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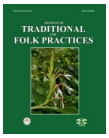

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Hepatoprotective activity of *Indianthus virgatus* (Roxb.) Suksathan & Borchs against carbon tetrachloride-induced liver damage in Wistar rats

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Abstract

Indianthus virgatus (Roxb.) Suksathan & Borchs, a medicinal herb belonging to the family Marantaceae, is traditionally used by tribal healers in Kerala to treat liver disorders. The present study evaluated the hepatoprotective effects of *I. virgatus* rhizome extract against carbon tetrachloride (CCl_4)-induced liver damage in Wistar rats. The ethanolic (SVEF) and crude (SV) extracts of *I. virgatus* rhizomes were administered at different doses (50, 100, and 150 mg/kg, p.o.) to Wistar rats to assess their hepatoprotective potential. Hepatic marker enzyme levels (AST, ALT and ALP), bilirubin levels, antioxidant enzyme activity (GSH, and CAT), and lipid peroxidation (MDA) were assessed. Histopathological examination of the liver sections was also performed. The results showed that CCl_4 administration significantly elevated the levels of hepatic marker enzymes and MDA while reducing the levels of antioxidant enzymes. Pre-treatment with SVEF (50 mg/kg) and SV (100 mg/kg) significantly ($P \leq 0.01$) restored the biochemical parameters to near-normal levels, demonstrating its hepatoprotective potential. Histopathological analysis further confirmed reduced hepatic damage in the pre-treated groups. The protective effects of *I. virgatus* may be attributed to its antioxidant and free-radical scavenging properties. These findings suggest that *I. virgatus* has potential as a natural hepatoprotective agent and warrants further pharmacological investigation.

Keywords: Antioxidant activity, Carbon tetrachloride, Hepatoprotective, Oxidative stress

1. Introduction

Indianthus virgatus (Roxb.) Suksathan & Borchs, a medicinal herb belonging to the family Marantaceae, is locally known as *Malamkoova*. It was formerly known as *Schumannianthus virgatus* (Roxb.) Rolfe. It is an erect herb that grows up to 4 m in height and has tuberous rootstocks. This plant is widely distributed in South India and Sri Lanka, with abundance in the Western Ghats of Kerala. Various pharmacological studies have reported its diuretic, antibacterial, antifungal, and antiviral properties (Goel *et al.,* 2002).

Traditional knowledge suggests that *I. virgatus* has hepatoprotective potential. The tribal communities of Kerala, particularly the *Muthuva* tribe of the Adimali region, use a paste of *I. virgatus*) to treat skin diseases (Suresh *et al.,* 2020). Additionally, the Kani tribe uses *I. virgatus* to treat liver disorders, highlighting its ethnomedicinal significance. However, this traditional knowledge is at risk of being lost due to poor documentation, necessitating the scientific validation of the medicinal properties of these plants.

The liver plays a crucial role in metabolism, detoxification, and homeostasis, however it is highly susceptible to damage owing to continuous exposure to toxins, drugs, and oxidative stress. Liver disorders, including hepatitis, cirrhosis, and liver failure, are a significant global health concern (Asrani *et al.,* 2019).

Despite advancements in synthetic drugs for liver diseases, many of these treatments have severe adverse effects. As a result, there is growing interest in exploring natural hepatoprotective agents, particularly from medicinal plants with antioxidant properties

"One widely accepted experimental model for studying liver damage is carbon tetrachloride (CCl_4)-induced hepatotoxicity. This potent hepatotoxin generates highly reactive free radicals, such as CCl_3O_2, which leads to lipid peroxidation and hepatocellular injury. The resulting oxidative stress mimics human liver diseases, making it a suitable model for evaluating hepatoprotective agents (Weber *et al.,* 2003). Earlier published reports suggest that plant-derived antioxidants can counteract liver damage caused by such toxins (Wu, 2014), making it important to assess the hepatoprotective potential of *I. virgatus*.

Considering these factors, the present study aimed to evaluate the hepatoprotective effects of *I. virgatus* rhizome extract against CCl_4-induced liver damage in Wistar rats. This study provides scientific validation of the traditional use and contributes to the growing body of knowledge on natural hepatoprotective agents.

