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Original Research Article

Hepatoprotective activity of Saraca indica leaves extract against ccl4-induced liver damage in rats

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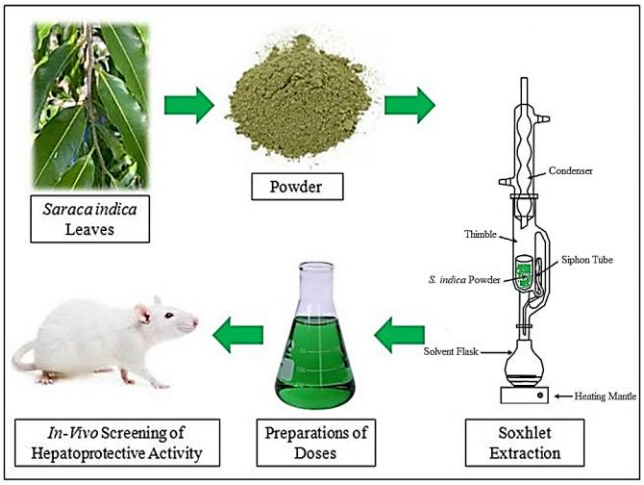
Abstract



The present study aimed to evaluate the hepatoprotective activity of ethanolic extract of Saraca indica leaves against carbon tetrachloride (CCl4) induced liver damage in rats. Induction of hepatotoxicity was produced by intraperitoneal injection of CCl4 and olive oil solution (1:1). The rats treated with the ethanolic extract of Saraca indica leaves at doses of 200 mg/kg and 400 mg/kg p.o. daily. A standard treated group received silymarin at 50 mg/kg for comparison. The higher dose 400 mg/kg significantly reduced serum levels of SGPT, SGOT, ALP, total bilirubin, and direct bilirubin compared to the untreated CCl4 control group. Histopathological analysis of liver tissue further supported these findings, showing a dose-dependent reduction in liver necrosis in treated groups. These results suggest that Saraca indica possesses significant hepatoprotective activity.

Keywords: Carbon tetrachloride, Hepatoprotection, Saraca indica, Silymarin

Received: 11-06-2025; **Accepted:** 28-08-2025; **Available Online**: 17-09-2025

1. Introduction

The liver is a body's largest and most crucial organ, plays a central role in numerous metabolic and detoxification processes. It is vital for the biotransformation of carbohydrates, proteins, and fats, and is also responsible for detoxifying harmful substances, producing bile, and storing vitamins. Consequently, maintaining normal liver function is

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https://doi.org/10.18231/j.ctppc.v.7.i.3.6

essential for overall physiological balance.[1] Despite its importance, liver disorders remain a significant global health challenge. The limited availability of effective drug treatments often leads to a poor prognosis and high mortality rates.[2] Liver diseases encompass a wide range of conditions, including viral hepatitis, alcoholic and non-alcoholic fatty liver diseases, autoimmune disorders, metabolic liver diseases, drug-induced liver injury, and gallstones. While the exact causes are often unknown, various infectious organisms and chemicals, such as alcohol, paracetamol, carbon tetrachloride (CCl4), and anabolic steroids, are known to cause hepatic damage.[3]

CCl4 is frequently used in animal studies to induce hepatic damage that mimics viral infections. When CCl4 reaches the liver, it is metabolized by cytochrome P-450 enzymes into two free radicals: trichloromethyl and trichloromethyl-peroxyl. These radicals initiate a process of lipid peroxidation, leading to significant liver damage.[4,5] Recent research highlights the critical role of antioxidants in reducing the risk of liver diseases and oxidative liver damage, spurring increased interest in natural remedie.[6,7]

