectively, confirming an appreciable phenolic-flavonoid content.

FTIR spectral analysis (Figure 3) revealed a broad O-H stretch at 3200.06 cm^{-1} (alcohols/phenolics), C-H stretching at 2922.89 and 2853.21 cm^{-1} (alkyl groups), C=C stretching at 1645.05 cm^{-1} (aromatic/alkene), N-O stretching at 1533.88 cm^{-1}, C-N stretching at 1411.86 and 1363.57 cm^{-1} (amines), strong C-O stretching at 1241.25, 1149.21, and 1079.05 cm^{-1} (ethers/alcohols), =C-H bending at 937.00 cm^{-1}, and C-Cl stretching at 571.12, 512.63, and 474.83 cm^{-1}. These bands collectively confirm the presence of phenolic, flavonoid, and other bioactive functional groups consistent with the phytochemical screening results.



Wavenumber (cm⁻¹)	Observed Band (cm⁻¹)	Intensity	Assignment	Functional Group	Vibrational Mode
3200.06	3200.06	Medium, broad	O–H stretch	Alcohol	Stretching
2922.89, 2853.21	2922.89, 2853.21	Medium	C–H stretch	Alkyl (–CH₂/–CH₃)	Asymmetric & symmetric stretching
1645.05	1645.05	Medium	C=C stretch	Alkene / Aromatic ring	Stretching
1533.88	1533.88	Medium-strong	N–O stretch	Nitro (–NO₂)	Asymmetric stretching
1411.86, 1363.57	1411.86, 1363.57	Medium	C–N stretch	Amine (–C–N)	Stretching
1241.25, 1149.21, 1079.05	1241.25, 1149.21, 1079.05	Strong	C–O stretch	Ether / Alcohol	Stretching
937.00	937.00	Medium	=C–H out-of-plane	Alkene	Out-of-plane bending
571.12, 512.63, 474.83	571.12, 512.63, 474.83	Strong	C–Cl stretch	Halo (alkyl chloride)	Stretching

Figure 3. FTIR spectrum and functional group interpretation of Coix lacryma-jobi seed extract

3.5. In Vitro Antioxidant Activity (DPPH Assay)

CLJSE exhibited concentration-dependent DPPH radical scavenging activity, with percentage inhibition increasing from 15.54% (20 µg/mL) to 66.84% (100 µg/mL), corresponding to an IC50 of 74.56 µg/mL. The reference standard, ascorbic acid, showed inhibition ranging from 26.42% to 82.90% over the same concentration range, with an IC50 of 59.23 µg/mL (Table 4, Figure 4). Although CLJSE showed comparatively lower potency than ascorbic acid, it demonstrated clear and reproducible free-radical scavenging capacity.

Table 4. DPPH radical scavenging activity of CLJSE and ascorbic acid

Concentration (µg/mL)	Ascorbic acid: % inhibition	CLJSE: % inhibition
20	26.42	15.54
40	32.12	24.87
60	50.77	40.93
80	57.51	55.44

Concentration (µg/mL)	Ascorbic acid: % inhibition	CLJSE: % inhibition
100	82.90	66.84
IC50 (µg/mL)	59.23	74.56

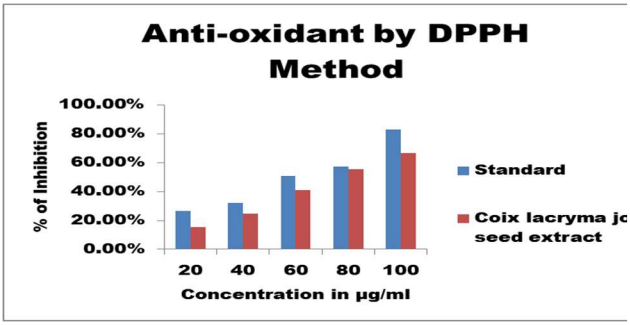


Figure 4. Concentration-dependent DPPH radical scavenging activity of CLJSE versus ascorbic acid

3.6. In Vitro Hepatoprotective Activity (HepG2/MTT Assay)

In the H2O2-induced HepG2 cytotoxicity model, silymarin produced 91.52-98.92% cell viability across the 20-100 µg/mL concentration range, while CLJSE produced a comparable concentration-dependent increase in viability, from 87.92% at 20 µg/mL to 94.46% at 100 µg/mL (Table 5, Figure 5). These findings indicate that CLJSE confers appreciable, though slightly lower, cytoprotection relative to the standard hepatoprotective agent.