2. Materials and methods

2.1. Chemicals and instruments

Analytical-grade solvents were procured from Merck India Pvt. Ltd. (Mumbai, India). All chemicals, including paracetamol, were purchased from Sigma Aldrich, USA. Biochemical kits were obtained from the Coral Clinical System (Goa, India). A rotary evaporator (Buchi R-215, Switzerland) and a spectrophotometer (Agilent 100 UV-Vis, Germany) were used in the study.

2.2. Plant material

Fresh rhizomes of *I. virgatus* were collected from Kulathupuzha, Kollam, Kerala, India. The plant was identified by a plant taxonomist at the institute, and a voucher specimen (TBGT 86803) was deposited in the herbarium.

2.3. Preparation of plant extract

Rhizomes were thoroughly washed with running tap water followed by distilled water to remove any adhering dust. The rhizomes were then shade-dried and powdered. A total of 100 g of powdered material was extracted with 1000 mL of ethanol using a Soxhlet apparatus. The solvent was removed under reduced pressure at a low temperature using a rotary evaporator, yielding the crude extract (SV). The plant extract (SV) was suspended in 0.5% Tween-80 at the required concentration for oral administration.

The rhizome powder of *I. virgatus* was first extracted with hexane using a Soxhlet apparatus. After drying, the remaining powder was sequentially extracted with chloroform and ethanol, yielding the hexane fraction (SVHF), chloroform fraction (SVCF), and ethanolic fraction (SVEF). Based on previous studies (Neethu *et al.,* 2016) the ethanolic fraction exhibited potent antioxidant activity and was therefore selected for hepatoprotective evaluation.

2.4. Animals

Wistar male albino rats (150–200 g) and Swiss albino male mice (20-25 gm.) were obtained from the animal house of the institute. The animals were housed under standard conditions and provided commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water *ad libitum*. The animals were acclimatised for one week before the experiment. All animal procedures followed the guidelines of the National Institute of Health guidelines and were approved by the Institute's Animal Ethics Committee (No: B-03/01/2013/12).

2.5. Carbon tetrachloride (CCl_4) - induced hepatotoxicity

Wistar rats were divided into nine groups (n=6 per group):

- Group I (Normal Control): Received 0.5% Tween-80 (1 mL p.o.) for 5 days and olive oil (2 mL/kg, s.c.) on days 2 and 3.
- Group II (CCl_4 Control): Received 0.5% Tween-80 (1 mL p.o.) for 5 days and CCl_4 : olive oil (1:1, 2 mL/kg, s.c.) on days 2 and 3.
- Groups III, IV, and V: SV (50, 100, and 150 mg/kg, b. w., p. o.) was administered for 5 days, with CCl_4: olive oil (2 mL/kg, s.c.) on days 2 and 3, 30 min after SV administration.
- Groups VI, VII, and VIII: Administered SVEF (50, 100, and 150 mg/kg, b.w., p.o.) for 5 days, with CCl_4: olive oil (2 mL/kg, s.c.) on days 2 and 3, 30 min after SVEF administration.
- Group IX (Standard Drug): Received silymarin (100 mg/ kg, p.o.) for 5 days, with CCl_4: olive oil (2 mL/kg, s.c.) on days 2 and 3, 30 min after silymarin administration.

On the 6th day, after 24 h of fasting, all animals were sacrificed using a carbon dioxide chamber. Blood samples were collected from the carotid artery for biochemical analysis, and liver tissues were collected for histopathological studies and antioxidant assays, including malondialdehyde (MDA) estimation, catalase (CAT) assay, and reduced glutathione (GSH) determination.

2.6. Experimental analysis

2.6.1. Estimation of Malondialdehyde (MDA): MDA levels in the liver were estimated using a modified version of the Ohkawa method (1979). Liver homogenate (10% w/v, 1 mL) was mixed with 100 µL of 8.1% SDS and 600 µL of 20% acetic acid solution and incubated, at room temperature for 2 min. Next, 600 µL of 0.8% TBA was added and heated at 95 º C for 60 min in a water bath. After cooling, a mixture of *n*-butanol and pyridine (15:1 v/v) was added, shaken vigorously, and centrifuged at 10,000 rpm for 5 min. The absorbance of the organic layer was measured at 532 nm. Lipid peroxidation was expressed as nmol/g of wet liver tissue.