Saraca indica (S. indica) is an evergreen plant that typically grows to a height of 7-10 cm. Its leaves are paripinnate, 15-20 cm long, and united at the petiole. The bark is dark brown and warty, and its golden-orange flowers have a petaloid calyx. The seeds are ellipsoid-oblong and compressed,[8] The plant is known for its diverse pharmacological activities. For instance, the flowers exhibit anticancer potential,[9] the leaves have anthelmintic and CNS depressant effects,[10,11] and the seeds possess antipyretic and anti-inflammatory activity.[12,13] Additionally, the bark has cardioprotective activity, and the roots are used to treat hemiplegia and paralysis.[14,15]

Bioactive compounds have been identified in the plant, with the leaves containing a higher concentration of gallic acid than the bark and stems.[16] The hepatoprotective activity of the leaves has not been evaluated. Therefore, this study aims to investigate the hepatoprotective activity of a S. indica leaves extract.

2. Materials and Methods

2.1. Chemicals

All organic solvents and chemicals of analytical grade were purchased from Labware Chemicals, Latur, Maharashtra, India.

2.2. Collection of plant material

Fresh leaves of *S. indica* were collected from Pune, Maharashtra, India. The plant material was identified and authenticated by Dr. C. S. Swami, Professor and Head, Department of Botany, Dayanand Science College, Latur.

2.3. Preparation of crude extract

The leaves of S. indica were thoroughly washed with distilled water and then shade-dried. After that the material was coarsely powdered using a mortar and pestle, and further grinded with an electric blender. The powder was stored in an airtight glass container for further process. A 50 g portion of the powdered leaves was subjected to continuous hot extraction using ethanol in a Soxhlet apparatus. After the extraction, the solvent was evaporated under reduced pressure using a vacuum evaporator.

2.4 Phytochemical screening

The phytochemical analysis of the extract was performed by following standard procedures.[17]

2.5 Experimental animals

All animal protocol experiments were carried out as per Committee for Control and Supervision of Experiments on Animals (CCSEA) guidelines and approved by Institutional Animal Ethics Committee (IAEC). Wistar albino rats weighing 180-200 g were procured from Crystal Biological Solutions, Pune. The rats were housed in polypropylene cages, with six rats per cage, in a controlled environment. The conditions were maintained at a temperature of 22-26°C and a relative humidity of 30-60%, with a 12-hour light-dark cycle. All animals provided free access to water ad libitum and a standard pellet diet.

2.6. Experimental procedure

Based on the methodology described by Mada et al.[18] the experimental procedure involved randomly dividing the rats into five groups of six animals each.

1. **Group I (Normal control):** Rats in this group received olive oil (1 mL/kg, p.o.) as a vehicle for 14 days.
2. **Group II (Disease control):** Rats were given CCl_4 1 mL/kg, i.p. in olive oil (1:1) on day 7 and day 14 to induce liver damage.
3. **Group III (Standard control):** Rats received silymarin (50 mg/kg, p.o.) for 14 days, along with the CCl_4 1 mL/kg, i.p. in olive oil (1:1) on day 7 and day 14.
4. **Group IV (EESI 200 mg/kg):** Rats were treated with ethanolic extract of *S. indica* leaves (EESI) at a dose of 200 mg/kg (p.o.) for 14 days, along with the CCl_4 1 mL/kg, i.p. in olive oil (1:1) on day 7 and day 14.
5. **Group V (EESI 400 mg/kg):** Rats received a higher dose of EESI (400 mg/kg, p.o.) for 14 days, along with the CCl_4 1 mL/kg, i.p. in olive oil (1:1) on day 7 and day 14.

On the 15^{th} day, blood samples were collected for biochemical analysis, and liver tissues were isolated for histopathological examination.

