HEPATOPROTECTIVE ACTIVITY OF POLYHERBAL EXTRACT AGAINST CCL4 INDUCED LIVER DAMAGE IN RATS

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KEYWORDS

E. crassipes, CCl_4, hepatoprotective, hepatotoxicity, histopathology

ABSTRACT

The current work evaluated the hepatoprotective effect of ethanolic extracts of *Eichhornia crassipes*, *Ipomoea aquatica*, and *Hydrilla verticillate* using silymarin against the CCl_4-induced in-vivo rat model at varying doses. Histological investigations were used to confirm the hepatoprotective action of EC, IP & HV, which was assessed by assessing serum biomarker levels. Rat's livers were effectively shielded from the damage caused by CCl_4 by the plant extract. Serum levels of bilirubin, total protein, alkaline phosphatase, serum glutamic-pyruvic transaminase and serum glutamic oxaloacetic transaminase were significantly decreased. In contrast to silymarin, the histological analysis showed a notable repair of liver structure. The traditional usage of polyherbs to comprehend its hepatoprotective potential has been scientifically validated by this investigation.
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INTRODUCTION

Metabolic and histological abnormalities i.e., vascular lesions, granulomas, cholestasis, hepatitis, and zonal necrosis are characteristics of hepatotoxicity, or liver damage, which accounts for 5% of all injuries and is hence common. As a result, over a million deaths are recorded annually, with hepatocellular cancer or liver deformity being the indirect cause [1]. We are protected against a variety of internal and external illnesses by conventional or synthetic medications. Unfortunately, the medications used to treat liver problems are inadequate since they might have major long-term negative effects and do not completely protect the organ [2–5]. Understanding the function of complementary and alternative medicine as a treatment option for liver disease is thus essential [6,7]. Because of its abundance of polyphenols, CAM is known to have good therapeutic effectiveness with few negative side effects [8, 9]. Therefore, foods high in polyphenols may reduce the risk of chronic illnesses [5, 10-14].

A monocotyledonous aquatic plant that floats freely, Eichhornia crassipes is a member of the Pontederiaceae family [15][16]. It has been used in wastewater treatment due to its ability to grow in polluted water and absorb heavy metals [17]. Both biofuels and biofertilizers are made from it. The plant has historically been used to treat intestinal worms, diarrhoea, vomiting, gas, and digestive problems [18]. Among the main metabolites that have been found from the different plant components are cellulose, hemicellulose, glycolipids, triacylglycerols, and heteropolysaccharides [19] [20]. Ponds, marshes, and other aquatic environments are home to its growth [21]. The phytochemical composition of several unusual green vegetables greatly inhibits glycoside hydrolases, including α-amylase and α-glucosidase [22][23].

Ipomoea aquatica or water spinach belongs to the Convolvulaceae family. It is also known as water spinach, river spinach, water morning glory, water convolvulus. It flourishes naturally in water which requires little care which commonly found in the banks of streams, ponds, ditches, lakes and marshes that usually floats or creeps along the shorelines but it can also climb over emergent vegetation, some also thrives in the water. It is used traditionally for the treatment of jaundice and liver diseases. The antioxidant property of water spinach is said to be due by the phytochemical, Flavonoid. [24].

Hydrilla verticillate is an aquatic plant with rapid growth and widespread dispersal. Particularly as water weeds, it may create problems in the water [25][26]. However, hydrilla plants have is a source of biomass for composting due to their vegetative reproductive systems (rhizomes and tubers), which may act as a place for reserves of carbohydrates [27]. Hydrilla verticillata is an aquatic plant that may act as a carbon source when it is fresh. For growth and development, plants need large quantities of the macroelements potassium, phosphorus, and nitrogen [28]. Denitrification is the process by which nitrogen gas is reduced during Hydrilla verticillata's breakdown. Waste will break down more quickly with bacterially aided phytoremediation [29]. In addition to the high expense of inorganic fertilizers and soil degradation caused by excessive use of chemical fertilizers, hydrilla plants may help address problems with natural resources that have not been fully used.

Hydrilla verticillata has a number of active compounds with antioxidant qualities. *Hydrilla verticillate* contains steroid [30]. The purpose of the research was to examine the hepatoprotective potential of ethanolic extract of polyherbs (EC IP & HV) on rats' liver damage caused by carbon tetrachloride.

MATERIAL AND METHODS

Plant collection and authentication

The plant species Eichhornia crassipes, Ipomoea aquatica, and Hydrilla verticillate were procured from Patna, Bihar, and authenticated by professionals in the Department of ICAR- National Bureau of plant genetic resources, Pusa campus, Delhi. On the basis of visual observations and detail of the specimen it was identified by Scientist/Botanist R.K. Pamarthi and authenticated, and authentication certificated had been issued (ref. no. AC-137/2023, AC-136/2023, and AC-138/2023 respectively).

Extraction of the plant

These were washed thoroughly and dried in shade. The dried plant materials were crushed into coarse powder and then extracted through Soxhlet using ethanol. After extraction, the obtained slurry was filtered with filter paper and dried using rotatory evaporator [31].

Animals and care

In this investigation, adult Wistar rats weighing 200-250g (either sex) were used. The Central animal facility at the All India Institute of Medical Sciences, New Delhi is where the animals were bought. With eight animals per cage, the animals were kept in normal polypropylene cages with bedding made of rice husk and standard climatic conditions (26±3°C, 65±1% RH, and 12-hour dark-light cycle. They were given regular pellet feed and distilled water as they acclimated for one to two weeks. The laws protecting animals used for scientific research were followed while conducting the animal studies.

Ethical statement

All experiments were conducted in rigorous compliance with established protocols. Ethical approval was secured from Innovative College of Pharmacy, Plot no. 6, Knowledge Park-2, Greater Noida U.P. India IAEC (1346/PO/Re/s/10CPCSEA) [32-34].

Acute toxicity study

The doses of extract utilized in this study were determined based on our prior findings from the acute toxicity assessment of the respective plants. The acute toxicity assessment was conducted in accordance with OECD guidelines 423. Administration of EV IP &HV at 2000mg/kg did not result in death of any animals. Therefore 250, 500 & 750mg/kg, 100, 200 & 400mg/kg & 50,75 & 100mg/kg respectively were chosen dose.

