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THERAPEUTIC POTENTIAL OF EUPHORBIA HIRTA AND LEPIDIUM SATIVUM, AND THEIR PHYTOCHEMICALS IN MITIGATING LIVER FIBROSIS: AN IN SILICO AND IN VIVO STUDY

AREEBA IMTIAZ[1*], SYED MUHAMMAD ALI SHAH[2], SULTAN AYAZ[3]

[1*]PHD SCHOLAR EASTERN MEDICINE, DEPARTMENT OF EASTERN MEDICINE GOVERNMENT COLLEGE UNIVERSITY FAISALABAD, PAK
[2]ASSISTANT PROFESSOR, DEPARTMENT OF EASTERN MEDICINE GOVERNMENT COLLEGE UNIVERSITY FAISALABAD, PAK
[3]ASSOCIATE PROFESSOR, DEPARTMENT OF EASTERN MEDICINE GOVERNMENT COLLEGE UNIVERSITY, FAISALABAD, PAK

*CORRESPONDING AUTHOR'S EMAIL: areeba.dahda247@gmail.com &, ORCID ID: 0009-0002-8377-2497

ABSTRACT

Background: Liver fibrosis, characterized by the excessive deposition of extracellular matrix proteins predominantly collagen, causes distortion of architecture and poor liver functioning. Existing anti-fibrotic treatment approaches are insufficient, and there is a need to investigate natural phytochemicals with hepatoprotective and regenerative effects.

Aims & Objectives: Aim was to evaluate hepatoprotective and anti-fibrotic properties of extracts of Euphorbia hirta and Lepidium sativum and their active phytochemicals, Kaempferol and Coumestrol, with CCL_4-induced hepatic fibrosis in rats.

Methodology: Combined in silico and in vivo method was used. Kaempferol and Coumestrol were identified as lead compounds in molecular docking of 69 phytochemicals against IL-6, AKT1, EGFR, and Caspase-3. Sixty-four male albino rats were stratified into control, intoxicated, standard drug, and treatment groups, administered with plant extracts and phytochemicals (50 and 100 mg/kg), and liver function tests, hematological parameters, and histopathology were evaluated.

Results: Histopathological results showed high-dose of Kaempferol and Coumestrol exhibited hepatic architecture restoration with no necrosis, hemorrhage, and fatty changes. Biochemical analysis showed improvement in liver functioning in all treatment groups, with decrease in levels of ALT, AST, ALP, and bilirubin compared to the intoxicated group. Hematologic outcomes revealed dose-related normalization of RBCs, hemoglobin, platelets, and WBCs with L. sativum extract. Overall, Coumestrol showed highest hepatoprotective effect, followed by Kaempferol, both had higher hepatoprotective effect than plant extracts.

Conclusion: Kaempferol and Coumestrol demonstrated significant hepatoprotective and anti-fibrotic properties, making these important as cost-effective alternative natural therapies against liver fibrosis.

Keywords: Anti-Fibrotic Agents; Coumestrol; Euphorbia Hirta; Kaempferol; Lepidium Sativum; Liver Fibrosis; Molecular Docking

INTRODUCTION

Liver fibrosis is the process in which chronic liver injury leads to the deposition of extracellular matrix (ECM) proteins mainly collagens around hepatocytes. This forms an abnormal tissue scaffold and distorts the liver's architecture. If untreated, fibrosis progresses and eventually leads to cirrhosis characterized by regenerative nodules and fibrous septa that significantly compromise liver function. Fibrosis is staged from F1- F4, with F4 representing cirrhosis (Somnay et al., 2024). A systematic review estimated the global prevalence of advanced liver fibrosis in the general population at 3.3% (95% CI: 2.4–4.2% (Zamani et al., 2025). In 2019, global burden showed that chronic liver diseases caused about 1.47 million deaths, up from 1.01 million in 1990. Death rates dropped to 18 per 100,000, highest in Africa (44.15) and lowest in Australasia (5.48). Men had 1.5 times higher risk (Wu et al., 2024).