2.7. Estimation of Serum biochemical parameters

Standard UV kinetic assay kits from Proton Biologicals India Private Limited were used to measure serum biochemical levels of SGPT, SGOT, ALP, total protein (TP), and total and direct bilirubin. All tests were conducted using a Shimadzu UV-1800 spectrophotometer, following the manufacturer's instructions included with the kits.[19]

2.8. Assessment of in-vivo antioxidant potential

2.8.1. Malondialdehyde (MDA) level

The level of malondialdehyde (MDA), a marker of lipid peroxidation, was determined by measuring thiobarbituric acid-reactive substances (TBARS). The reaction mixture was prepared by combining 100 µL of 10% tissue homogenate with 15 µL of 8.1% SDS, 60 µL of 2.5M acetic acid buffer (pH 3.4), and 115 µL of 0.81% thiobarbituric acid. This mixture was then heated in a 95°C water bath for 120 minutes. After cooling to room temperature, the absorbance was measured at 532 nm.[20.21]

The concentration of MDA, expressed in nmol/mg of protein, was calculated using the following formula:

$$\text{Concentration} = A \times (V/E) \times P$$

Where:

1. A = Absorbance
2. V = Volume of the solution
3. E = Extinction coefficient (1.56×10^5 m^{-1} cm^{-1})
4. P = Protein content of the tissue (mg of protein/g of tissue)

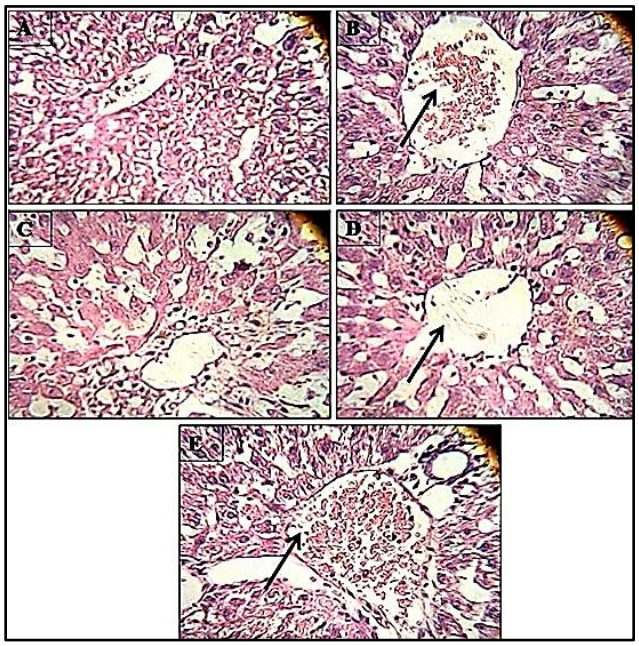

Figure 1: Histopathology of liver tissues in experimental groups of rats: **(A)** Normal control Group I. **(B)** Disease control Group II. **(C)** Silymarin treated Group III. **(D)** EESI 200 mg/kg treated Group IV. (E) EESI 400 mg/kg treated Group V.

2.8.2. Catalase (CAT) level

25 µL sample or standard was added to duplicate wells, with an additional 25 µL of assay buffer used for zero standards. The reaction was initiated by adding 25 µL of hydrogen peroxide reagent to each well. After 30 minutes incubation at room temperature, 25µL of a substrate was introduced, followed by the addition of 25 µL of HRP reagent to begin the reaction. The plate was incubated for another 15 minutes at room temperature, and the optical density was then measured at 560 nm.[22.23]

2.8.3. Reduced glutathione (GSH) level

A glutathione (GSH) assay was performed by mixing a tissue homogenate with a 10% w/v trichloroacetic acid (TCA) solution at a 1:1 ratio. The mixture was then centrifuged at 1000 rpm for 10 minutes at 4°C. A 0.5mL aliquot of the resulting supernatant was combined with 2mL of 0.3 M disodium hydrogen phosphate. Following this, 0.25mL of freshly prepared 0.01M DTNB (dissolved in 1% w/v citric acid) was added. The absorbance of the final mixture was measured at 412 nm using UV-spectrophotometer. A standard curve was generated with reduced glutathione concentrations from 5 to 50 µM, and the final results are expressed as micromoles of reduced glutathione per milligram of protein.[24]