Experimental design and drug administration

Rodents were kept separately in 6 groups 4^{th}, 5^{th} & 6^{th} group divided in 3 subgroups (12) (n= 8) as follows.

Group I (control) given normal diet.

Group II (disease control) given CCl_4 1ml/kg. i.p. (mixed in olive oil 1:1) 2 times in 3 weeks) [11].

Group III (CCl_4 & Sily group) given CCl_4 1ml/kg, i.p. (mixed olive oil 1:1) + silymarin (50mg/kg, orally) for 21 days.

Group IV-VI given CCl_4 1ml/kg, i.p. (olive oil 1:1) and at different doses of ethanolic extracts of all 3 plants orally (250, 500 & 750mg/kg, 100, 200 & 400mg/kg & 50,75 & 100mg/kg) for 3 weeks [35].

Preparation of liver tissue samples

All rats were fasted for 12 hours, were weighed and euthanized under proper anaesthesia using a combination of 0.15 ml/100 g xylazine (30 mg/ kg BW) and 0.3 ml/100 g i.p ketamine (300 mg/kg BW) as proposed by Committee for the Purpose of Control and Supervision of Experiments on Animals (CCSEA). The blood was collected by retro-orbital puncture in an EDTA containing tube from all the experimental rats. The livers of each rat were removed, weighed and perfused in the ice-cold phosphate buffer of pH 7.0. A portion of the liver was preserved in 10% formaldehyde solution for histopathological evaluation.

Biochemical assays in blood

Alanine amino transaminase, aspartate amino transaminase, and alkaline phosphatase were quantified using an automated analyzer, alongside various liver markers such as total protein and albumin, adhering to established standard procedures [36].

Histopathological studies

A little section of the liver was removed, cleaned with regular saline, and prepared independently for histopathological analysis. The liver tissues were first preserved for at least 48 hours in 10% buffered

neutral formalin, then gradually dried in ethanol (50–100%), cleaned in xylene, and finally embedded in paraffin. A microtome was used to create the 4 μm slices. The liver slices were then rinsed with distilled water for five minutes after being dewaxed in xylene and rehydrated in a succession of alcohol grades. The liver slices were counterstained with acidic stain eosin for 20 s (H-E) dye after being stained for 40 s with basic stain hematoxylin [37]. Using an Olympus microscope, the sections were viewed at 400X magnification to check for any histopathological alterations, such as vacuolation, fatty changes, or necrosis of the cells.

Statistical analysis

The data are presented as mean ± SE. A one-way ANOVA was conducted to compare different groups, followed by Tukey's multiple comparison tests using GraphPad Prism. A p-value of <0.05, $p<0.01$, and $p<0.001$ was taken as statistically significant.

RESULTS AND DISCUSSION

% Yield

Percentage yield of EV IP & HV extract isolated was found to be 18.35% 21.6 & 20.01% w/w.

Effect of ethanolic extract of EC HV & IP on biochemical parameters

Effect of ethanolic extract of EC HV & IP on the indices of hepatotoxicity based on liver marker enzymes

Administration of CCl4 markedly increased the levels of serum hepatic enzymes such as ALT, AST and ALP in the disease-induced group as compared to the control group (Table 1). The increase in these enzyme activities was significantly decreased with the treatment with extract of EV IP & HV. The high concentration group of EV IP & HV showing better results than the low concentration. These findings suggest a dose-dependent response for the EC and IP extracts, while the HV extracts exhibit less consistent effectiveness in reducing ALT, AST and ALP levels.

Table 1. Effect of plant extract on ALP ALT &AST level in CCl4 induced hepatoxic animals

Treatment	ALP (U/L) level	AST (U/L) level	ALT (U/L) level
Normal saline	49.31± 3.82	13.65± 1.22	18.1±1.276
CCl_4 (1ml/kg, *i. p.*)	130.2± 6.76	91.08± 2.90	54.05±2.901
Silymarin (50mg/kg) + CCl4	52.77± 3.64	17.38± 1.21	21.73±1.214
EC 250 mg/kg + CCl4	81.67± 6.26	44.81± 2.08	36.77±2.08
EC 500 mg/kg + CCl4	71.86± 4.51	39.75± 1.50	28.09±1.50
EC 750 mg/kg + CCl4	60.06± 5.54	36.13± 1.50	24.16±1.84
IP 100 mg/kg + CCl4	101.4± 6.43	50.53± 2.14	42.49±2.14
IP 200 mg/kg + CCl4	98.84± 7.92	46.91± 2.64	38.87±2.64
IP 400 mg/kg + CCl4	80.03± 5.18	41.35± 1.91	30.81±1.72
HV 50 mg/kg + CCl4	105.4± 6.43	69.51± 2.44	43.85±2.14
HV 75 mg/kg + CCl4	97.45± 5.63	64.35± 2.40	41.19±1.87
HV 100 mg/kg + CCl4	101.6± 6.27	57.4± 2.09	37.99±2.09

EC - *Eichhornia crassipes*, IP - *Ipomoea aquatica*, HV - *Hydrilla Verticillate*