Table 5. HepG2 cell viability following treatment with silymarin and CLJSE under H2O2 challenge

Concentration (µg/mL)	Silymarin: % viability	CLJSE: % viability
20	91.52	87.92
40	93.46	89.43
60	94.54	91.45
80	96.55	93.17
100	98.92	94.46

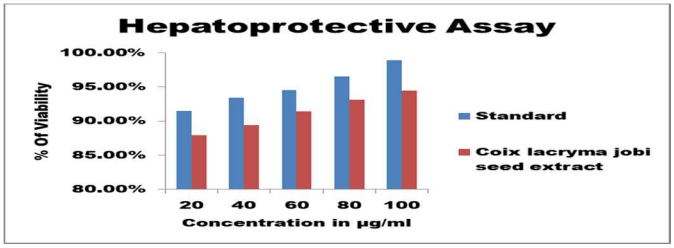
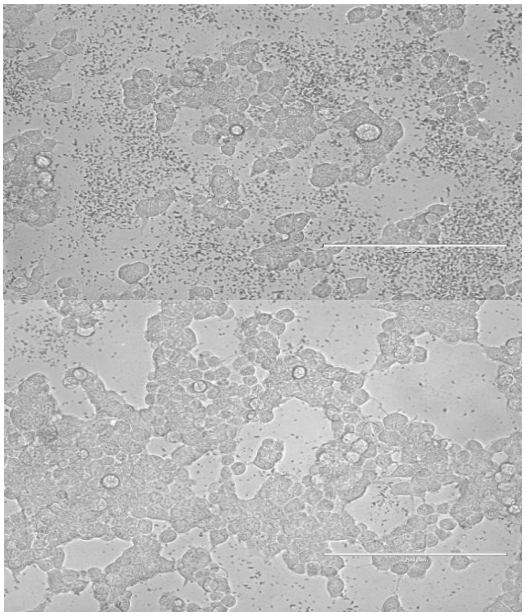
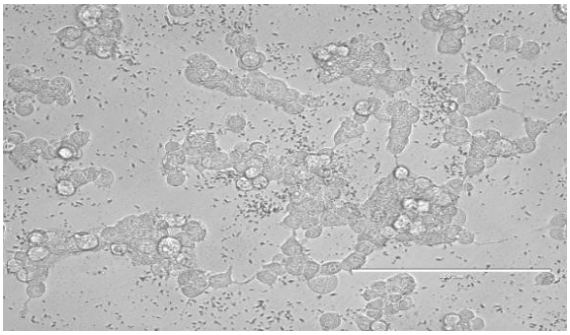


Figure 5. Hepatoprotective effect of CLJSE on H2O2-induced cytotoxicity in HepG2 cells (MTT assay)

Images:-



Control **Seed Extract**



Standard

3.7. Acute Oral Toxicity

A single oral dose of CLJSE (2000 mg/kg) produced no mortality, and no clinically significant abnormalities in behaviour, posture, motor coordination, or autonomic function over the 14-day observation period; mild laxative action was the only notable finding, consistent with the saponin content of the extract. Body weight gain in the treated group (202.5 ± 1.89 g to 215 ± 1.50 g) did not differ significantly from the control group (199 ± 0.8 g to 211 ± 0.96 g), confirming a favourable acute safety margin for CLJSE.