2.9. Histopathology studies

The livers were removed from the rats and preserved in a 10% formalin solution. The liver tissue was embedded in paraffin and sliced into 6µm thick sections, which were then mounted on glass slides. The sections were stained with eosin and hematoxylin and examined under a light microscope. The hepatoprotective effects were accessed by looking for signs of lymphocyte infiltration, centrilobular necrosis, fatty infiltration, and fibrosis.[25]

2.10. Statistical analysis

All data are presented as the mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test and p-value < 0.05 was considered statistically significant.

3. Results

3.1. Phytochemical analysis of the extract

Preliminary phytochemical analysis of the extract showed the presence of several key compounds, including carbohydrates, proteins, terpenoids, flavonoids, tannins, saponins, polyphenols, and glycosides (**Table 1**).

3.2. Effects of the EESI on body weight and liver weight

Upon CCl4 administration, the disease control group (Group II) experienced a significant decrease in body weight, falling

to 184 ± 2.42 g, which was a substantial reduction compared to the normal control group (Group I) at 206.67 ± 3.24 g ($p<0.001$). This indicates that CCl4 induced a state of physiological stress or illness.

However, treatment with the EESI at both 200 mg/kg (Group IV) and 400 mg/kg (Group V) doses significantly decreased this effect. Body weights in these groups increased to 196.83 ± 2.35 g ($p<0.01$) and 200.83 ± 2.76 g ($p<0.001$), respectively, when compared to the disease control group. Similarly, the standard treatment, Silymarin (Group III), also significantly improved body weight to 199.83 ± 2.60 g ($p<0.001$). These findings suggest that both the EESI and Silymarin treatments helped to mitigate the CCl4-induced weight loss.

Regarding liver health, the CCl4-treated rats in Group II showed a significant elevation in liver weight (7.02 ± 0.18 g, $p<0.001$) compared to the normal control group (4.89 ± 0.22 g). This increase in liver weight is a common indicator of liver injury, such as inflammation, cellular swelling, or fatty infiltration.

Treatment with Silymarin (Group III) effectively reduced liver weight to 5.23 ± 0.16 g ($p<0.001$), bringing it close to the normal range. The EESI-treated groups also showed a dose-dependent reduction in liver weight. At 200 mg/kg, the liver weight was reduced to 6.38 ± 0.15 g ($p<0.05$), while the 400 mg/kg dose resulted in a more substantial reduction to 5.80 ± 0.09 g ($p<0.001$).

The relative liver weight, which accounts for the animal's overall body weight, showed a similar pattern. It was significantly increased in the disease control group (3.81 ± 0.24 g, $p<0.001$) compared to the normal control group (2.36 ± 0.12 g). Silymarin treatment significantly decreased this ratio to 2.61 ± 0.11 g ($p<0.001$). Both doses of EESI also significantly lowered the relative liver weight to 3.24 ± 0.16 g ($p<0.05$) and 2.89 ± 0.07 g ($p<0.001$) for the 200 mg/kg and 400 mg/kg doses, respectively. The significant reductions in both liver weight and relative liver weight in the EESI-treated groups indicate that the extract possesses hepatoprotective properties that help to reduce CCl4-induced liver enlargement and damage (**Table 2**).

3.3. Serum biochemical parameters

All serum biochemical parameters are detailed in **Table 3**.

3.3.1. Liver enzyme and bilirubin levels

In the disease control group (Group II), CCl_4 administration led to a significant increase in key liver enzymes compared to the normal control group (Group I) ($p<0.001$). SGPT levels rose to 116.62 ± 16.23 IU/L (from 38.41 ± 6.22 IU/L in Group I), SGOT to 289.43 ± 18.22 IU/L (from 86.12 ± 5.38 IU/L), and ALP to 690.11 ± 7.63 IU/L (from 180.31 ± 12.76 IU/L). This drastic increase in liver enzymes is a clear sign of severe liver damage.