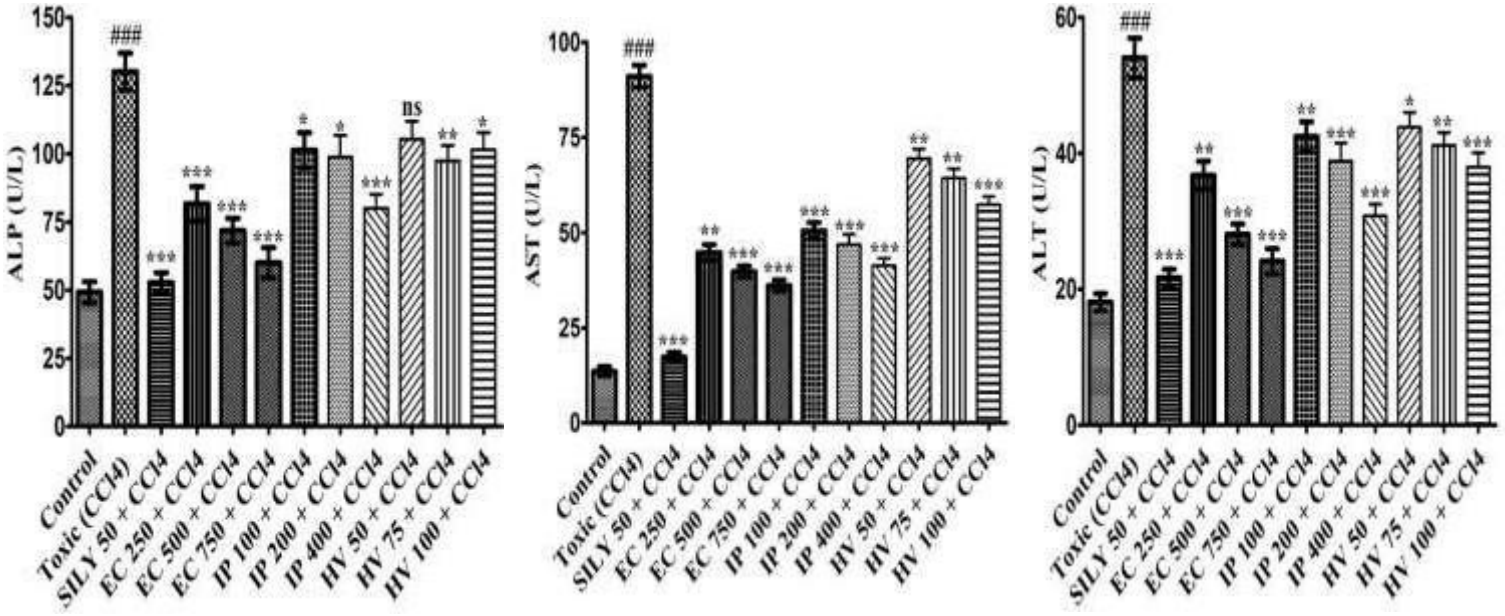

Figure 1 Level of ALP AST ALT level in animals
Data are presented as mean ± SD, with significant differences indicated at *p<0.05, **p<0.01, and ***p<0.001 when compared to the control group

Effect of ethanolic extract of EC HV & IP on total protein and albumin level

As shown in Table 2, CCl4 administration decreased the albumin (to 1.618 g/dL,) and total protein level (to 3.268 g/dL) as compared to the control. The albumin level was increased on treatment with extract of EV IP & HV at a high dose and silymarin (4.001 g/dL) significantly increased the albumin to 3.356 g/dL, 3.622 g/dL, and 3.803 g/dL, respectively, as compared to the control (4.683 g/dL). The total protein level was increased on treatment with extract of EV IP & HV at a high dose and silymarin (6.601 g/dL) significantly increased the albumin to 6.265 g/dL, 5.01 g/dL, and 4.825 g/dL, respectively, as compared to the control (6.783 g/dL). Treatments with Silymarin, as well as various doses of EC and IP extracts, demonstrate potential in increasing albumin & total protein levels and restoring liver function. The findings suggest a dose-dependent response for the EC and IP extracts, while the HV extracts exhibit less consistent effectiveness in increasing albumin & total protein levels.

Table 2. Effect of plant extract on albumin & Total Protein level in CCl4 induced hepatoxic animals

Treatment	Total protein (mg//dL) level	Albumin (g/dl)
Normal saline	6.78±0.42	4.68± 0.25
CCl_4 (1ml/kg, *i. p.*)	3.26±0.31	1.61± 0.15
Silymarin (50mg/kg) + CCl4	6.60±0.28	4.00± 0.30
EC 250 mg/kg + CCl4	5.76±0.35	3.35± 0.26

EC 500 mg/kg + CCl4	5.97±0.36	3.62± 0.30
EC 750 mg/kg + CCl4	6.26±0.26	3.80± 0.26
IP 100 mg/kg + CCl4	5.45±0.20	3.00± 0.09
IP 200 mg/kg + CCl4	5.30±0.34	3.20± 0.34
IP 400 mg/kg + CCl4	5.01±0.38	3.74± 0.30
HV 50 mg/kg + CCl4	3.93±0.23	2.16± 0.17
HV 75 mg/kg + CCl4	4.42±0.29	2.50± 0.19
HV 100 mg/kg + CCl4	4.82±0.29	2.85± 0.22

EC - *Eichhornia crassipes*, IP - *Ipomoea aquatica*, HV - *Hydrilla Verticillate*

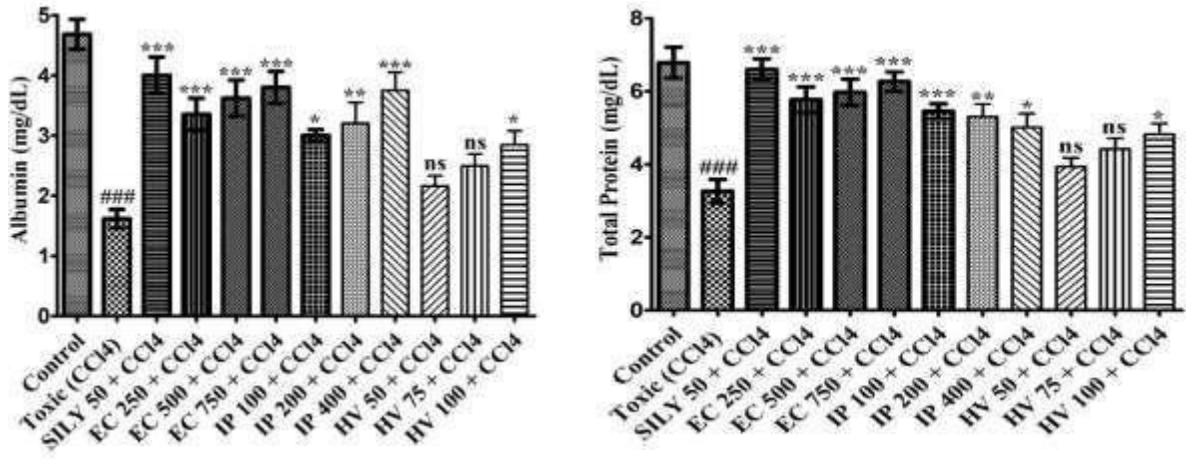

Figure 2 Albumin & Total protein level in animals
Data are presented as mean ± SD, with significant differences indicated at *p<0.05, **p<0.01, and ***p<0.001 when compared to the control group

Effect of ethanolic extract of EC HV & IP on bilirubin level

The administration of CCl4 elevated the levels of serum bilirubin (0.1848 mg/dL) as compared to the control group (Table 3). After the treatment with ethanolic extract of EC HV & IP at higher dose bilirubin was lessened to (0.2837, 0.7589 & 1.243 mg/dL) respectively and 0.2298 mg/dL (with silymarin) as compared to control (0.1848 mg/dL). These findings suggest a dose-dependent response for the EC and IP extracts, while the HV extracts exhibit less consistent effectiveness in reducing bilirubin levels.