2.6.2. Assay of Catalase (CAT): Catalase activity was measured using the Aebi method (1974). To 0.9 mL of phosphate buffer (0.01M, pH 7.0), 0.1 mL of liver homogenate (10% w/v) and 0.4 mL of H_2O_2 (0.2 M) were added. After 60 seconds, 2 mL of dichromate-acetic acid reagent (5%) was added. The tubes were heated in a boiling water bath for 10 min, and the developed colour was read at 620 nm. Catalase activity was expressed as U/ mg of protein.

2.6.3. Determination of Reduced Glutathione (GSH): Liver homogenates (10% w/v, 0.2 mL) were mixed with 1.8 mL of 1 mM EDTA solution, followed by the addition of 3.0 mL of precipitating reagent (containing meta-phosphoric acid, EDTA disodium salt, and sodium chloride). The mixture was incubated for 5 min and centrifuged. The supernatant (2 mL) was mixed with 4.0 mL of 0.3 M disodium hydrogen phosphate solution and 1.0 mL of DTNB reagent. The absorbance was measured at 412 nm. GSH content was determined using a standard curve.

2.6.4. Statistical Analysis: All data are expressed as the mean ± SD. Statistical significance among groups was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Graph Pad Prism version 5.00 (Graph Pad Software, San Diego, CA, USA) was used for the analysis.

3. Results and discussion

3.1. Biochemical analysis

In the present study, the hepatoprotective activity of crude and ethanolic extracts of *I. virgatus* (SV and SVEF) was assessed by analysing biochemical parameters indicative of hepatic damage. In CCl_4-administered experimental rats, the levels of liver marker enzymes - Aspartate Amino Transferase (AST), Alanine transaminase (ALT), Alkaline Phosphatase (ALP), and Gamma Glutamyl Transferase (GGT)-were significantly elevated ($P \leq 0.01$), indicating hepatocellular injury. The mean AST, ALT, ALP, and GGT levels in CCl_4-treated rats were 145.3 ± 8.5 U/L, 122.6 ± 7.4 U/L, 285.2 ± 10.2 U/L, and 9.3 ± 0.8 U/L, respectively, compared to the control group (78.2 ± 5.1 U/L, 65.7 ± 4.6 U/L, 198.5 ± 7.8 U/L, and 4.5 ± 0.5 U/L, respectively). Pre-treatment with SV/SVEF (50, 100, and 150 mg/kg b.w., p. o.) significantly ($P \leq 0.01$) hepatoprotection by reducing these elevated levels. Among all the doses studied, SV at 100 mg/kg b.w. and SVEF at 50 mg/kg b.w. showed the most pronounced hepatoprotective effect, with AST, ALT, ALP, and GGT levels restored to near-normal values of 88.5 ± 6.2, 72.3 ± 5.2, 210.8 ± 8.4, and 5.2 ± 0.6 U/L, respectively.

Furthermore, serum bilirubin (SB) levels were significantly elevated in CCl4-treated rats (2.1 ± 0.2 mg/ dL) compared to those in the control group (0.6 ± 0.05 mg/dL). Pre-treatment with SV/SVEF significantly reduced bilirubin levels to 0.85 ± 0.08 mg/dL, further confirming hepatoprotective activity. The results are summarised in Table 1.

3.2. Antioxidant defense system

The activities of GSH and CAT were markedly decreased in CCl4-treated rats, indicating oxidative stress-induced hepatotoxicity. GSH levels were reduced from 6.4 ± 0.5 μM/mg protein in controls to 2.1 ± 0.3 μM/mg protein in the CCl4 group. Similarly, CAT activity was lowered from 58.3 ± 4.7 U/mg protein in the control group to 22.5 ± 2.3 U/mg protein. Pre-treatment with SV (100 mg/kg b.w.) and SVEF (50 mg/ kg b.w.) significantly restored GSH and CAT levels to 5.8 ± 0.4 μM/mg protein and 50.6 ± 3.8 U/mg protein, respectively (Fig. 1).