Treatment with the ethanolic extract of *S. indica* leaves (EESI) significantly mitigated this damage in a dose-dependent manner.

1. The 200 mg/kg dose (Group IV) significantly reduced SGPT to 85.4 ± 12.24 IU/L ($p<0.01$), SGOT to 170.3 ± 20.52 IU/L ($p<0.05$), and ALP to 350.25 ± 10.55 IU/L ($p<0.01$).
2. The 400 mg/kg dose (Group V) provided an even greater protective effect, lowering SGPT to 78.9 ± 5.80 IU/L ($p<0.001$), SGOT to 130.40 ± 13.28 IU/L ($p<0.001$), and ALP to 297.83 ± 12.68 IU/L ($p<0.001$).
3. Similarly, the standard drug Silymarin (Group III) also caused a significant reduction in these enzyme levels ($p<0.001$), with SGPT at 70.10 ± 5.35 IU/L, SGOT at 114.00 ± 12.43 IU/L, and ALP at 209.93 ± 8.33 IU/L.
4. Notably, there were no significant differences in these enzyme levels between the EESI-treated groups and the Silymarin-treated group.

3.3.2. Total protein and bilirubin levels

In addition to enzyme elevation, the disease control group (Group II) showed a significant decrease in serum total protein (TP) to 3.58 ± 0.28 g/dL ($p<0.001$), a marker of impaired liver synthesis. Concurrently, total and direct bilirubin levels were significantly elevated to 4.45 ± 0.18 mg/dL and 3.83 ± 0.21 mg/dL, respectively, compared to the normal control group ($p<0.001$). This indicates compromised liver function and an inability to properly process and excrete bilirubin.

Again, treatment with EESI showed significant improvement:

1. Both EESI-treated groups (IV and V) significantly improved TP levels ($p<0.05$ and $p<0.001$, respectively) and significantly reduced total and direct bilirubin levels ($p<0.01$ for all).
2. The higher dose of EESI (400 mg/kg) was more effective, with TP levels reaching 6.80 ± 0.25 g/dL and total and direct bilirubin at 2.41 ± 0.06 mg/dL and 1.16 ± 0.11 mg/dL, respectively.
3. Silymarin (Group III) was also highly effective ($p<0.001$), restoring TP to 7.2 ± 0.18 g/dL and significantly reducing bilirubin levels.
4. Similar to the enzyme results, there were no significant differences in TP, total bilirubin, or direct bilirubin levels between the EESI and Silymarin groups, suggesting that the plant extract has a comparable therapeutic effect on these markers of liver function.

Table 1: Phytochemical investigation of ethanolic extract of *Saraca indica* leaves.

Sr. No.	Phytochemical	Result
1	Alkaloids	–
2	Terpenoids	+
3	Polyphenols	+
4	Tannins	+
5	Flavonoids	+
`6	Saponins	+
7	Steroids	–
8	Glycosides	+

***Note:** (+) indicates Presence, (–) indicates Absence.

Table 2: Effect of EESI on body weight, change in body weight and absolute and relative liver weight.

Group	Body Weight (g)		Change in Body Weight	Liver Weight (g)	Relative Liver Weight (%)
	Initial	Final			
Normal control	195.83 ± 2.82	206.67 ± 3.24	10.83 ± 3.58	4.89 ± 0.22	2.36 ± 0.12
Disease control (CCl_4)	193.16 ± 1.55	184.00 ± 2.42$^{###}$	-9.16 ± 1.62$^{###}$	7.02 ± 0.18$^{###}$	3.81 ± 0.24$^{###}$
Standard (Silymarin + CCl_4)	189.67 ± 2.45	199.83 ± 2.60***	10.16 ± 1.35***	5.23 ± 0.16***	2.61 ± 0.11***
EESI 200 mg/kg + CCl_4	191.33 ± 1.92	196.83 ± 2.35**	5.50 ± 1.93 ***	6.38 ± 0.15*	3.24 ± 0.16*
EESI 400 mg/kg + CCl_4	190.50 ± 2.72	200.83 ± 2.76***	10.33 ± 2.13***	5.80 ± 0.09***	2.89 ± 0.07***

All values are represented as mean ± SEM, n = 6.
###P < 0.001 compared with the normal control group. ***P < 0.001 to **P < 0.01 and to *P < 0.05 compared with the disease control group (CCl_4).