Table 3. Effect of plant extract on bilirubin level in CCl4 induced hepatoxic animals

Treatment	Bilirubin (mg//dL) level
Normal saline	0.18±0.04
CCl_4 (1ml/kg, *i. p.*)	1.9±0.07
Silymarin (50mg/kg) + CCl4	0.22±0.02
EC 250 mg/kg + CCl4	0.35±0.04
EC 500 mg/kg + CCl4	0.32±0.03
EC 750 mg/kg + CCl4	0.28±0.07
IP 100 mg/kg + CCl4	0.96±0.07

IP 200 mg/kg + CCl4	0.87±0.04
IP 400 mg/kg + CCl4	0.75±0.10
HV 50 mg/kg + CCl4	1.5±0.11
HV 75 mg/kg + CCl4	1.3±0.15
HV 100 mg/kg + CCl4	1.2±0.13

EC - *Eichhornia crassipes*, IP - *Ipomoea aquatica*, HV - *Hydrilla Verticillate*

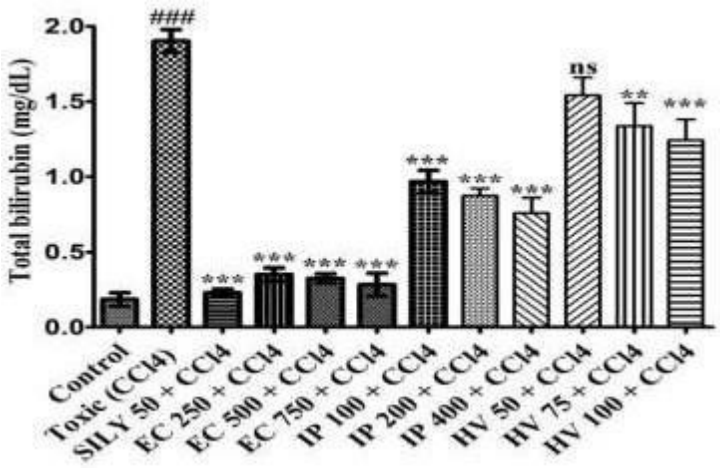

Figure 3. Bilirubin level in animals
Data are presented as mean ± SD, with significant differences indicated at *p<0.05, **p<0.01, and ***p<0.001 when compared to the control group

Table 4. Effect of plant extract on liver weight to body weight ratio of animals

Groups	Body weight	Liver weight	Ratio
Control	227.48±11.61	4.69±0.88	0.020
CCl_4 (1ml/kg, *i. p.*)	177.82±7.64	7.76±0.88	0.004
Silymarin (50mg/kg) + CCl4	219.17±7.37	4.59±0.88	0.021
EC 250 mg/kg+ CCl4	185.66±9.39	5.72±0.88	0.031
EC 500 mg/kg+ CCl4	189.02±7.64	5.58±0.89	0.029
EC 750 mg/kg+ CCl4	194.87±5.87	5.48±0.88	0.028
IP 100 mg/kg+ CCl4	191.59±7.39	5.45±0.89	0.028
IP 200 mg/kg+ CCl4	196.00±6.36	5.16±0.88	0.026
IP 400 mg/kg+ CCl4	200.13±0.28	4.96±0.88	0.024
HV 50 mg/kg+ CCl4	199.08±5.23	4.02±0.88	0.020
HV 75 mg/kg+ CCl4	205.42±7.45	4.14±0.54	0.020
HV 100 mg/kg+ CCl4	210.21±4.48	4.01±0.88	0.019

EC - *Eichhornia crassipes*, IP - *Ipomoea aquatica*, HV - *Hydrilla Verticillate*

As table 4 provides data on the liver weight to body weight ratio for animals in different treatment groups, which is a key metric for assessing the effects of plant extracts on liver health. This ratio helps evaluate liver hypertrophy or damage by comparing liver size relative to body weight. A higher ratio could indicate liver enlargement, which may be a response to toxicity, while a lower ratio could suggest liver shrinkage or other forms of liver dysfunction.

Histopathological evaluation of the rat livers (Table 4; Fig. 4) showed that the hepatocytes of a healthy rat (from the control group) had a normal architecture (Fig. 4a), whereas, in contrast, CCl4 induced severe hepatocyte necrosis, inflammation, and hemorrhage (Fig. 4b). After treatment with EV IP & HV, the severity of CCl4-induced liver intoxication was reduced in a dose-dependent manner (Fig. 4d,e,f and 4g,h,i and 4j,k,l), although the treatment with silymarin showed better result (Fig. 4c).
The table highlights how different plant extracts at varying dosages can influence the liver weight to body weight ratio, indicating that some extracts (like EC) may increase liver size, while others (like HV) might reduce it or have little effect compared to the control. These differences are important for understanding the potential liver-protective or liver-modifying properties of the extracts tested.