3.8. Effect of CLJSE on Serum Biochemical Markers of Hepatotoxicity

Isoniazid administration produced marked hepatocellular injury in the toxic control group, evidenced by significant elevations in serum AST (71.16 ± 0.70 U/mL), ALT (104.0 ± 1.70 U/mL), ALP (21.10 ± 0.39 KA units/dL), total bilirubin (1.52 ± 0.02 mg/dL), and liver weight (7.56 ± 0.08 g) compared with the normal control group ($p < 0.01$; Table 6, Figure 6). Co-treatment with silymarin (100 mg/kg) and CLJSE at both 200 mg/kg and 400 mg/kg significantly attenuated these elevations relative to the toxic control ($p < 0.01$). The high-dose CLJSE group (400 mg/kg) produced the greatest reduction among the test groups, with biochemical values approaching those of the silymarin-treated standard group, indicating dose-dependent hepatoprotection.

Table 6. Effect of CLJSE on serum biochemical parameters and liver weight in isoniazid-induced hepatotoxicity (Mean ± SEM, n = 6)

Parameter	Normal control	Toxic control (INH)	INH + Silymarin 100	INH + CLJSE 200	INH + CLJSE 400
AST (U/mL)	43.0 ± 1.07	71.16 ## ± 0.70	66.33** ± 0.88	53.33** ± 1.45	51.16** ± 0.87
ALT (U/mL)	35.5 ± 2.01	104.0 ## ± 1.70	65.33** ± 1.45	45.0** ± 1.83	40.16** ± 1.41
ALP (KA units/dL)	7.85 ± 0.26	21.10 ## ± 0.39	17.04** ± 0.43	15.35** ± 0.28	11.94** ± 0.32
Total bilirubin (mg/dL)	0.45 ± 0.20	1.52### ± 0.02	0.57** ± 0.03	0.77** ± 0.03	0.65** ± 0.01
Liver weight (g)	4.50 ± 0.09	7.56### ± 0.08	6.31** ± 0.04	6.18** ± 0.04	5.15** ± 0.03

##p < 0.01 vs. normal control; **p < 0.01 vs. toxic control (one-way ANOVA followed by Dunnett's test).

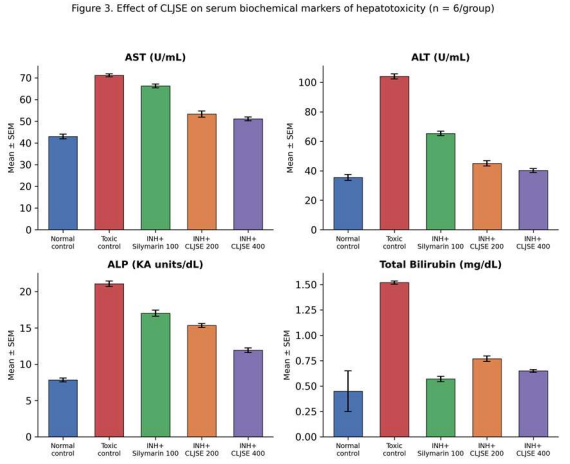


Figure 6. Effect of CLJSE treatment on serum biochemical markers of isoniazid-induced hepatotoxicity (n = 6/group; error bars represent SEM)

3.9. Effect of CLJSE on Hepatic Antioxidant Status

Isoniazid-induced hepatotoxicity was accompanied by significant oxidative stress, characterized by elevated hepatic MDA (0.672 ± 0.015 nmol/mg protein) and

depleted GSH (2.95 ± 0.08 nmol/mg protein), SOD (3.45 ± 0.10 U/mg protein), and CAT (26.00 ± 0.58 µmol H2O2/min/mg protein) compared with normal control ($p < 0.01$; Table 7, Figure 7). Treatment with CLJSE at both doses significantly reduced MDA and restored GSH, SOD, and CAT activities relative to the toxic control ($p < 0.01$), with the 400 mg/kg dose producing the greatest restoration, approaching values observed in the silymarin-treated group.

