

Evaluation of *Cissampelos pareira* L. on Chemical-Induced Hepatocellular Carcinoma in Rats

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Article Info:

Received: December 2025

Accepted: January 2026

Published online:

January 2026

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ABSTRACT:

Hepatocellular carcinoma (HCC), the largest cause of cancer death, is linked to oxidative stress, inflammation, and fibrosis. The N-nitroso diethylamine (NDEA) + carbon tetrachloride (CCl₄) model is a common preclinical method for assessing chemopreventive effects. *Cissampelos pareira* L., a medicinal plant high in isoquinoline alkaloids and flavonoids, has antioxidant, anti-inflammatory, and anticancer properties. This work used an NDEA-induced HCC model with CCl₄ in rats treated with a standardised *Cissampelos pareira* L. Serum biochemical markers (ALT, AST, ALP, bilirubin), antioxidant enzyme activities (SOD, CAT, GPx), lipid peroxidation, haematological measures (RBC, WBC, Hb), and histopathology were all used to assess outcomes. *Cissampelos pareira* L. improved liver biomarkers, antioxidant enzyme activity, and lipid peroxidation in NDEA + CCl₄-induced HCC, demonstrating chemopreventive and hepatoprotective properties. Histopathology revealed improved liver architecture, including decreased necrosis, fibrosis, and nodular lesions. The extract's effects are most likely mediated by its antioxidant and anticancer properties, suggesting its promise as an alternative treatment for HCC.

Keywords: *Cissampelos pareira* L.; Hepatocellular Carcinoma; NDEA; CCl₄.

Please Cite this article as: Reddy GD, Kartik R, Gupta A, Rao CV, Unnikrishnan MK, Pushpangadan P. Evaluation of *Cissampelos pareira* L. on Chemical-Induced Hepatocellular Carcinoma in Rats. Int. Pharm. Acta. 2025; 8(1):e10.

DOI: <https://doi.org/10.22037/ipa.v9i1.51064>

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common primary liver cancers globally, causing significant morbidity and mortality [1]. HCC aetiology is linked to chronic inflammation, oxidative stress, fibrosis, and genetic changes that promote uncontrolled cell growth [2]. Several chemical carcinogens have been utilised to imitate human HCC in rodents. N-nitrosodiethylamine (NDEA) is extensively used as an initiator of hepatocarcinogenesis, whereas carbon tetrachloride (CCl₄) promotes fibrosis and oxidative stress [3].

Dysregulation of signalling pathways such as NF-κB, STAT3, and p53 can worsen hepatic damage and promote malignant transformation [1, 4]. As a result, treatment methods that improve antioxidant defence, reduce inflammation, and induce apoptosis are

considered promising for HCC chemoprevention and management [5].

Medicinal plants have been widely studied for their hepatoprotective and anticancer properties due to their availability of bioactive phytochemicals such as alkaloids, flavonoids, and triterpenoids [6-9]. *Cissampelos pareira* L. (Menispermaceae), which has long been utilised in Ayurveda and folk medicine, has attracted attention for its broad range of pharmacological activities [10, 11]. Phytochemical studies reveal the existence of isoquinoline alkaloids such as hayatinin, cissamine, and pareirubrine, as well as flavonoids and tannins with antioxidant, anti-inflammatory, immunomodulatory, and anticancer effects [12, 13].

This study aimed to assess the chemopreventive and hepatoprotective effects of *Cissampelos pareira* L. extract in animals with NDEA + CCl₄-induced HCC. The

study examined biochemical markers of liver function, antioxidant enzyme activity, and histopathological changes, in contrast to conventional reference medicines such as Cisplatin [14].

2. Materials & Methods

2.1. Plant material

Cissampelos pareira L. plant material was gathered from the Botanical Garden of the National Botanical Research Institute in Lucknow, India (NBRI). Dr. Sayeeda Khatoon, a taxonomist, validated the plant material, and the voucher specimens (NAB2B04293, 200495, and 200492) were deposited in the departmental herbarium and the Institutional Museum for future reference.