MDA levels, a marker of lipid peroxidation, were significantly increased in the CCl4-treated group (4.8 ± 0.4 nmol/mg protein) compared to the control group (1.7 ± 0.2 nmol/mg protein). Pre-treatment with SV/SVEF significantly ($P \leq 0.01$) reduced MDA levels to 2.2 ± 0.3 nmol/mg protein, indicating a reduction in lipid peroxidation.

3.3. Histopathological analysis

Liver sections from normal control rats exhibited a well-preserved hepatic architecture with intact hepatocytes and no fatty changes. In contrast, CCl_4-treated rats showed severe degenerative changes around the central veins, including fatty infiltration, lobular ballooning degeneration, necrotic cells, mononuclear infiltration, and loss of cellular boundaries. The liver architecture was significantly distorted, with vacuolisation and sinusoidal expansion.

However, pre-treatment with *I. virgatus* (SV/SVEF) markedly improved hepatic histology, as evidenced by reduced fatty infiltration, minimal necrosis, and normal hepatic cords. The histopathological findings strongly support the results of the biochemical analysis (Fig. 2).

Exposure to environmental toxins, including CCl_4, leads to liver injury due to oxidative stress and lipid peroxidation. CCl_4 metabolism produces reactive oxygen species (ROS), which induce membrane leakage of cytoplasmic enzymes, resulting in elevated ALT, AST, ALP, and bilirubin levels (Singh *et al.,* 2023). The observed increase in these enzymes in CCl_4-treated rats in this study is consistent with that of a previous report (Recknagel *et al.,* 1989).

The hepatoprotective effect of *I. virgatus* extract is likely due to its antioxidative properties, which counteract oxidative stress-induced liver injury. The significant reduction in ALT, AST, and ALP levels in pretreated groups suggests hepatocyte membrane stabilisation and repair of the hepatic tissue. Similar hepatoprotective mechanisms have been observed in other medicinal plants, such as *Silybum marianum* (L.) Gaertn. (milk thistle) and *Andrographis paniculata* (Burm.f.) Wall. ex Nees (Ghosh *et al.,* 2011; Singh *et al.,* 2023).

S. marianum has been documented to protect against liver disorders through antioxidant and anti-inflammatory

Table 1. Effect of *Indianthus virgatus* (Roxb.) Suksathan & Borchs, ethanolic extract (SV) and ethanolic fraction (SVEF) on serum markers of hepatic injury after CCl_4 administration

Sl. No.	Treatment groups	Parameters							
		AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (U/L)	SB (mg/dl)	Total Cholesterol (TC) (mg/dl)	Triglycerides (TGL) (mg/dl)	Total Protein (TP) (g/dl)
1	Normal control	45.50± 3.22	67.51± 2.66	62.05± 4.45	13.55± 2.69	0.34± 0.06	52.56± 3.80	56.14± 6.30	5.93± 0.12
2	CCl_4 (1 mL/kg)	149.98± 4.44 ***	176.34± 6.29 ***	137.73± 11.28 ***	31.93± 5.55 ***	3.94± 0.39***	101.75± 5.80***	116.43± 7.60 ***	3.67± 0.13
3	CCl_4+SV (50 mg/kg)	80.25± 6.80 ***	100.68± 8.58 ***	68.29± 6.39 ns	28.17± 3.23 ***	0.86± 0.05 **	64.56± 5.24 *	75.80± 10.0 0*	4.34± 0.15 **
4	CCl_4+SV (100 mg/kg)	52.32± 3.20 ns	72.10± 7.15 ns	65.76± 6.04ns	16.17± 3.20 ns	0.38± 0.06 ns	60.94± 4.43 ns	63.61± 4.99 ns	4.74± 0.12 **
5	CCl_4+SV 150 mg/kg)	49.40± 3.80 ns	74.15± 5.26ns	72.93± 10.23 ns	24.27± 3.49**	0.50± 0.05 ns	67.56± 5.61 **	77.05± 5.60 *	5.16± 0.17 **
6	CCl_4+SVEF (50 mg/kg)	54.58± 2.40 ns	68.90± 4.37 ns	67.50± 7.19 ns	13.93± 2.60 ns	0.37± 0.05ns	55.47± 4.60 ns	61.62± 6.80 ns	4.76± 0.13 **
7	CCl_4+SVEF (100 mg/kg)	68.33± 5.83 ***	90.77± 5.24 **	82.17± 7.53 ns	24.70± 4.50 **	0.56± 0.06 ns	69.42± 1.84 **	70.06± 4.47 ns	5.18± 0.13 **
8	CCl_4+SVEF (150 mg/kg)	72.53± 3.05 ***	100.21± 7.08 ***	90.73± 3.58 ns	23.73± 2.78*	0.56± 0.11 ns	68.96± 2.87**	82.25± 5.12 **	5.47± 0.14 **
9	CCl_4+Silymarin (100 mg/kg)	52.78± 4.22 ns	59.76± 6.57 ns	60.02± 4.88 ns	16.52± 3.94 ns	0.27± 0.02 ns	60.68± 5.05 ns	60.74± 9.69 ns	5.66± 0.09 **