Table 7. Effect of CLJSE on hepatic antioxidant parameters in isoniazid-induced hepatotoxicity (Mean ± SEM, n = 6)

Parameter	Normal control	Toxic control (INH)	INH + Silymarin 100	INH + CLJSE 200	INH + CLJSE 400
MDA (nmol/mg protein)	0.120 ± 0.006	0.672 ± 0.015	0.240 ± 0.006	0.415 ± 0.008	0.275 ± 0.008
GSH (nmol/mg protein)	7.75 ± 0.08	2.95 ± 0.08	6.55 ± 0.08	4.85 ± 0.08	6.05 ± 0.08
SOD (U/mg protein)	8.48 ± 0.09	3.45 ± 0.10	7.25 ± 0.08	5.45 ± 0.08	6.85 ± 0.08
CAT (µmol/min/mg protein)	62.00 ± 0.58	26.00 ± 0.58	52.50 ± 0.76	38.50 ± 0.76	48.50 ± 0.76

All toxic-control values differ significantly from normal control ($p < 0.01$); all treated-group values differ significantly from toxic control ($p < 0.01$); one-way ANOVA followed by Dunnett's test.

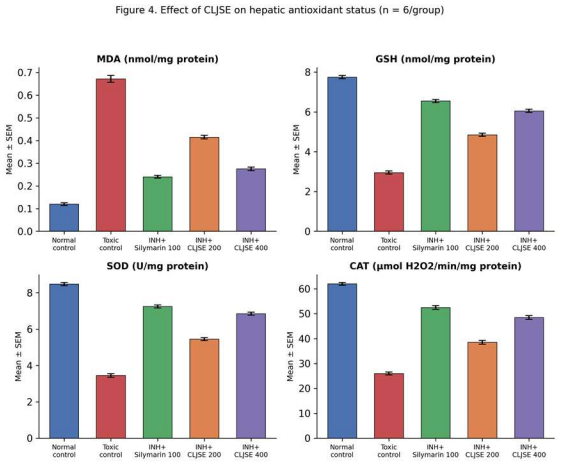


Figure 7. Effect of CLJSE treatment on hepatic antioxidant status in isoniazid-induced hepatotoxicity (n = 6/group; error bars represent SEM)

3.10. Histopathological Findings

Histopathological examination corroborated the biochemical and antioxidant findings. Liver sections from the normal control group showed well-preserved hepatic architecture with normal hepatocytes, prominent nuclei, intact sinusoidal spaces, and no evidence of necrosis or inflammation (Figure 8A). The toxic control group exhibited severe centrilobular necrosis, hepatocellular degeneration, sinusoidal congestion, fatty change, and dense inflammatory cell infiltration (Figure 8B), consistent with the marked biochemical derangement observed in this group. Animals treated with silymarin (100 mg/kg) showed substantial restoration of normal lobular architecture with well-preserved hepatocytes and only minimal residual inflammatory infiltration (Figure 8C). The CLJSE 200 mg/kg group showed moderate histological improvement, with reduced but still discernible hepatocellular degeneration and focal inflammatory infiltration (Figure 8D), corresponding to its intermediate biochemical recovery. The CLJSE 400 mg/kg group displayed near-normal hepatic architecture with well-preserved hepatocytes, minimal sinusoidal congestion, and an absence of significant necrosis (Figure 8E), closely paralleling the silymarin-treated group and providing morphological confirmation of the superior hepatoprotection observed at this dose.

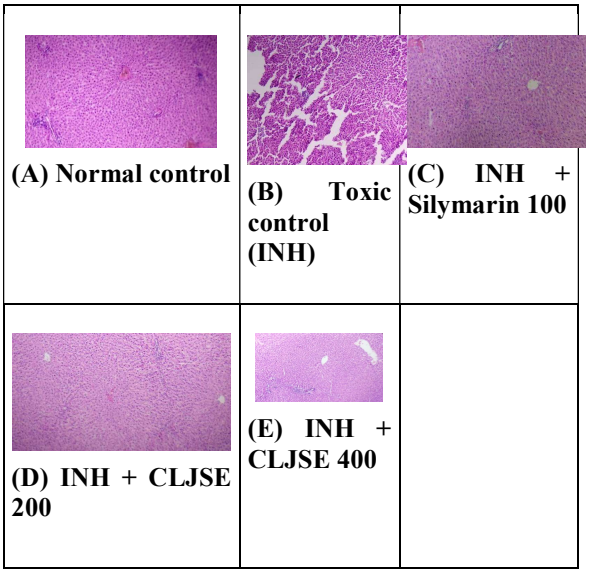


Figure 8. Representative photomicrographs of liver sections (H&E, ×400) across all experimental groups. (A) Normal control showing normal