2.2. Preparation of plant extract

Cissampelos pareira L. plant material was washed with distilled water to remove dirt and soil. Air-dried ($30 \pm 20^\circ\text{C}$) powdered *Cissampelos pareira* L. (100g) was exhaustively extracted by overnight maceration with 10 ml of ethanol and centrifugation at 10000 rev/min on a Rota evaporator (Buchi, USA), followed by drying in a lyophilizer (Labconco, USA) under reduced pressure. The extract was then exposed to phytochemical and pharmacological tests.

2.3. HPTLC analysis

To standardise the extract, the 50% ethanolic extract of *Cissampelos pareira* L. was fingerprinted using HPTLC. Finely ground plant material (5 g) was refluxed with ethanol, concentrated under decreased pressure, and 25 mg of extract was dissolved in ethanol. Samples (10 μL) were put on silica gel 60 F₂₅₄ plates using a CAMAG Linomat IV and developed in a mobile phase of ethyl acetate: formic acid: acetic acid: water (100:11:11:27) to a distance of 9 cm. Plates were visualised at UV 254 nm, derivatised with anisaldehyde-sulfuric acid, and heated at 110°C for 10 minutes. A CAMAG TLC scanner was used for densitometric scanning, and a peak at $R_f = 0.51$ was chosen as the standardisation marker for the extract [15].

2.4. In vivo study

2.4.1. Experimental animals

Adult Wistar rats weighing 140-160 g were used in the investigation. Animals were obtained from the Central Drug Research Institute's (CDRI) toxicity control animal facility in Lucknow and from animals raised at the department's animal house. Rats were housed in polyacrylic cages (a maximum of six animals per cage) under conventional laboratory conditions (temperature

$25 \pm 2^\circ\text{C}$, relative humidity 50-60%, and a 12-hour light/dark cycle). The animals were given free access to a conventional pellet meal (Amrut Laboratory Animal Feed, India) and water as needed.

All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) and were conducted in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India (IAEC Reg. No. 221/2000/CPCSEA).

2.4.2. Induction of hepatocellular carcinoma and experimental design

After acclimatization, animals were randomly divided into six groups ($n = 6$ per group). Hepatocellular carcinoma was induced using N-nitrosodiethylamine (NDEA) followed by carbon tetrachloride (CCl_4) as per the standard protocol.

- Group I (Normal control)

Rats received 0.9% normal saline intraperitoneally and served as the normal control.

- Group II (HCC control)

Rats received a single intraperitoneal injection of N-nitrosodiethylamine (NDEA) at a dose of 200 mg/kg body weight, followed by carbon tetrachloride (CCl_4) administration to promote hepatocarcinogenesis.

- Group III (*Cissampelos pareira* L.– low dose)

HCC-induced rats were treated with a 50% ethanolic extract of *Cissampelos pareira* L. at a dose of 100 mg/kg body weight, administered intraperitoneally.

- Group IV (*Cissampelos pareira* L.– medium dose)

HCC-induced rats were treated with a 50% ethanolic extract of *Cissampelos pareira* L. at a dose of 200 mg/kg body weight, administered intraperitoneally.

- Group V (*Cissampelos pareira* L.– high dose)

HCC-induced rats were treated with a 50% ethanolic extract of *Cissampelos pareira* L. at a dose of 400 mg/kg body weight, administered intraperitoneally.

- Group VI (Standard control)

HCC-induced rats received the standard anticancer drug cisplatin at a dose of 6 mg/kg body weight, administered intraperitoneally.

All treatments were administered using the same route (intraperitoneal) and vehicle (50% ethanol for extract-treated groups) to ensure experimental consistency. Animals were monitored throughout the study period for changes in body weight, clinical signs, and mortality.

2.5. Laboratory investigations

Body weights were measured on the day of receipt (before randomisation), on the day of dosing, and weekly thereafter for the treatment and recovery groups, and food and water consumption were tracked daily and

reported weekly. All animals had blood samples drawn for haematology and clinical biochemistry. Animals were placed in metabolic cages and fasted overnight before blood sampling, with unlimited access to water. Blood was drawn from the retro-orbital plexus using a micro-hematocrit heparinised glass capillary tube. Potassium EDTA was employed as an anticoagulant during haematology examinations. To obtain serum aliquots, blood samples were maintained in serum tubes at room temperature for around 30 minutes. Following clotting, the blood tubes were centrifuged at 3000 rpm for 15 minutes. The supernatants were decanted and kept at 70°C for further analysis.