*Values are expressed as mean ± SD of six values, one-way ANOVA followed by Dunnet's multiple comparison test, * P≤ 0.05, * * P≤ 0.01, *** P≤ 0.001, ns= not significant compared to normal control*

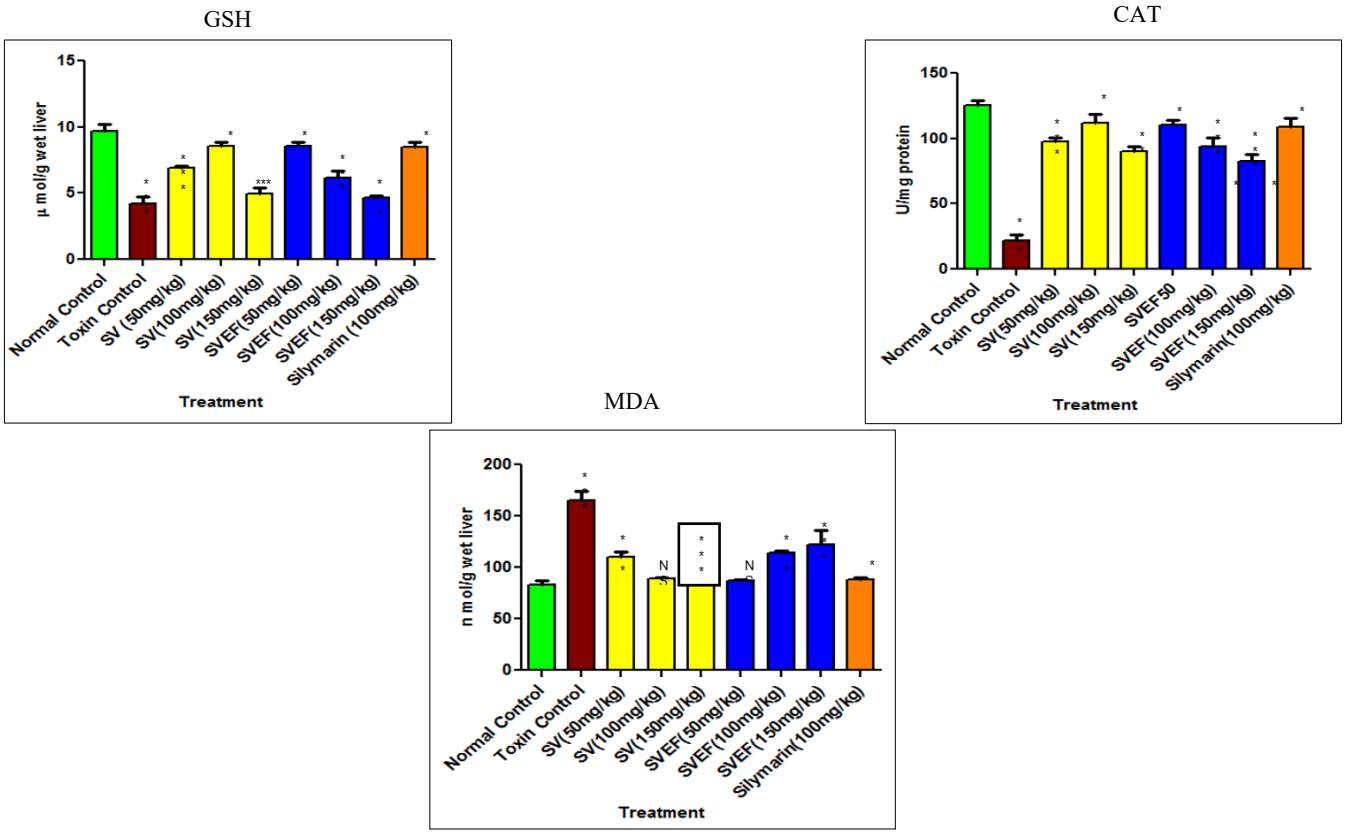

Fig. 1. Effect of *Indianthus virgatus* (Roxb.) Suksathan & Borchs ethanolic extract (SV)/ethanolic fraction (SVEF) on hepatic GSH, CAT, MDA after CCl_4 administration *(values are expressed as mean ± SD of six values, one-way ANOVA followed by Dunnet's multiple comparison test, * P≤ 0.05, **P≤ 0.01, *** P≤ 0.001, ns= not significant compared to normal control)*