hepatic architecture; (B) Toxic control (isoniazid alone) showing hepatocellular degeneration, sinusoidal congestion, and inflammatory infiltration; (C) Isoniazid + silymarin (100 mg/kg) showing near-complete restoration of normal architecture; (D) Isoniazid + CLJSE (200 mg/kg) showing moderate improvement with mild residual degeneration; (E) Isoniazid + CLJSE (400 mg/kg) showing near-normal hepatic architecture comparable to the standard-treated group.

3.11. Ex Vivo Pharmacological Screening

On isolated chicken ileum tissue challenged with isoniazid, the mean contractile response in the isoniazid-alone (toxic control) group was 1.20 ± 0.04 cm (Table 8, Figure 9). Co-incubation with the ethanolic extract (E-CLJSE, 100 mg) and the aqueous extract (Aq-CLJSE, 100 mg) reduced the mean contractile response to 0.97 ± 0.03 cm and 1.02 ± 0.09 cm, respectively, corresponding to reductions of approximately 19.3% and 15.1% relative to the isoniazid-alone group, consistent with a protective, spasmolytic-type action of both extracts on isoniazid-induced tissue contraction. Silymarin (10 µg/mL), included as the reference hepatoprotective standard, produced a mean contractile response of 1.54 ± 0.09 cm in this particular ex vivo assay - higher than the isoniazid-alone group - indicating that, unlike its consistent hepatoprotective performance in the biochemical and histopathological endpoints, silymarin did not show a comparable inhibitory action on isoniazid-induced ileal smooth-muscle contraction under the present experimental conditions. One-way ANOVA confirmed a statistically significant difference among the four groups ($p < 0.05$), with the extract-treated groups differing significantly from the isoniazid-alone group.

Table 8. Mean contractile response of isolated chicken ileum following isoniazid challenge with and without co-treatment (Mean ± SEM)

Group	Mean contractile response (cm)	% change vs. isoniazid alone
Isoniazid alone (toxic control)	1.20 ± 0.04	-
Isoniazid + Silymarin (10 µg/mL)	1.54 ± 0.09	+27.9%
Isoniazid + E-CLJSE (100 mg)	0.97 ± 0.03	−19.3%
Isoniazid + Aq-CLJSE (100 mg)	1.02 ± 0.09	−15.1%

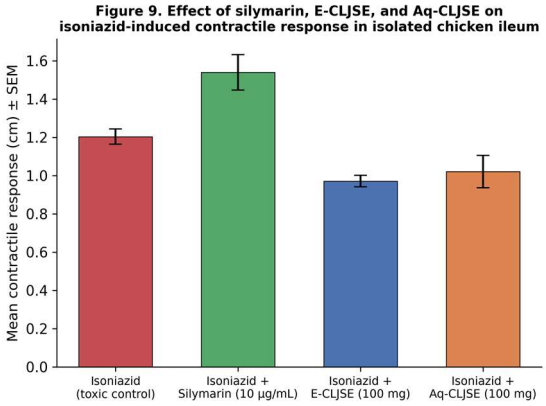


Figure 9. Effect of silymarin, E-CLJSE, and Aq-CLJSE on isoniazid-induced contractile response in isolated chicken ileum (mean ± SEM; error bars represent SEM).

DISCUSSION

This study provides an integrated phytochemical and pharmacological evaluation of *Coix lacryma-jobi* seed extract (CLJSE), demonstrating significant hepatoprotective activity against isoniazid-induced hepatotoxicity across in vitro, ex vivo, and in vivo systems, consistent with its traditional ethnomedicinal use in liver disorders.