2.6. Biochemical marker estimation

Biochemical markers such as SGOT (U/l), SGPT (U/l), SALP (U/l), Bilirubin level (U/l), and Gamma-glutamyltransferase (GGT; U/l) were determined for both control and treated groups using a standard biochemical method [16].

2.7. Estimation of free radical generation

The liver homogenate (5%) in ice-cold phosphate buffer was centrifuged at 800X g for 10 minutes, followed by centrifugation of the supernatant at 12,000X g for 15 minutes to obtain the mitochondrial fractions [17], which were used to measure lipid peroxidation (LPO), superoxide dismutase activity (SOD), catalase activity (CAT), reduced glutathione (GSH), and glutathione peroxidase (GPX).

2.8. Hematological estimation

Red blood cell counts, White blood cell counts, and hemoglobin were estimated using a hematology analyzer (Medonic CA620, Boule, Sweden) [16].

2.9. Histopathological analysis

The livers were taken immediately upon autopsy for histological analysis, and the tissues were preserved in 10% formalin for at least 24 hours. The paraffin sections were subsequently processed (Automatic Tissue Processor, Lipshaw) and sliced into 5 µm thick sections using a rotary microtome. The sections were then stained with haematoxylin-eosin (Merck) and mounted in Canada balsam. The histopathology slides were inspected and photographed using a picture zoom microscope (3.2X10 and 10X10) [18].

2.10 Statistical analysis

Experimental data are shown as mean ± SEM for six mice per group (n = 6). To analyse differences across groups, a one-way ANOVA was used, followed by Tukey's multiple-comparison post hoc test. A p-value of <0.05 was judged statistically significant. Data analysis was performed using GraphPad Prism software version [19].

3. Results & Discussion

3.1. HPTLC analysis of 50% EtOH extract of *Cissampelos pareira* L.

The derivatised plates displayed many well-defined bands of varying intensities, indicating the presence of various phytoconstituents. The chromatographic profile was recorded after densitometric scanning at 254 nm with a CAMAG TLC scanner (Figure 1). Among the resolved peaks, a distinct, reproducible band with an R_f of approximately 0.51 was consistently observed across repeated analyses. This peak, which has a somewhat higher area %, was chosen as the marker peak for extract standardisation, confirming the identification, consistency, and repeatability of the *Cissampelos pareira* L. extract utilised in the biological research.

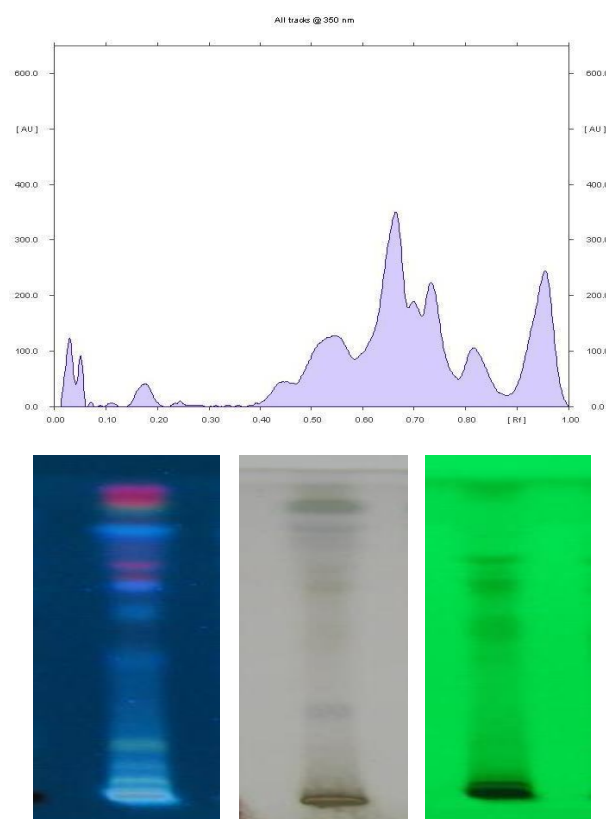


Figure 1: HPTLC fingerprint of ethanolic extract of *Cissampelos pareira* L. - EtOAcToluene: EtOAc: Formic acid (5:5:1).