The pathogenesis of liver fibrosis is complex and involves multiple cell types and signaling pathways. Causes of chronic injury are viral hepatitis (HBV, HCV), alcohol (alcoholic liver disease), non-alcoholic fatty liver disease (NAFLD/NASH), cholestatic liver diseases (e.g., biliary obstruction, primary biliary cholangitis, primary sclerosing cholangitis), and genetic or metabolic disorders. Injured hepatocytes release damage-associated molecular patterns (DAMPs), reactive oxygen species (ROS), and other signals that trigger inflammation. Inflammation and immune

activation are caused by Kupffer cells (liver macrophages) and infiltrating immune cells (macrophages, neutrophils) which release cytokines such as TGF-β and PDGF. These amplify injury, recruit further immune cells, and stimulate stellate cells (Berumen et al., 2021).

In healthy liver, hepatic stellate cells (HSCs) are quiescent (vitamin A-storing). Injury activates them to become myofibroblast-like cells that proliferate and secrete ECM proteins (collagen types I & III), driving fibrosis. ECM imbalance is caused by deposition of ECM increases, while degradation (by MMPs) is suppressed due to excess inhibitors (TIMPs). Angiogenesis and vascular changes cause sinusoidal endothelial cells lose fenestrations, capillarization occurs, and vascular remodeling leads to portal hypertension. At earlier stages, if the injurious stimulus is removed (e.g., viral clearance, abstaining from alcohol, metabolic control), fibrosis can regress. In some cases, even early cirrhosis may improve ((Hernandez-Gea & Friedman et al., 2011). However, once severe architectural distortion and decompensation are present, reversal is very difficult (D'Amico et al., 2006). Currently, there is no FDA-approved drug that universally reverses fibrosis. Available treatments target underlying causes, reduce inflammation, and prevent further injury. Research on anti-fibrotic drugs is ongoing (Pei et al., 2023).

Herbal plant extracts have shown promise in reversing liver fibrosis, a reversible condition preceding cirrhosis and HCC, by mainly targeting hepatic stellate cells (HSCs) and the TGF-β pathway. While TGF-β is crucial for normal physiology, targeting downstream effectors like MERTK offers a safer anti-fibrotic strategy. Several active compounds—including Silymarin, Curcumin, EGCG, and Salvianolic acid have been identified, though many plant extracts remain uncharacterized. Clinical trials with formulations like Silybin-phospholipids plus vitamin E (SPV) and FZHY demonstrate efficacy in humans, highlighting the potential of herbal therapies as safer, cost-effective alternatives for managing liver fibrosis and MAFLD (Mungamuri et al., 2025).

Euphorbia hirta and Lepidium sativum have long been used for treatment of different ailments, including liver related disorders. Euphorbia hirta is known for its antioxidant and anti-inflammatory properties, and it contains bioactive compounds like flavonoids, tannins, saponins, and phenolic compounds (Rai et al., 2021 and Khursheed et al., 2022). Lepidium sativum is also known for its digestive and anti-inflammatory properties. It is rich in flavonoids, glucosinolates, and phenolic compounds (Al-Snafi et al., 2019). Coumestrol is the most significant and biologically important phytochemical among all the other phytochemicals present in Lepidium sativum (Abdul-Nabie et al., 2025). The systemic evaluation of both the plants and their respective phytochemicals is very limited despite of the fat that they have been used traditionally as medicinal agents. The current study focuses on combination of computational and experimental approaches to assess the hepatoprotective effects and anti-fibrotic potential of Euphorbia hirta and Lepidium sativum, and their best suitable phytochemicals, Kaempferol and Coumestrol, against CCL_4-induced liver fibrosis in animal model.

MATERIALS AND METHODS

In silico molecular docking study: To identify the most potent anti-fibrotic compounds, an in silico screening was conducted, it included the following steps:

- **Retrieval of bioactive compounds and proteins:** Sixty nine phytochemicals were retrieved from Euphorbia hirta and Lepidium sativum using databases (TCMSP, IMPPAT, PubChem). Four key proteins involved in hepatic fibrosis, including IL-6 (PDB ID: 1alu), AKT1 (PDB ID: 2uvm), EGFR (PDB ID: 5gty), and Caspase-3 (PDB ID: 2cdr) were selected as targets and their crystal structures were obtained from Protein Data Bank (Althagafi et al., 2019 and Dhorajiwala et al., 2019).
- **Docking and visualization:** Molecular docking was performed using PyRx Virtual Screening software, which uses AutoDock Vina. The interactions between the lead compounds and the amino acid residues in the binding pockets were visualized and analyzed using Discovery Studio (Hussain et al., 2023).