Table 3: Effects of EESI on serum biochemical parameters.

Group	SGPT (IU/L)	SGOT (IU/L)	Bilirubin (mg/dl)		ALP (IU/L)	Total Protein (g/dL)
			Total	Direct		
Normal control	38.41 ± 6.22	86.12 ± 5.38	0.65 ± 0.08	0.09 ± 0.05	180.31 ± 12.76	7.9 ± 0.27
Disease control (CCl4)	116.62 ± 16.23###	289.43 ± 18.22###	4.45 ± 0.18###	3.83 ± 0.21###	690.11 ± 7.63###	4.2 ± 0.23###
Standard (Silymarin + CCl4)	70.10 ± 5.35***	114.0 ± 12.43***	1.16 ± 0.09***	1.12 ± 0.12***	200.93 ± 8.33***	7.2 ± 0.18***
EESI 200 mg/kg + CCl4	85.4 ± 12.24**	170.3 ± 20.52*	4.20 ± 0.14**	2.8 ± 0.24**	350.25 ± 10.55**	5.3 ± 0.32*
EESI 400 mg/kg + CCl4	78.9 ± 5.82***	130.4 ± 13.28***	2.41 ± 0.06**	1.16 ± 0.11**	297.83 ± 12.68***	6.8 ± 0.25***

All values are represented as mean ± SEM, n = 6.
###P < 0.001 compared with the normal control group. ***P < 0.001 to **P < 0.01 and to *P < 0.05 compared with the disease control group (CCl_4).

Table 4: Effects of EESI on MDA, CAT and GSH levels in CCl_4 treated rats.

Group	MDA (nmole/mg of tissue protein)	CAT (U/mL)	GSH (µmol/mg of tissue protein)
Disease control (CCl4)	**0.479 ± 1.121###**	**1.49 ± 0.04###**	**46.66 ± 1.66###**
Standard (Silymarin + CCl4)	0.211 ± 0.012***	2.42 ± 0.02***	53.67 ± 1.63***
EESI 200 mg/kg + CCl4	0.328 ± 0.003*	2.20 ± 0.04**	50.52 ± 1.21*
EESI 400 mg/kg + CCl4	0.230 ± 0.002**	2.33 ± 0.06**	51.81 ± 1.71**

All values are represented as mean ± SEM, n = 6.
$^{###}$P < 0.001 compared with the normal control group. ***P < 0.001 to **P < 0.01 and to *P < 0.05 compared with the disease control group (CCl_4).

3.3. Estimation of malondialdehyde, catalase and glutathione in CCl4 treated rats

The effects of EESI on hepatic levels of MDA, CAT, and GSH are showed in **Table 4**. In rats treated with CCl4 (Group II), researchers observed significant signs of oxidative stress and liver damage compared to the healthy control group (Group I). Specifically, the CCl4 group showed a drastic reduction in antioxidant levels, with catalase (CAT) activity dropping from 2.10 to 1.49 U/mL and glutathione (GSH) levels decreasing from 55.39 to 46.66 µmol/mg. There was a dramatic increase in lipid peroxidation, indicated by a rise in malondialdehyde (MDA) levels from 0.185 to 0.479 nmole/mg.