The plant material was extracted with 50% ethanol, resulting in a 6.26% (w/w) extractive value (Table 1). The developed HPTLC fingerprint profile, together with the selection of a marker R_f peak, provides a credible chemical basis for quality control and allows for the use of the standardised extract in subsequent in vivo studies.

Table 1: HPTLC values.

Peak	Start Rf	Height %	End Rf	Area %
1	0.07	12.01	0.09	7.93
2	0.09	17.99	0.11	9.48
3	0.11	8.41	0.15	6.47
4	0.18	6.67	0.25	6.52
5	0.28	6.26	0.31	5.62
6	0.31	6.39	0.35	6.05
7	0.45	7.98	0.52	8.30
8	0.52	12.84	0.63	18.44
9	0.63	19.19	0.76	30.74
10	0.77	13.05	0.82	10.49
11	0.82	13.21	0.90	14.04
12	0.91	13.73	0.98	14.40
13	0.98	27.26	1.05	26.53

3.2. Effect of 50% ethanolic extract of *Cissampelos pareira* L. on body weight, liver weight, and average liver weight in (NDEA+CCl₄) induced HCC rat

50% ethanolic extracts of *Cissampelos pareira* L. at a dose of 100, 200 mg, and 400 mg once daily for 28 days, and standard Cisplatin at a dose of 6 mg/kg were studied to determine the body weight, liver weight, and average liver weight in HCC rats. The study showed that liver weights were significantly increased from 19.3,15.33 to

22.8,15.78 in the NDEA+CCl₄ group to 21.1,15.36 to 19.8,10.66 in the *Cissampelos pareira* L. treated animal. whereas the standard drug, Cisplatin 6 mg/kg, showed a significant reduction in liver weight compared with the NDEA+CCl₄ group (Figure 2).

3.3. Effect of 50% ethanolic extract of *Cissampelos pareira* L. SGOT, SGPT, SALP, BL, and GGT against NDEA+CCl₄ induced HCC

Table 2 summarizes the effect of the 50% ethanolic extract of *Cissampelos pareira* L. on serum biochemical markers in NDEA + CCl₄-induced hepatocellular carcinoma in rats. The HCC control group showed a significant elevation of SGOT, SGPT, SALP, bilirubin, and GGT levels compared with the normal control group ($p < 0.001$). Treatment with *Cissampelos pareira* L. extract at doses of 100, 200, and 400 mg/kg significantly reduced these elevated markers in a dose-dependent manner. The highest dose (400 mg/kg) restored biochemical parameters to near-normal levels and showed effects comparable to those of the standard drug, Cisplatin ($p < 0.001$).

3.4. Effect of 50% ethanolic extract of *Cissampelos pareira* L. on LPO, SOD, CAT, GPX, GST, and GSH against NDEA+CCl₄ induced HCC

Table 3 shows the effect of the 50% ethanolic extract of *Cissampelos pareira* L. on antioxidant parameters in NDEA + CCl₄-induced hepatocellular carcinoma in rats.