Collection and extraction of plant material: The medicinal plants, Euphorbia hirta (whole plant) and Lepidium sativum (seeds), were collected and taxonomically confirmed and identified. Ethanolic extracts were prepared with the help of maceration method (Harborne et al., 1998).

In vivo study of medicinal plants: The therapeutic potential of selected plant drugs and phytochemicals was evaluated through the experimental study for 12 weeks. For this study, 64 male albino rats, weighing about 120-150g, were housed in a well-organized (12 hours light and 12 hours darkness) and clean wire cage, with a temperature of 20-25ºC, and were given a diet during experimentation. The rats were distributed into seven groups in the following manner:

- **Group 1** (Healthy group): Only diet was given.
- **Group 2** (Intoxicated group): 50% CCL_4 (2ml/kg).
- **Group 3** (Standard drug group): 50% CCL_4 + Silymarin
- **Group 4a & 4b:** 50% CCL_4 + Euphorbia hirta (Low dose 50ml/kg and high dose 100mg/kg)
- **Group 5a & 5b:** 50% CCL_4 + Lepidium sativum (Low dose 50ml/kg and high dose 100mg/kg)
- **Group 6a & 6b:** 50% CCL_4 + Kaempferol (Low dose 50ml/kg and high dose 100mg/kg)

- **Group 7a & 7b:** 50% CCL_4 + Coumestrol (Low dose 50ml/kg and high dose 100mg/kg)

The treatment continued for 12 weeks, where CCL_4 was administered for first 6 weeks, followed by 6 weeks of treatment with plant extracts and phytochemicals.

Blood and organs collection and analyses: After completion of the monitoring period, the animals were sacrificed; blood was collected for hematological analysis and analysis of liver function tests, including ALP, ALT, AST, and bilirubin. Liver organs of the experimental animals were stored for histopathological examination using H & E staining. (Bancroft et al., 2008 and Rahmioglu et al., 2009).

Statistical analysis: Data were analyzed using one-way ANOVA test, and GraphPad Prism software was used for it. A p-value<0.05 was considered significant (Neelamegam et al., 2022).

RESULTS

In silico study and phytochemical selection:

Molecular docking of 69 phytochemicals was performed against the selected proteins, IL-6, AKT1, EGFR, and Casp-3. It identified Kaempferol and Coumestrol as the lead candidates based on their high binding energies and interactions with target proteins at the binding site. (Table 1) Coumestrol exhibited higher binding affinities for three of the target proteins, AKT1 (-6.4 kcal/mol), EGFR (-7.1 kcal/mol), and CASP3 (-7.1 kcal/mol).

Table 1: Binding energies of phytochemicals with target proteins as shown by molecular docking studies

Sr. No.	Target Protein	PDB ID	Phytochemical	Binding Energy (kcal/mol)
1	IL-6	1ALU	Kaempferol	-6.3
			Coumestrol	-6.3
2	AKT1	2UVM	Kaempferol	-6
			Coumestrol	-6.4
3	EGFR	5GTY	Kaempferol	-6.7
			Coumestrol	-7.1
4	CASP3	2CDR	Kaempferol	-6.4
			Coumestrol	-7.1

Measurement of Liver Function Tests and Bilirubin: CCL_4 intoxication significantly elevated the levels of liver enzymes (ALT, AST, ALP) and altered bilirubin levels compared to the healthy control group (Group 1), indicating severe hepatocellular damage. Treatment with the plant extracts and phytochemicals resulted in a dose-dependent significant reduction in these elevated enzyme levels. The high-dose groups of phytochemicals (Group 6b and Group 7b) showed the most significant reductions in level of liver enzymes, nearing the efficacy of Silymarin. (Table 2) (Figure 1)

Table 2: Effect of compounds and standard drug on liver function tests on male rats