Figure 4 Histopathology of plant extract treated CCL4-induced hepatotoxic rats

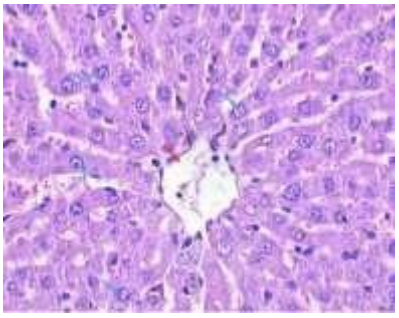

Fig. 4a Control group

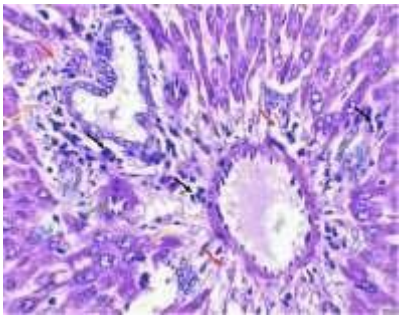

Fig. 4b CCl_4

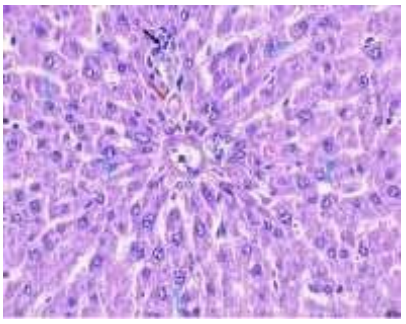

Fig. 4c Silymarin + CCl4

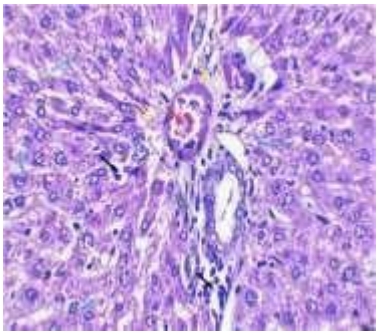

Fig. 4d EC 250 mg/kg+ CCl4

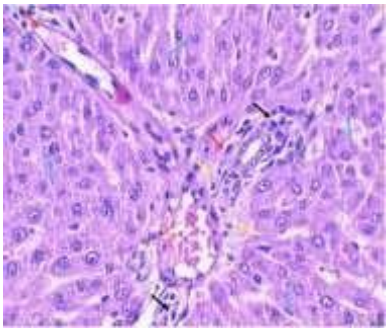

Fig. 4e EC 500 mg/kg+ CCl4

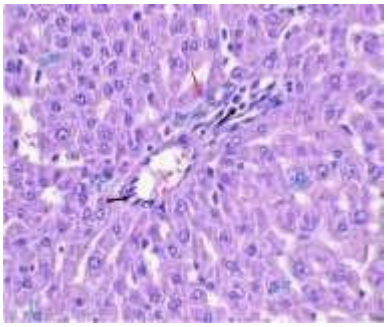

Fig. 4f EC 750 mg/kg+ CCl4

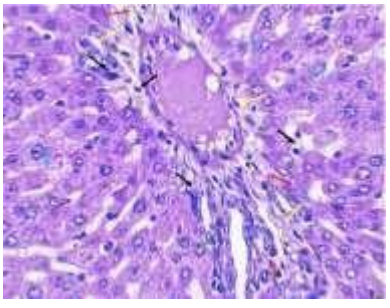

Fig. 4g IP 100mg/kg+ CCl4

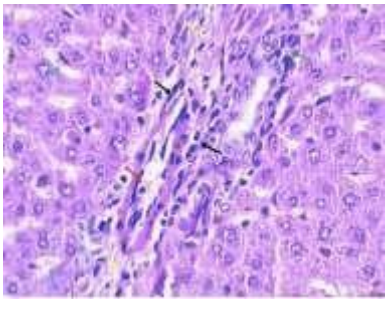

Fig. 4h IP 200mg/kg+ CCl4

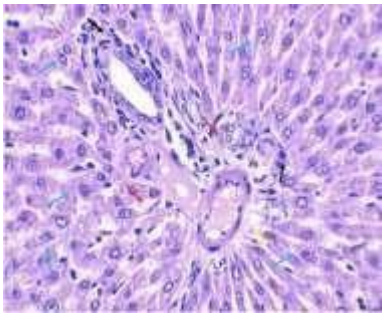

Fig. 4i IP 400mg/kg+ CCl4

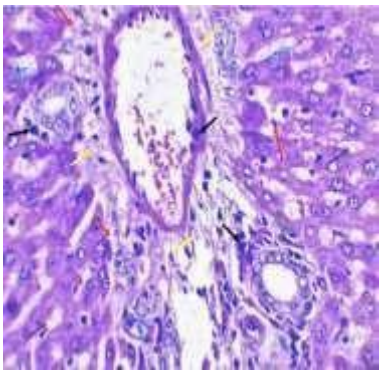

Fig.j HV 50 mg/kg+ CCl4

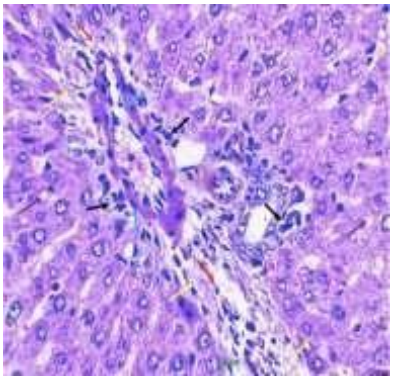

Fig.k HV 75 mg/kg+ CCl4

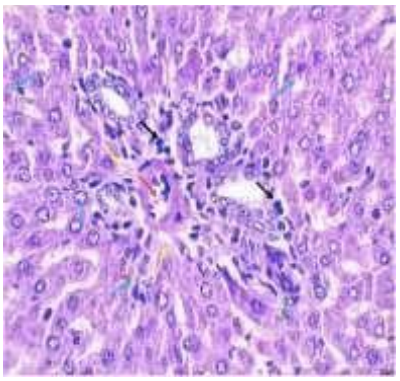

Fig. 4 l HV 100 mg/kg+ CCl4

Toxic group shows significantly damaged hepatocytes (blue arrow). High cellular density, dense cytoplasm, and significant fatty changes (yellow arrow) and vacuolation (purple arrow) are seen. Marked cellular disintegration (red arrow), pyknosis (black arrow), inflammatory infiltrate (green arrow) are seen. The histomorphological characteristics manifest marked hepatic toxicity. (H&E, 400X). Std. group shows minimal histological damage to liver tissue. No congestion in the central vein (CV) is seen. The section shows normal and trabecular-arranged hepatocytes, which are polygonal in shape (blue arrow). No fatty changes, non-dilated sinusoids, well-placed Kuffer's cells, no lipoid vacuoles, and minimal cellular disintegration (red arrow) along with mild pyknosis (black arrow) and no evidence of inflammatory infiltration were seen (H&E, 400X). EC 250 shows moderate histological damage to liver tissue. Moderate congestion in the central vein (CV) is seen (red arrow). The section shows mildly damaged hepatocytes (blue arrow). Moderate fatty changes (yellow arrow), non-dilated sinusoids, well-placed Kuffer's cells, mild lipoid vacuoles (white arrow) and severe cellular disintegration along with mild pyknosis (black arrow) and mild inflammatory cells (green arrow) were seen (H&E, 400X). EC 500 shows minimal histological damage to liver tissue. Mild congestion in the central vein (CV) is seen (orange arrow). The section shows normal and trabecular-arranged hepatocytes, which are polygonal in shape (blue arrow). Minimal fatty changes (yellow arrow), non-dilated sinusoids, well-placed Kuffer's cells, minimal lipoid vacuoles (white arrow), and minimal cellular disintegration (red arrow) along with mild pyknosis (black arrow) and no evidence of inflammatory infiltration were seen (H&E, 400X). EC 750 shows minimal histological damage to liver tissue. No congestion in the central vein (CV) is seen. The section shows normal and trabecular-arranged hepatocytes, which are polygonal in shape (blue arrow). No fatty changes, non-dilated sinusoids, well-placed Kuffer's cells, no lipoid vacuoles, and minimal cellular disintegration (red arrow) along with mild pyknosis (black arrow) and no evidence of inflammatory infiltration were seen (H&E, 400X). IP 100 shows significantly damaged hepatocytes (blue arrow). High cellular density, dense cytoplasm, and significant fatty changes (yellow arrow) and vacuolation (purple arrow) are seen. Marked cellular disintegration (red arrow), pyknosis (black arrow), inflammatory infiltrate (green arrow) are seen. The histomorphological characteristics manifest marked hepatic toxicity. (H&E, 400X). IP 200 shows moderate histological damage to liver tissue. Moderate congestion in the central vein (CV) is seen (red arrow). The section shows mildly damaged hepatocytes (blue arrow). Moderate fatty changes (yellow arrow), non-dilated sinusoids, well-placed Kuffer's cells, mild lipoid vacuoles (white arrow) and severe cellular disintegration along with mild pyknosis (black arrow) and mild inflammatory cells (green arrow) were seen (H&E, 400X). IP 400 shows mild histological