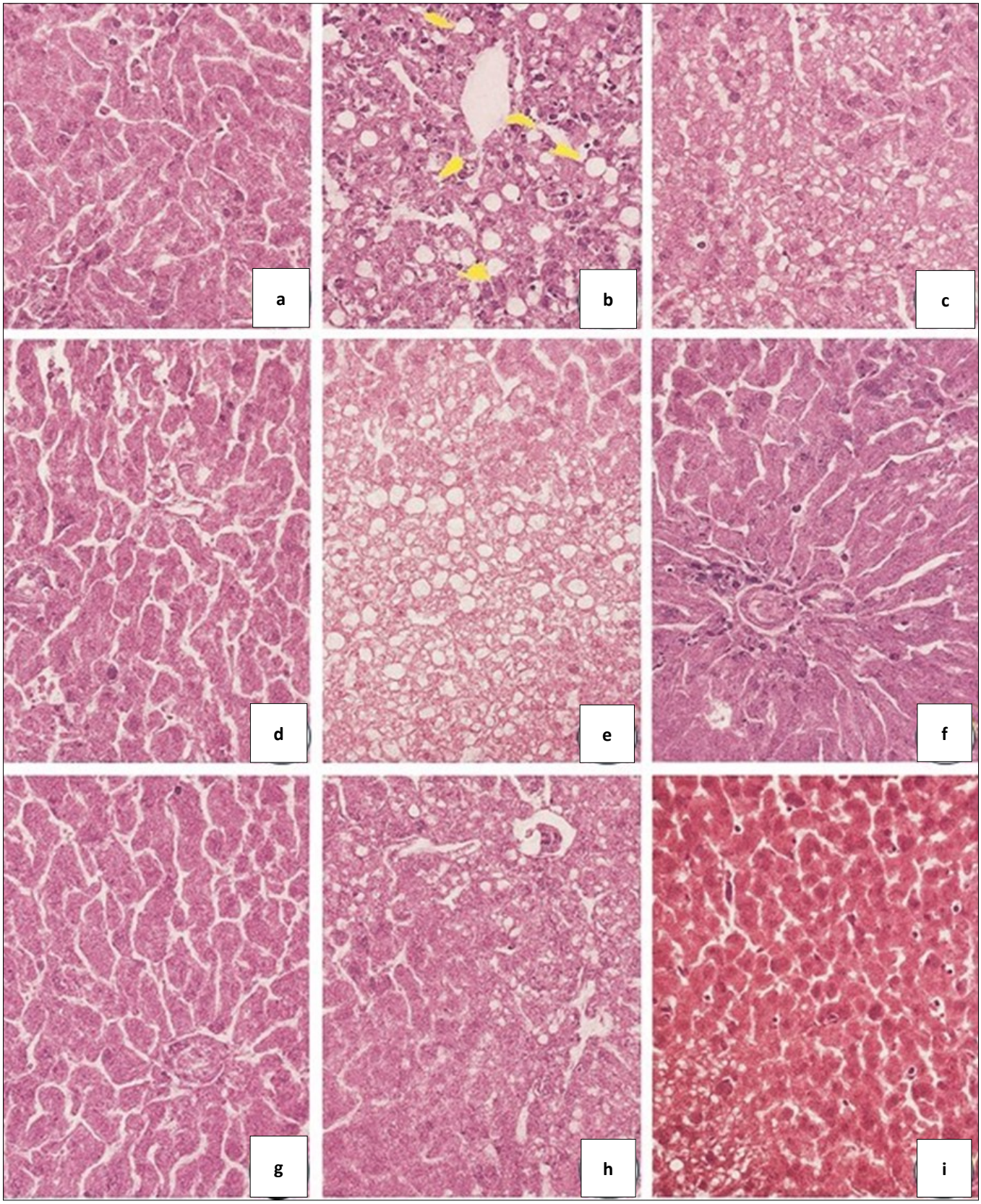

Fig. 2. Effect of SV and SVEF on liver histopathological damage induced by CCl_4 in Wistar rats: a. Normal control: rat liver histology showing normal hepatic architecture; b. Toxin control rat liver showing macro and microvesicular steatosis and ballooning degeneration; c. SV 50 mg/kg; d. SV 100 mg/kg; e. SV 150 mg/kg; f. SVEF 50 mg/kg; g. SVEF 100 mg/kg; h. SVEF 150 mg/kg (c to h, all showing improved liver architecture); i. Liver histology of silymarin-treated animals showing normal hepatic architecture

activities, regulation of cell permeability, membrane stabilization, stimulation of liver regeneration, and inhibition of collagen fiber deposition, which may lead to cirrhosis. *A. paniculata* has also demonstrated hepatoprotective activity, which is attributed to its antioxidative constituents (Ghosh *et al.,* 2011).

Antioxidants play a crucial role in scavenging ROS and mitigating hepatic injury. A significant decrease in GSH and CAT levels in CCl_4-treated rats suggests oxidative stress-mediated depletion of these enzymes. However, pre -treatment with *I. virgatus* restored antioxidant enzyme levels, indicating its protective role in the management of oxidative stress. These findings align with previous studies demonstrating the hepatoprotective effects of flavonoid-rich plant extracts (Halliwell and Gutteridge, 1999; Sreelatha and Padma, 2009).

Lipid peroxidation is another hallmark of CCl_4-induced liver damage. The increased MDA levels in toxin-treated rats highlight excessive peroxidative damage. The significant reduction in MDA levels following *I. virgatus* pre-treatment indicates its potent antilipid peroxidative effect, which may be attributed to its polyphenolic and flavonoid contents (Tiwari, 2001).