Treatment with the extract EESI in doses of both 200 mg/kg (Group IV) and 400 mg/kg (Group V) effectively reversed these negative effects. Both doses significantly increased CAT and GSH levels while lowering MDA levels, demonstrating an improvement in the rats' antioxidant status. The higher dose of EESI (400 mg/kg) was more effective than the lower dose, providing greater protection against CCl4-induced damage.

A standard control group treated with Silymarin (Group III) also showed strong antioxidant effects, significantly increasing CAT activity and GSH levels while reducing MDA. The results suggest that while EESI is a promising agent for combating oxidative stress, the 400 mg/kg dose was still slightly less effective than Silymarin at 50 mg/kg.

3.4. Histopathological studies

Histopathological analysis of liver tissues from the normal control group (Group I) revealed intact hepatic architecture and well-defined vesicular nuclei. In contrast, the CCl4-induced group (Group II) showed marked hepatocellular necrosis and degeneration. Rats treated with Silymarin (Group III) displayed normal liver histology, with well-preserved nuclei and architecture.

The EESI 200 mg/kg treated group (Group IV) showed mild degenerative changes in the regenerating hepatocytes, along with evident widening of intercellular spaces. The EESI 400 mg/kg treated group (Group V), however, exhibited normal liver histology, with a marked reduction in hepatocyte degeneration and well-preserved nuclei and architecture, similar to the Silymarin-treated group (Group III).

The CCl4-induced liver damage in Group II was further characterized by prominent lymphocytic infiltration, centrilobular cell degeneration, and extensive necrosis. These pathological changes were significantly reduced in Groups IV and V compared to the Silymarin-treated Group III (**Figure 1**).

4. Discussion

This study evaluated the hepatoprotective potential of the EESI in CCl4-induced liver damage in rats, supporting the traditional use of plant remedies for managing liver disorders. CCl4 induced liver damage by causing centrilobular hemorrhagic necrosis in both humans and experimental animals,[26] and its hepatotoxicity model is commonly used to evaluate the hepatoprotective potential of medicinal plant extracts. The effects of plants were measured by serum biochemical markers and supported through histopathological analysis of liver tissues.

CCl4 undergoes hepatic metabolism converts into trichloromethyl radicals, which in the presence of reactive oxygen species (ROS) converted into trichloromethyl peroxy radicals. These highly reactive species bind to lipid membranes and shows disruption of liver microsomal membranes, and finally lead to loss of membrane integrity as well as cellular damage.[27]

In the present study, EESI trated rats showed significantly attenuated the elevated biochemical parameters induced by CCl4 administration. This improvement is likely related to the extract's ability to counteract early liver damage and restore cytochrome P450 function in the endoplasmic reticulum. The normalization of serum SGOT, SGPT, ALP, and bilirubin levels suggests the onset of hepatic regeneration. Histopathological findings further confirmed the hepatoprotective activity of EESI: while Group I (normal control) displayed intact liver architecture, Group II (CCl4-treated) showed marked hepatocyte necrosis and degeneration. EESI treatment 200 and 400 mg/kg doses reduced the CCl4-induced liver damage in a dose-dependent manner. The liver sections from CCl4-intoxicated rats also showed features such as centrilobular necrosis and karyolysis,[28] which were markedly reduced by EESI treatment.

The antioxidant potential of EESI was evaluated by in vitro assays. Elevated levels of MDA and other lipid peroxidation products, such as conjugated dienes and thiobarbituric acid reactive substances, are indicators of oxidative stress.[29] In this study, EESI significantly decreased MDA levels in CCl4-treated rats. Moreover, it restored the levels of key antioxidant enzymes glutathione (GSH) and catalase (CAT), suggesting potent free radical scavenging activity. These findings suggest that EESI helps to replenish endogenous antioxidant defenses by preventing the depletion of detoxifying enzymes, thereby mitigating oxidative stress.[30]

Free radical-induced oxidative damage is a central mechanism in the development of numerous diseases. The numerous phytochemicals including flavonoids, terpenoids, tannins, polyphenols, steroids, and glycosides are known for their strong antioxidant and hepatoprotective properties.[31,32] Preliminary phytochemical screening of EESI revealed the presence of carbohydrates, proteins, terpenoids, polyphenols,

tannins, flavonoids, saponins, and glycosides. The observed hepatoprotective activity of S. indica therefore attributed due to the presence of bioactive constituents such as flavonoids, terpenoids, tannins, polyphenols, and glycosides.