damage to liver tissue. No congestion in the central vein (CV) is seen. The section shows normal and trabecular-arranged hepatocytes, which are polygonal in shape (blue arrow). Minimal fatty changes (yellow arrow), non-dilated sinusoids, well-placed Kuffer's cells, no lipoid vacuoles (white arrow), and minimal cellular disintegration (red arrow) along with mild pyknosis (black arrow) and no evidence of inflammatory infiltration were seen (H&E, 400X). HV 50 shows significantly damaged hepatocytes (blue arrow). High cellular density, dense cytoplasm, and significant fatty changes (yellow arrow) and vacuolation (purple arrow) are seen. Marked cellular disintegration (red arrow), pyknosis (black arrow), and inflammatory infiltrate (green arrow) are seen. The histomorphological characteristics manifest marked hepatic toxicity. (H&E, 400X). HV 75 shows significantly damaged hepatocytes (blue arrow). High cellular density, dense cytoplasm, and

significant fatty changes (yellow arrow) and vacuolation (purple arrow) are seen. Marked cellular disintegration (red arrow), pyknosis (black arrow), inflammatory infiltrate (green arrow) are seen. The histomorphological characteristics manifest marked hepatic toxicity. (H&E, 400X). HV 100 shows moderate histological damage to liver tissue. Moderate congestion in the central vein (CV) is seen (red arrow). The section shows mildly damaged hepatocytes (blue arrow). Moderate fatty changes (yellow arrow), non-dilated sinusoids, well-placed Kuffer's cells, mild lipoid vacuoles and severe cellular disintegration along with mild pyknosis (black arrow) and mild inflammatory cells (green arrow) were seen (H&E, 400X).

Conclusion

Liver damage can result in inflammation, fibrosis, and necrosis, ultimately leading to liver failure [38]. Various plants have been utilized for their medicinal properties across numerous regions globally [39–42]. Silymarin, a highly potent phytochemical, is utilized in the treatment of hepatic diseases [34]. EP, classified within the polyphenol category, serves as a powerful antioxidant that combats ROS-induced oxidative stress, making it a valuable agent in the management of liver diseases [43]. CCl4 is frequently utilized in studies of liver toxicity involving experimental animal models, as it induces lipid peroxidation through the generation of free radicals [44]. CCl4 serves as an exemplary animal model known for its association with free radical-induced hepatotoxicity caused by xenobiotics [45]. This analysis demonstrates that CCl4 induced considerable hepatic damage and oxidative stress in animals, as indicated by changes in liver function tests and antioxidant enzyme levels [46, 47]. This study's findings demonstrated that CCl4 exposure led to hepatotoxicity and oxidative stress in rats. The intraperitoneal injection of CCl4 results in an increase in the activity of ALT, AST, and ALP, while simultaneously decreasing the levels of TP and serum albumin, as well as reducing the level of CYP450. The administration of EV IP & HV and SILY effectively mitigates all harmful effects observed in the livers of rats. These factors diminish oxidative stress, inhibit the infiltration of inflammatory cells, enhance the regenerative potential of injured tissues, and decrease liver apoptosis. Therefore, it is our assertion that incorporating natural products like EV IP & HV and SILY may help mitigate the toxic effects associated with exposure to xenobiotics, including CCl4 and other harmful substances.

In conclusion, the ethanolic extract has shown the capacity to preserve the liver's functioning state. The ethanolic extract of E. crassipes has been shown to be one of the herbal treatments for liver disease, according to the above early investigation.

Abbreviations

CCl4: Carbon tetrachloride
SILY: Silymarin
ALT: Alanine amino transaminase
AST: Aspartate amino transaminase
ALP: Alkaline phosphatase
TP: Total protein
EV: Eichhornia crassipes
IP: Ipomoea aquatica
HV: Hydrilla verticillate
CYP450: Cytochrome P450