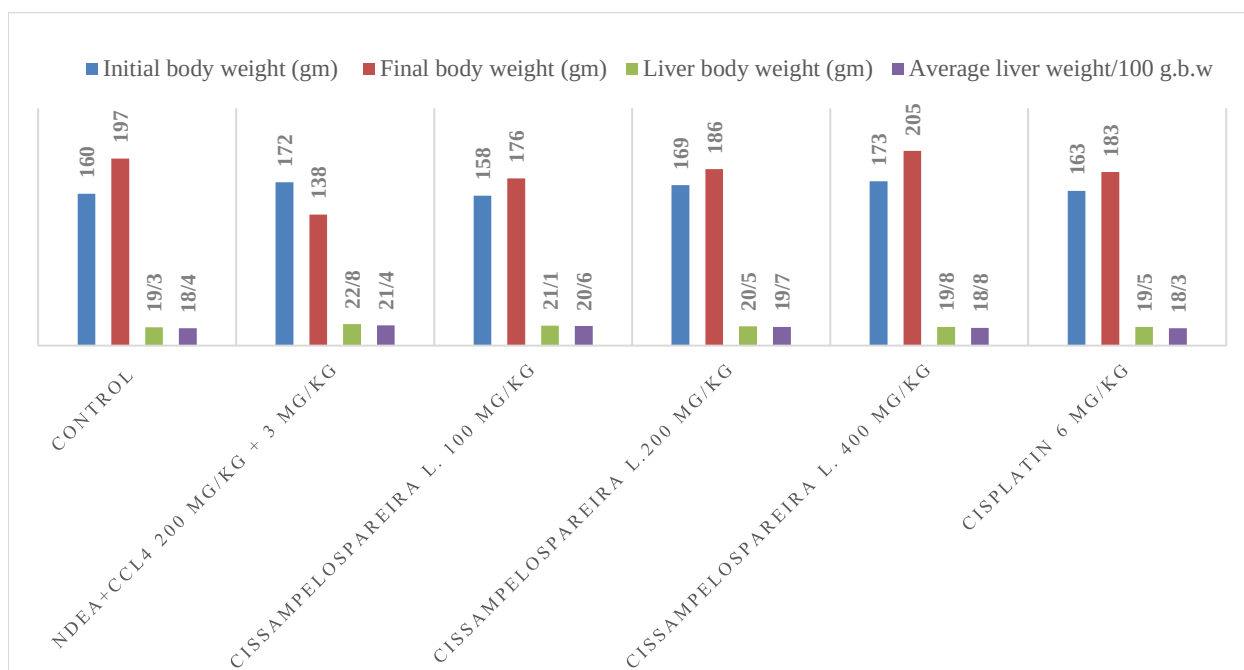


Figure 2: Effect of 50% ethanolic extract of *Cissampelos pareira* L. on body weight, liver weight, and average liver weight in (NDEA+CCl₄) induced HCC rat. Values are the mean $\pm$ SEM for 6 rats per group.

Table 2: Effect of the 50% ethanolic extract of *Cissampelos pareira* L. SGOT(U/l), SGPT(U/l), SALP, Bilirubin level (U/l) and Gamma glutamyl transpeptidase, GGT (U/l) in serum of rat.

Group	Treatment	Dose	SGOT (U/L)	SGPT (U/L)	SALP (U/L)	Bilirubin (mg/dL)	GGT (U/L)
I	Normal Control	-	208.22 ± 1.52	96.22 ± 1.64	246.12 ± 8.29	0.88 ± 0.04	43.4 ± 5.9
II	NDEA + CCl ₄	200 mg/kg (NDEA) + 3 mL/kg (CCl ₄)	374.62 ± 24.39 ^z	396.48 ± 28.26 ^z	452.39 ± 19.34 ^z	1.44 ± 0.04 ^z	171.4 ± 9.1 ^z
III	<i>Cissampelospareira</i> L.+ NDEA + CCl ₄	100 mg/kg	294.36 ± 21.52 ^a	291.31 ± 26.24 ^a	419.29 ± 22.32 ^a	1.31 ± 0.05 ^a	162.2 ± 8.2 ^a
IV	<i>Cissampelospareira</i> L.+ NDEA + CCl ₄	200 mg/kg	258.32 ± 23.31 ^b	204.11 ± 29.28 ^c	362.39 ± 20.52 ^b	1.02 ± 0.09 ^c	126.9 ± 4.1 ^c
V	<i>Cissampelospareira</i> L.+ NDEA + CCl ₄	400 mg/kg	214.72 ± 21.96 ^c	112.02 ± 30.39 ^c	258.86 ± 22.24 ^c	0.90 ± 0.11 ^c	69.4 ± 3.5 ^c
VI	Cisplatin + NDEA + CCl ₄	6 mg/kg	206.21 ± 19.01 ^c	104.28 ± 31.46 ^c	248.29 ± 22.62 ^c	0.92 ± 0.12 ^b	62.3 ± 3.9 ^c

Values are expressed as mean ± SEM (n = 6). ^z p < 0.001 compared with the normal control group (Group I). ^a p < 0.05, ^b p < 0.01, ^c p < 0.001 compared with HCC control group (Group II). Statistical analysis was performed using one-way ANOVA followed by a post hoc multiple-comparison test.

Table 3: Effect of 50% Ethanolic Extract of *Cissampelos pareira* L. on Antioxidant Parameters in NDEA + CCl₄- Induced Hepatocellular Carcinoma in Rats.