This study investigated the potential of an ethanolic extract of S. indica (EESI) to protect against liver damage induced by CCl4 in rats, supporting the traditional use of the plant for managing liver disorders. The effects were measured using serum biochemical markers and confirmed by histopathological analysis of liver tissues.

The CCl4-induced hepatotoxicity model is commonly used to evaluate the hepatoprotective effects of medicinal plants. CCl4 causes liver damage by inducing centrilobular hemorrhagic necrosis in both humans and experimental animals.[26] During hepatic metabolism, CCl4 is converted into highly reactive trichloromethyl radicals. These radicals, in the presence of reactive oxygen species (ROS), are further converted into trichloromethyl peroxy radicals. These reactive species bind to and disrupt liver microsomal membranes, leading to a loss of membrane integrity and cellular damage.[27]

In this study, EESI treatment significantly attenuated the elevated biochemical parameters induced by CCl4 administration. This improvement is likely due to the extract's ability to counteract early liver damage and restore cytochrome P450 function in the endoplasmic reticulum. The normalization of serum SGOT, SGPT, ALP, and bilirubin levels suggests the onset of hepatic regeneration.

Histopathological findings further confirmed the hepatoprotective activity of EESI. While the normal control group (Group I) showed intact liver architecture, the CCl4-treated group (Group II) displayed marked hepatocyte necrosis and degeneration. EESI treatment at doses of 200 and 400 mg/kg reduced the CCl4-induced liver damage in a dose-dependent manner. Liver sections from CCl4-intoxicated rats also showed centrilobular necrosis and karyolysis.[28] which were markedly reduced by EESI treatment.

EESI's antioxidant potential was evaluated through in vitro assays. Elevated levels of malondialdehyde (MDA), a key indicator of oxidative stress, were significantly decreased in CCl4-treated rats after EESI administration.[29] Moreover, EESI restored the levels of key antioxidant enzymes, glutathione (GSH) and catalase (CAT), suggesting potent free radical scavenging activity. These findings indicate that EESI helps replenish endogenous antioxidant defenses and mitigates oxidative stress by preventing the depletion of detoxifying enzymes.[30]

Free radical-induced oxidative damage is a central mechanism in many diseases. Numerous phytochemicals, including flavonoids, terpenoids, tannins, polyphenols, steroids, and glycosides, are known for their strong antioxidant and hepatoprotective properties.[31,32] Preliminary phytochemical screening of EESI revealed the presence of carbohydrates, proteins, terpenoids, polyphenols, tannins, flavonoids, saponins, and glycosides. Therefore, the observed hepatoprotective activity of S. indica is attributed to the presence of these bioactive constituents.

5. Conclusion

The ethanolic extract of *S. indica* leaves posses potent hepatoprotective activity. The results of this study provide scientific validation for the traditional use of this plant in the treatment of liver disorders. However, further studies are required to isolate, characterize, and evaluate the specific bioactive compounds responsible for these effects and to elucidate their precise mechanisms of action.

6. Conflict of Interest

None.

7. Source of Funding

None.

8. Ethical Committee Approval

CPCSEA/CBPL/AH/80.

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Cite this article. Manke MB, Maharaj GH, Jadhav SJ, Giram PS, Bhusnure OG. Hepatoprotective activity of Saraca indica leaves extract against ccl4-induced liver damage in rats. *Curr Trends Pharm Pharm Chem.* 2025;7(3):111-118.