4.1. Phytochemical and Physicochemical Basis of Activity

Preliminary phytochemical screening confirmed that CLJSE is rich in flavonoids, phenolic compounds, tannins, saponins, glycosides, proteins, carbohydrates, and steroids, while alkaloids were notably absent. FTIR analysis corroborated these findings, identifying O-H stretching characteristic of phenolics/alcohols, C=C stretching of aromatic/flavonoid rings, and C-O vibrations of ethers and alcohols. Flavonoids and phenolic acids are well-established free-radical scavengers and modulators of endogenous antioxidant enzyme systems [13,14], providing a plausible phytochemical basis for the antioxidant and hepatoprotective activities observed in this study. The physicochemical parameters obtained (loss on drying, ash values, extractive values) fell within standard reference ranges, confirming the quality and reproducibility of the extract used for pharmacological evaluation.

4.2. In Vitro Antioxidant and Cytoprotective Activity

The concentration-dependent DPPH scavenging activity of CLJSE (IC50 = 74.56 µg/mL), although lower than that of ascorbic acid (IC50 = 59.23 µg/mL), is consistent with previous reports describing appreciable antioxidant activity in phenolic-rich *C. lacryma-jobi* extracts [17,18]. Because reactive oxygen species generated during isoniazid biotransformation are central to the pathogenesis of hepatocellular injury [8-10], this radical-scavenging capacity is mechanistically relevant to the hepatoprotective effect subsequently observed in vivo.

The HepG2/MTT assay extended this observation to a human hepatocellular model, in which CLJSE conferred 94.46% cell viability against H2O2-induced cytotoxicity at 100 µg/mL, closely approaching the 98.92% viability achieved with silymarin. This cytoprotective effect in a human-derived cell line strengthens the translational relevance of the subsequent rodent findings and is consistent with the known cytoprotective actions of flavonoid- and phenolic-rich plant extracts against oxidative hepatocellular injury.

4.3. Safety Profile

The absence of mortality or significant toxic signs at 2000 mg/kg in the acute toxicity study, together with the lack of significant body-weight differences between treated and control animals, indicates that CLJSE is practically non-toxic under the conditions of OECD Guideline 423, affording a wide safety margin relative to the therapeutic doses (200-400 mg/kg) used in the hepatoprotective study. The mild laxative effect observed at the highest dose is consistent with the saponin content identified during phytochemical screening and is unlikely to limit therapeutic use.

4.4. Biochemical and Antioxidant Evidence of Hepatoprotection

Repeated intraperitoneal administration of isoniazid produced a robust and reproducible model of hepatotoxicity, reflected in significant elevations of serum AST, ALT, ALP, and total bilirubin, alongside increased liver weight, consistent with the established mechanism of isoniazid bioactivation via N-acetyltransferase 2 and cytochrome P450 enzymes to reactive hydrazine intermediates [8-10]. Elevated transaminases reflect loss of hepatocellular membrane integrity, while the rise in bilirubin indicates impaired hepatic excretory function.

Treatment with CLJSE produced a clear dose-dependent attenuation of these biochemical derangements, with the 400 mg/kg dose producing the most pronounced normalization, approaching the efficacy of silymarin. This pattern suggests that CLJSE limits hepatocellular enzyme leakage, most plausibly through membrane-stabilizing and antioxidant mechanisms attributable to its flavonoid and phenolic constituents.

This interpretation is reinforced by the hepatic antioxidant data. The toxic control group exhibited a substantial increase in MDA, a stable end-product of lipid peroxidation, together with marked depletion of GSH, SOD, and CAT, confirming oxidative injury as a central mechanism of isoniazid-induced hepatotoxicity. CLJSE treatment significantly reduced MDA while restoring GSH, SOD, and CAT activities in a dose-dependent manner, with the high-dose group again producing the greatest restoration. Preservation of hepatic GSH is of particular mechanistic importance, since glutathione is directly involved in the detoxification of reactive isoniazid metabolites; its conservation by CLJSE may reduce covalent binding of these metabolites to cellular macromolecules and thereby limit downstream necroinflammatory injury.