Group	Treatment	Dose	SOD	CAT	LPO	GPx	GST	GSH
I	Normal Control	—	126.8 ± 8.1	39.4 ± 1.6	10.51 ± 0.03	14.14 ± 0.02	11.61 ± 0.18	13.56 ± 0.36
II	NDEA + CCl ₄	200 mg/kg (NDEA) + 3 mL/kg (CCl ₄)	48.43 ± 8.22 ^z	15.38 ± 2.40 ^z	15.71 ± 1.19 ^z	11.37 ± 0.01 ^z	10.54 ± 0.04 ^z	13.10 ± 0.33
III	<i>Cissampelospareira</i> L. + NDEA + CCl ₄	100 mg/kg	52.24 ± 5.37	21.89 ± 1.40	14.56 ± 1.09	12.81 ± 0.04 ^c	10.62 ± 0.02	13.21 ± 0.34
IV	<i>Cissampelospareira</i> L. + NDEA + CCl ₄	200 mg/kg	80.89 ± 7.56 ^a	25.95 ± 2.75 ^a	12.10 ± 0.93 ^a	12.94 ± 0.02 ^c	10.89 ± 0.05 ^c	13.25 ± 0.33
V	<i>Cissampelospareira</i> L. + NDEA + CCl ₄	400 mg/kg	103.58 ± 8.24 ^c	33.32 ± 1.91 ^c	11.28 ± 0.68 ^b	13.68 ± 0.01 ^c	10.99 ± 0.04 ^c	13.34 ± 0.35
VI	Cisplatin + NDEA + CCl ₄	6 mg/kg	106.35 ± 6.20 ^c	35.99 ± 2.13 ^c	11.03 ± 0.51 ^b	13.93 ± 0.04 ^c	11.12 ± 0.10 ^c	13.36 ± 0.34

Values are expressed as mean ± SEM (n = 6). ^z p < 0.001 compared with the normal control group (Group I). ^a p < 0.05, ^b p < 0.01, ^c p < 0.001 compared with HCC control group (Group II). Statistical analysis was performed using one-way ANOVA followed by a post hoc multiple-comparison test.

The HCC control group showed a significant reduction in antioxidant enzymes (SOD, CAT, GPx, GST, and GSH) and a marked increase in lipid peroxidation compared with the normal control group (p < 0.001). Treatment with *Cissampelos pareira* L. extract significantly restored antioxidant enzyme levels and reduced lipid peroxidation in a dose-dependent manner, with the highest dose (400 mg/kg) showing effects comparable to Cisplatin.

3.5 Effect of 50% ethanolic extract of *Cissampelos pareira* L. on haematological parameters

Table 4 depicts the effect of the 50% ethanolic extract of *Cissampelos pareira* L. on hematological parameters in NDEA + CCl₄-induced hepatocellular carcinoma in rats. The HCC control group showed a significant reduction in RBC count and hemoglobin levels, along with a

marked elevation in WBC count compared with the normal control group. Treatment with *C. pareira* extract at doses of 100–400 mg/kg significantly restored RBC and hemoglobin levels and normalized WBC counts in a dose-dependent manner. The highest dose (400 mg/kg) produced hematological protection comparable to that of the standard drug.

3.6. Histopathological analysis

The control group's liver sections exhibited normal hepatic architecture, including well-arranged hepatocyte cords, intact sinusoids, and no indications of necrosis or inflammation. In contrast, rats treated with NDEA + CCl₄ showed severe histopathological changes, such as disruption of lobular architecture, hepatocyte ballooning, inflammatory cell infiltration, bile duct hyperplasia, preneoplastic nodules, nuclear pleomorphism,

Table 4: Effect of *Cissampelos pareira* L. on Hematological Parameters in NDEA + CCl₄-Induced Hepatocellular Carcinoma in Rats.