REFERENCES

1. Ishibashi H, Nakamura M, Komori A, Migita K, Shimoda S (2009), "*Liver architecture cell function and disease*". Seminars Immunopathol 31:399–409
2. Cicek T, Gokturk HS, Unler GK (2015), "*Acute hepatocellular drug induced liver injury probably by alfuzosin"*. Case Rep Urol. Artcile ID: 101062
3. Chaudhury A, Duvoor C, Dendi R, Sena V, Kraleti S, Chada A, Ravilla R, Marco A, Shekhawat NS, Montales MT, Kuriakose K (2017), "*Clinical review of antidiabetic drugs: implications for type 2 diabetes mellitus management"*. Front Endrocrinol 8:6
4. Giacometti J, Muhvić D, Pavletić A, Đudarić L (2016), "*Cocoa polyphenols exhibit antioxidant anti-infammatory anticancerogenic and anti-necrotic activity in carbon tetrachloride-intoxicated mice"*. J Funct Food 23:177–187
5. Kamala A, Middha SK, Karigar CS (2018), "*Plants in traditional medicine with special reference to Cyperus rotundus L"*.: a review. 3 Biotech 8:309
6. Madrigal-Santillán E, Madrigal-Bujaidar E, Álvarez-González I, Sumaya-Martínez MT, Gutiérrez-Salinas J, Bautista M, Morales González ÁY, González-Rubio MGL, Aguilar-Faisal JL, Morales González JA (2014, "*Review of natural products with hepatoprotective effects"*. World J. Gastroentero: WJG 20(40):147
7. Quan M, Li Q, Zhao P, Tian C (2018), "*Chemical composition and hepatoprotective effect of free phenolic extract from barley during malting process"*. Sci Rep 8(1):1–9
8. Dey SK, Middha SK, Usha T, Brahma BK, Goyal AK (2018), "*Antidiabetic activity of giant grass Bambusa tulda".* Bangladesh J Pharmacol 13(2):134–135
9. Goyal AK, Middha SK, Usha T (2020) Baccaurea ramiflora Lour, "*a comprehensive review from traditional usage to pharmacological evidenc".* Adv Tradit Med. Doi: 10.1007%2Fs13596-020-00489-9
10. Goyal AK, Middha SK, Usha T, Sen A (2017), "*Analysis of toxic antidiabetic and antioxidant potential of Bambusa balcooa Roxb leaf extracts in alloxan-induced diabetic rats*". 3 Biotech 7(2):120
11. Ma T, Sun X, Tian C, Zheng Y, Zheng C, Zhan J (2015), "*Chemical composition and hepatoprotective effects of polyphenols extracted from the stems and leaves of Sphallerocarpus gracilis*." J Funct Food 8:673–683
12. Middha SK, Usha T, Basistha BC, Goyal AK (2019), "*Amelioration of antioxidant potential, toxicity, and antihyperglycemic activity of Hippophae salicifolia D. Don leaf extracts in alloxan-induced diabetic rats"*. 3 Biotech 9:308
13. Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L (2005), "*Dietary polyphenols and the prevention of diseases*" Crit Rev Food Sci 45(4):287–306
14. Usha T, Pradhan S, Goyal AK, Dhivya S, Kumar HP, Singh MK, Joshi N, Basistha BC, Murthy KS, Selvaraj S, Middha SK (2017) "*Molecular simulation-based combinatorial modeling and antioxidant activities of zingiberaceae family rhizomes"*. Phcog Mag 13(Suppl 3): S715
15. Su, W., Sun, Q., Xia, M., Wen, Z., and Yao, Z. (2018). "*The Resource Utilization of Water Hyacinth (Eichhornia crassipes [Mart.] Solms) and its Challenges"*. Resources 7 (3), 46.

16. Yan, S. H., Song, W., and Guo, J. Y. (2017). "*Advances in Management and Utilization of Invasive Water Hyacinth (Eichhornia crassipes) in Aquatic Ecosystems - a Review"*. Crit. Rev. Biotechnol. 37 (2), 218–228.
17. Dersseh, M. G., Melesse, A. M., Tilahun, S. A., Abate, M., and Dagnew, D. C. (2019). "*Water Hyacinth: Review of its Impacts on Hydrology and Ecosystem Services-Lessons for Management of Lake Tana*," in Extreme Hydrology and Climate Variability. Editors A. M. Melesse, W. Abtew, and G. Senay (Amsterdam, The Netherlands: Elsevier) v 237–251.
18. Mustafa, H. M., and Hayder, G. (2021), "*Recent Studies on Applications of Aquatic weed Plants in Phytoremediation of Wastewater*": A Review Article. Ain Shams Eng. J. 12 (1), 355–365.
19. Malik, A. (2007). Environmental challenge Vis a Vis Opportunity: the Case of Water Hyacinth. Environ. Int. 33 (1), 122–138.
20. Balasubramanian, D., Arunachalam, K., Das, A. K., and Arunachalam, "*A. (2012). Decomposition and Nutrient Release of Eichhornia crassipes (Mart.) Solms. Under Different Trophic Conditions in Wetlands of Eastern Himalayan Foothills"*. Ecol. Eng. 44, 111–122.
21. Kangkon Saikia, Saurav Dey, Shabiha Nudrat Hazarika, Gautam Kumar Handique, Debajit Thakur, Arun Kumar Handique. "*Chemical and biochemical characterization of Ipomoea aquatica: genoprotective potential and inhibitory mechanism of its phytochemicals against α-amylase and α-glucosidase"*. Front Nutr., 2023 Dec 21;10:1304903.
22. Ranilla LG, Kwon YI, Apostolidis E, Shetty K." *Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America*". Bioresour Technol. (2010) 101:4676–89.
23. aat nahi karenge kyaSun L, Gidley MJ, Warren FJ. "*The mechanism of interactions between tea polyphenols and porcine pancreatic alpha-amylase: analysis by inhibition kinetics, fluorescence quenching, differential scanning calorimetry and isothermal titration calorimetry*". Mol Nutr Food Res. (2017) 61:324.
24. Alkiyumi, S. S., Abdullah, M. A., Alrashdi, A. S., Salama, S. M., Abdelwahab, S. I., Hamid, A., Hadi, A. (2012), "*Ipomoea aquatica Extract Shows Protective Action Against Thioacetamide-Induced Hepatotoxicity"*,Molecules 2012, 17, 6146-6155. (DOI:10.3390/molecules17056146)
25. Gettys, L.A. and R.G. Leon. 2021. "*A population genetics approach for the study of fluridone resistance in hydrilla. Special Issue"* Proceedings of the 21st Int. Conf. on Aquatic Invasive species. Aq. Inv. Vol.16. Issue 1: 28-42.
26. Marwan, H. Naima, M.Y. Sitti. 2017. "*Pemanfaatan Hydrilla verticillata (L.f) Royle sebagai pupuk hijau untuk memacu pertumbuhan bibit cacao (Theobroma cacao L.)*". Journal TABARO, Vol. 1(1): 1-10.
27. Danlu, S., K. Zhuang, Y. Chen, Z. Hu, Z. Shen. 2021. "*Phytotoxicity and accumulation of Cu in mature and young leaves of submerged macrophyte Hydrilla verticillata (L.f.) Royl"e. 2021*. Ecotoxicology and Environment Safety.