4.5. Histopathological Correlation

Histopathological findings provided morphological confirmation of the biochemical and antioxidant data. The severe centrilobular necrosis, inflammatory infiltration, and sinusoidal congestion observed in the toxic control group are characteristic features of isoniazid-induced hepatocellular injury. The near-normal hepatic architecture observed in the CLJSE 400 mg/kg group, closely paralleling the silymarin-treated group, corroborates the biochemical evidence

of dose-dependent hepatoprotection and strengthens the overall weight of evidence supporting the protective efficacy of the extract.

4.6. Comparison with Existing Literature

The hepatoprotective efficacy of CLJSE observed here is consistent with earlier reports describing protective activity of ethanolic *C. lacryma-jobi* seed extract against carbon-tetrachloride-induced hepatotoxicity [19], and with evidence that coixol, a bioactive lactone isolated from this plant, attenuates inflammatory liver injury through suppression of inflammatory signalling. The present findings extend this evidence base to a clinically relevant isoniazid-induced model and to human HepG2 cells, while also providing a complete pharmacognostic and phytochemical standardization profile that has been comparatively limited in earlier reports. Collectively, these results align with a broader literature describing hepatoprotective activity of structurally related antioxidant-rich plant extracts (e.g., Picrorhiza kurroa, Phyllanthus niruri, Andrographis paniculata) against antitubercular drug-induced liver injury, reinforcing the plausibility of an antioxidant-mediated, multi-targeted mechanism of hepatoprotection [reviewed in references cited].

4.7. Limitations

This study has certain limitations that should be acknowledged. The bioactive constituents responsible for the observed hepatoprotective effect were not individually isolated or quantified; the precise molecular mechanism (e.g., effects on specific signalling pathways such as Nrf2/Keap1 or NF-κB) was not investigated; and the duration of the in vivo study was limited to 28 days. Pharmacokinetic and chronic toxicity data are also currently unavailable. These aspects warrant further investigation, as outlined below.

5.CONCLUSION

The present study demonstrates that the ethanolic seed extract of *Coix lacryma-jobi* possesses significant, dose-dependent hepatoprotective activity against isoniazid-induced hepatotoxicity, supported by convergent evidence from in vitro antioxidant and cytoprotective assays, an acute toxicity study confirming a favourable safety margin, and an in vivo model demonstrating restoration of serum biochemical markers, hepatic antioxidant enzyme activity, and liver histoarchitecture. These protective effects are most plausibly mediated through free-radical scavenging, inhibition of lipid peroxidation, restoration of endogenous antioxidant defences, and stabilization of hepatocellular membranes, attributable to the flavonoid and phenolic constituents identified in the extract. The 400 mg/kg dose consistently produced effects approaching those of the standard hepatoprotective agent, silymarin. These findings provide scientific validation for the traditional hepatoprotective use of *C. lacryma-jobi* and support its further development as a natural adjuvant agent for the prevention of drug-induced liver injury during long-term antitubercular therapy.

6.FUTURE SCOPE

Future investigations should focus on the isolation, structural characterization, and quantification of the specific bioactive phytoconstituents responsible for the hepatoprotective activity of CLJSE, together with mechanistic studies examining relevant molecular pathways (e.g., Nrf2-mediated antioxidant response, inflammatory cytokine signalling). Evaluation of CLJSE in additional models of chronic liver injury, including hepatic fibrosis and non-alcoholic fatty liver disease, would help establish the breadth of its hepatoprotective potential. Pharmacokinetic profiling, sub-chronic and chronic toxicity studies, and the development of standardized, bioavailability-enhanced formulations are necessary steps toward eventual clinical translation as an adjuvant phytotherapeutic agent in patients receiving long-term antitubercular therapy.

7. DECLARATIONS

Ethics Approval

All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC), RJS College of Pharmacy (Approval No. IAEC-I/RJSCOP/2025-2026/05), and were conducted in accordance with OECD Guidelines 423 and 407 and institutional norms for the care and use of laboratory animals.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

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