Histopathological analysis further confirmed these biochemical findings. Liver sections of CCl_4-treated rats exhibited severe hepatocellular damage, whereas pre-treatment with *I. virgatus* minimised histological alterations and preserved the hepatic architecture. The observed hepatoprotective effect suggests that *I. virgatus* functions similarly to established hepatoprotective agents such as silymarin (Muriel and Rivera-Espinoza, 2008).

4. Conclusion

The study demonstrated that *I. virgatus* rhizome extract possesses significant hepatoprotective activity against CCl_4-induced liver damage. The hepatoprotective effect is attributed to its antioxidative and free radical scavenging properties. The ability of *I. virgatus* to restore liver enzyme levels, enhance antioxidant defences, and reduce lipid peroxidation highlights its potential as a therapeutic agent for treating liver disorders.

Histopathological analysis confirmed that *I. virgatus* mitigated hepatic damage and preserved liver architecture. The presence of bioactive compounds in *I. virgatus* may contribute to its protective effects, suggesting the need for further phytochemical investigations to isolate and identify its active constituents.

Future research should focus on elucidating the precise molecular mechanisms of *I. virgatus* in liver protection, conducting clinical trials to validate its efficacy and safety in humans, and exploring its potential applications in pharmaceutical formulations for liver disorders. The promising results of this study pave the way for the development of *I. Virgatus*-based hepatoprotective therapeutics, which could serve as an alternative to conventional liver-protective drugs.

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