28. Jain, M.S. and A.S. Kalamdhat. 2019. "*Drum composting of nitrogen-rich Hydrilla verticillata with carbon-rich agents: effects on composting physics and kinetics"*. Journal of Environment Management Vol. 231: 770-779.
29. Wilson, T.Z.S., S.M. Thomaz, K.J. Murphy. 2010. "*Response of native Egeria najas Planch and invasive Hydrilla verticillata (L.f.) Royle to altered hydroecological in a subtropical river".* Journal Aquatic Botany 92 (2010): 40-48.
30. Fasya, A., S. Amalia, D. Megawati, F. Salima, V. Kusuma, B. Purwantoro. 2020. "*Isolation, identification, and bioactivity of steroids isolates from Hydrilla verticillata petroleum ether fraction", Conference Proceedings*. IOP Conference Series: Earth and Environmental Science (2020) 456(1).
31. Fattahi S, Ardekani AM, Zabihi E, et al. "*Antioxidant and apoptotic effects of an aqueous extract of Urtica dioica on the MCF-7 human breast cancer cell lin"e*. Asian Pac J Cancer Prev. 2013;14:5317–23.
32. Lalitha P, Shubashini. Sripathi K and Jayanthi P, "*Acute toxicity study of extracts of Eichhornia Crassipes (Mart.) Solms*", Asian Journal of Pharmaceutical and Clinical Research, Vol 5, Issue 4, 2012.
33. Pal Dilipkumar, Balasaheb Satish Nimse, Khatun Samina, and Bandyopadhyay Kumar Pranab,"*CNS Activities of the Aqueous Extract of Hydrilla verticillata in Mice*", Natural Product Sciences, Vol. 12, No. 1, 2006.
34. Choudhury Urmi, Lihite J Ratan , Singha Binita, Lahkar Mangala, "*Hypolipidaemic effect of ethanolic extract of, Ipomoea aquatic Trigonella foenum graecum Bryophyllum pinnatum and in experimental animals"*, Asian Journal of Pharmacy and Pharmacology 2018; 4(5): 692-696.
35. Khadijah B. Alkinani, Ehab M.M, Ali, Turki M. Al-shaikh, jalaluddin A. Awliaaa Khan, Tahani M. Al-naomasi, soad s. Ali Asaad A, Abduljawad, Osamas F. Mosa & tariq A. Zafar, "*Hepatoprotective effect of Epicatechin in CCl4- induced toxicity model aremediated via modulation of oxidative stress markers in Rats*", Evidence Based Complementary and Alternative Medicine, Volume 2021, Article ID 4655150, 12 pages.
36. S. Uraz, V. Tahan, C. Aygun et al., "*Role of ursodeoxycholic acid in prevention of methotrexate-induced liver toxicity*," Digestive Diseases and Sciences, vol. 53, no. 4, pp. 1071–1077, 2008.
37. Middha SK, Usha T, Babu D, Misra AK, Lokesh P, Goyal AK (2016) *Evaluation of antioxidative analgesic and anti-inflammatory activities of methanolic extract of Myrica nagi leaves-an animal model approach*. Symbiosis 70(1–3):179–184
38. D. Schuppan and N. H. Afdhal, "*Liver cirrhosis*," Fe Lancet, vol. 371, no. 9615, pp. 838–851, 2008.
39. M. F. Akhtar, M. O. Mehal, A. Saleem et al., "*Attenuating effect of Prosopis cineraria against paraquat-induced toxicity in prepubertal mice, Mus musculus*," Environmental Science and Pollution Research International, pp. 1–17, 2021.
40. A. T. Khalil, M. Ovais, J. Iqbal et al., "*Microbes-mediated synthesis strategies of metal nanoparticles and their potential role in cancer therapeutics*," in Seminars in Cancer Biology, Elsevier, Amsterdam, Netherlands, 2021.

41. M. Ayaz, A. Wadood, A. Sadiq, F. Ullah, O. Anichkina, and M. Ghufran, "*In-silico evaluations of the isolated phytosterols from polygonum hydropiper L against BACE1 and MAO drug targets*," Journal of Biomolecular Structure and Dynamics, pp. 1–9, 2021.
42. M. Ghufran, A. U. Rehman, M. Shah, M. Ayaz, H. L. Ng, and A. Wadood, "*In-silico design of peptide inhibitors of K-Ras target in cancer disease*," Journal of Biomolecular Structure and Dynamics, vol. 38, no. 18, pp. 5488–5499, 2020.
43. G. Karimi, M. Vahabzadeh, P. Lari, M. Rashedinia, and M. Moshiri, "*Silymarin", a promising pharmacological agent for treatment of diseases*," Iranian Journal of Basic Medical Sciences, vol. 14, no. 4, pp. 308–317, 2011.
44. M. L. Salem, A. A.-H. Khamis, A.-H. A.-H. Mostafa, and E. M. Ali, "*Antitumor potential of some selective medicinal plants on experimental tumor ascites*" Journal of Investigational Biochemistry, vol. 6, 2017.
45. K. R. Ritesh, A. Suganya, H. V. Dileepkumar, Y. Rajashekar, and T. Shivanandappa, "A single acute hepatotoxic dose of CCl4 causes oxidative stress in the rat brain," Toxicology Reports, vol. 2, pp. 891–895, 2015.
46. R. A. R. Elgawish, H. G. A. Rahman, and H. M. A. Abdelrazek, "*Green tea extract attenuates CCl4-induced hepatic injury in male hamsters via inhibition of lipid peroxidation and p53- mediated apoptosis*," Toxicology Reports, vol. 2, pp. 1149–1156, 2015.
47. H.-M. Lin, H.-C. Tseng, C.-J. Wang, J.-J. Lin, C.-W. Lo, and F.-P. Chou, "*Hepatoprotective effects of Solanum nigrum Linn extract against CCl4-induced oxidative damage in rats*," Chemico-Biological Interactions, vol. 171, no. 3, pp. 283–293, 2008.