Group	Treatment	Dose	RBC (million/mm ³)	WBC (million/mm ³)	Hb (g/dL)
I	Normal Control	—	18.26 ± 0.74	16.32 ± 0.08	21.23 ± 0.40
II	NDEA + CCl ₄	200 mg/kg (NDEA) + 3 mL/kg (CCl ₄)	15.72 ± 0.50 ^z	19.76 ± 0.80 ^y	17.54 ± 0.30 ^z
III	<i>Cissampelospareira</i> L. + NDEA + CCl ₄	100 mg/kg	16.67 ± 0.53	18.51 ± 0.69	17.94 ± 0.68
IV	<i>Cissampelospareira</i> L. + NDEA + CCl ₄	200 mg/kg	17.04 ± 0.58	17.68 ± 0.54	18.36 ± 0.45
V	<i>Cissampelospareira</i> L. + NDEA + CCl ₄	400 mg/kg	17.82 ± 0.78	16.89 ± 0.40 ^b	19.87 ± 0.82 ^a
VI	Cisplatin + NDEA + CCl ₄	6 mg/kg	17.91 ± 0.48 ^a	16.43 ± 0.32 ^b	20.85 ± 0.81 ^b

Values are expressed as mean ± SEM (n = 6). ^zp<0.001 compared with the normal control group (Group I). ^yp<0.01 compared with the normal control group (Group I). ^a p < 0.05, ^b p < 0.01 compared with HCC control group (Group II). Statistical analysis was performed using one-way ANOVA followed by a post hoc multiple-comparison test

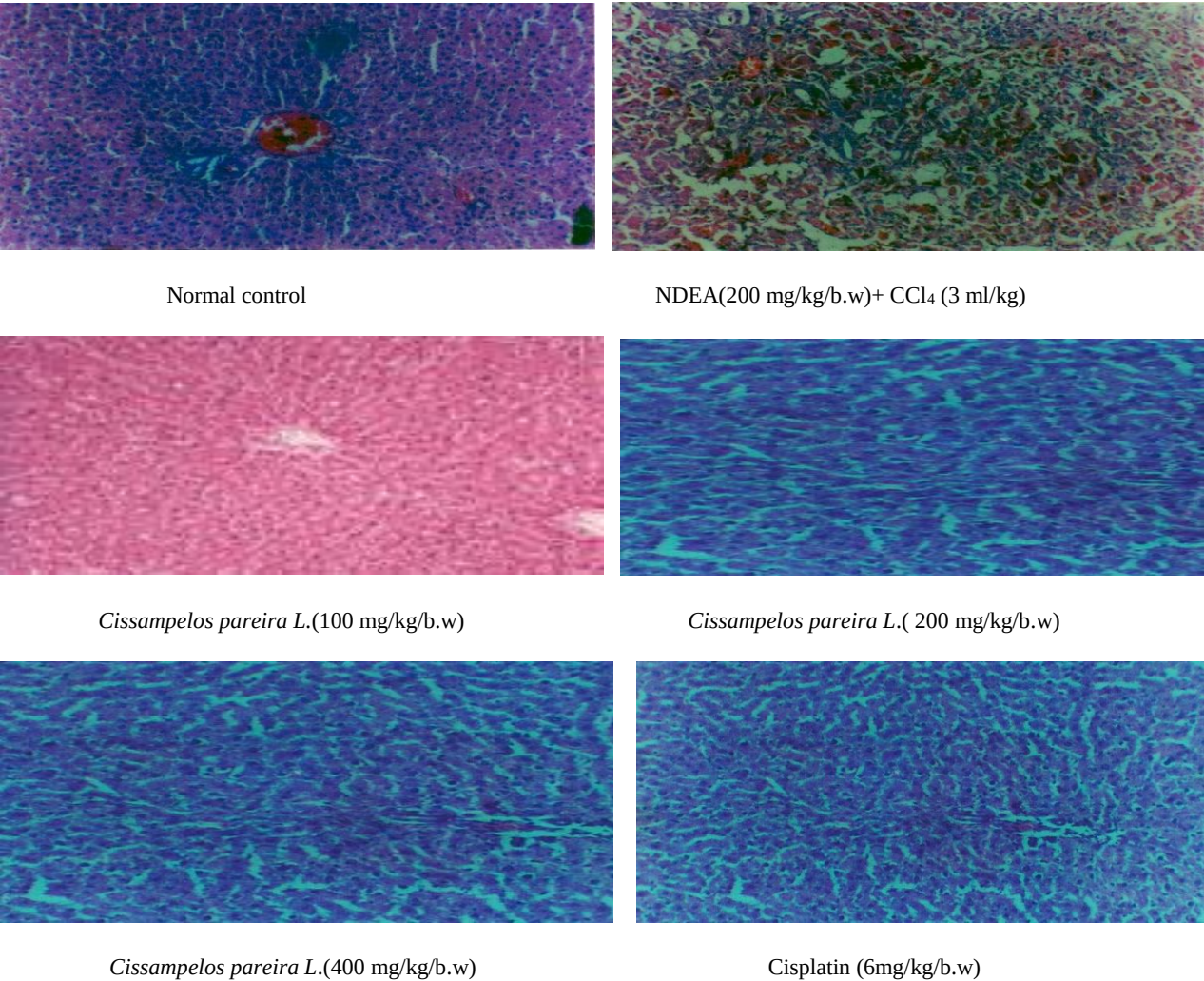


Figure 3: Histopathological analysis of *Cissampelos pareira* L. against NDEA+CCl₄ induced HCC.

hyperchromasia, and bridging fibrosis, indicating hepatocarcinogenesis. Treatment with a 50% ethanolic extract of *Cissampelos pareira* L. (100, 200, and 400 mg/kg) significantly improved these pathological

alterations in a dose-dependent manner. Compared with the HCC control group, extract-treated groups showed restored hepatic architecture, reduced inflammation and fibrosis, fewer preneoplastic lesions, and improved nuclear morphology. The cisplatin-treated group had nearly normal liver histology with minimal residual changes.

Animals were randomly assigned to experimental groups, and histopathological evaluations were performed in a blinded manner to ensure unbiased assessment (Figure 3).

This study found that *Cissampelos pareira* L. had strong hepatoprotective and chemopreventive effects against NDEA + CCl₄-induced hepatocellular carcinoma (HCC) in rats. Treatment with the 50% ethanolic extract successfully reduced hepatic injury and tumour progression, as demonstrated by normalisation of serum liver function markers, restoration of antioxidant enzyme levels, and improvement in histological architecture [20]. Normalisation of ALT, AST, ALP, and bilirubin levels indicates hepatic membrane integrity, but enhanced SOD, CAT, and GPx activity, along with decreased lipid peroxidation, emphasise oxidative stress reduction as a crucial protective mechanism. Histopathological observations supported these biochemical results, revealing reduced necrosis, fibrosis, and preneoplastic alterations in extract-treated groups [21].

HPTLC fingerprint profiling verified the presence of multiple reproducible phytoconstituents and ensured the chemical consistency of the extract used for biological evaluation. The well-resolved chromatographic pattern provides a phytochemical basis for the observed biological benefits, as the alkaloids and polyphenolic compounds identified by HPTLC are known to contribute to antioxidant and hepatoprotective properties. Thus, HPTLC research demonstrates a direct relationship between extract standardisation and in vivo efficacy [22]. This study's NDEA + CCl₄ model closely matches human hepatocarcinogenesis, including both initiation and promotion phases. *Cissampelos pareira* L. has the ability to alleviate biochemical, oxidative, and histological changes in this paradigm, demonstrating its translational significance and chemopreventive potential. To summarise, *Cissampelos pareira* L. has promising protective benefits against chemically induced HCC, as evidenced by biochemical recovery, antioxidant restoration, histological preservation, and phytochemical standardisation. Further research into isolating active ingredients and verifying molecular targets is required to increase its medicinal potential.

4. Conclusion

Cissampelos pareira L. extract effectively prevents and protects against hepatocellular cancer induced by

NDEA+CCl₄. The extract successfully restored serum biochemical indicators, increased endogenous antioxidant defence, decreased lipid peroxidation, and improved hepatic histoarchitecture. These findings emphasise *Cissampelos pareira* L.'s Therapeutic promise as a plant-based intervention for liver cancer and support further research focused on extracting its bioactive compounds, understanding molecular pathways, and verifying safety and efficacy in clinical settings.

Acknowledgements

We thank all the Ethno Pharmacology and Administrative staff at NBRI (CSIR), Lucknow, for their support and the timely completion of the studies.

Ethical Statment

All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) and were conducted in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India (IAEC Reg. No. 221/2000/CPCSEA).

Conflict of interest

There is no conflict of interest.

Funding

This study received no specific grant from any funding agency.

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