Groups	ALT (U/L) Mean ± SE	AST (U/L) Mean ± SE	ALP (U/L) Mean ± SE	Bilirubin (mg/dl) Mean ± SE
Group 1 (Healthy group)	40.3±3.51	32.6±2.51	200±12	2.17±0.01
Group 2 (Intoxicated group)	81.6±9.07	91.6±2.51	736.6±15.27	1.38±0.45
Group 3 (standard drug group)	57.6±4.04	67.6±4.5	465±5.03	1.91±0.02
Group 4a (Euphorbia hirta extract LD)	64.3±4.5	67±3	522.26±26.1	1.89±0.07
Group 4b (Euphorbia hirta extract HD)	59±4.58	62±4	406±10.39	1.91±0.04
Group 5a (Lepidium sativum extract LD)	57±3.6	65.3±4.04	439±16.52	1.22±0.10

Group 5b (Lepidium sativum extract HD)	57.6±5.5	60±8	371±5.68	1.48±0.05
Group 6a (Kaempferol LD)	48.6±4.5	54.3±1.15	289.6±10.69	1.41±0.03
Group 6b (Kaempferol HD)	41±3	49.9±5.03	313.3±6.11	1.45±0.02
Group 7a (Coumestrol LD)	44±3	54.6±5.03	276±24.87	1.43±0.06
Group 7b (Coumestrol HD)	38.6±2.51	43.3±0.57	241.6±26.76	1.44±0.12

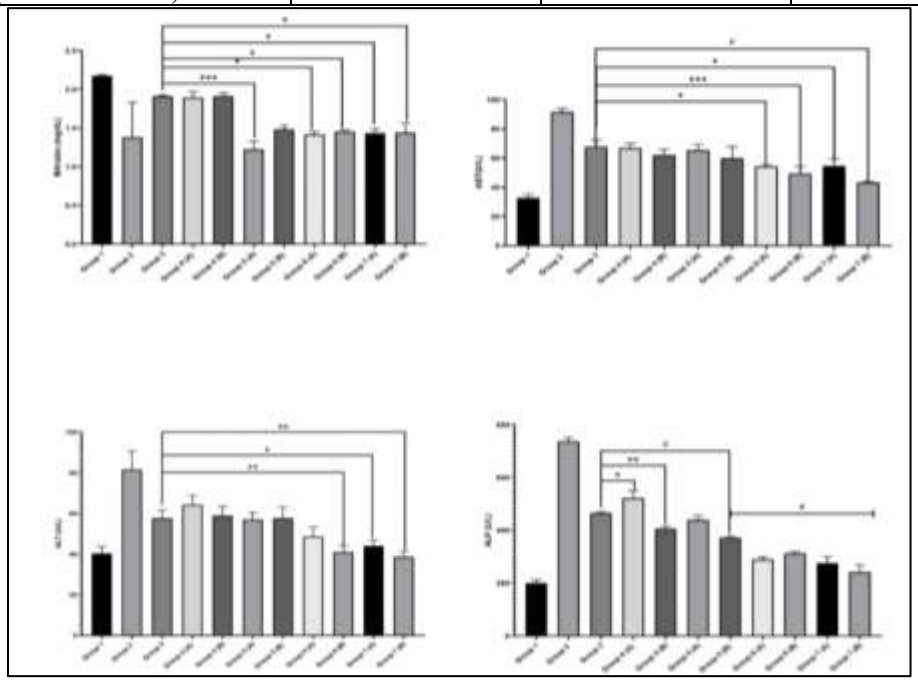

FIG. 1: EFFECT OF STANDARD DRUG AND DRUG CANDIDATES ON LIVER ENZYMES AND TOTAL BILIRUBIN

H**ematological Analysis:** CCL_4 intoxication caused major hematological disruptions, like reduced RBCs, haemoglobin, and platelets, and increased WBCs compared to the healthy group. Treatment with plant extracts restored these parameters towards normalcy in a dose-dependent manner. Lepidium sativum high-dose (Group 5b) and Coumestrol high-dose (Group 7b) remarkably restored the hemoglobin levels. (Table 3) (Figures 2 and 3)

Table 3: Effect of standard drug and drug candidates on hematology in male rats.

GROUPS	**WBCS (10^3/UL) MEAN ± SE**	**RBCS (10^6/UL) MEAN ± SE**	**HB (G/DL) MEAN ± SE**	**PLTS (10^3/UL) MEAN ± SE**	**LYM (%) MEAN ± SE**
Group 1	8.7 ± 0.10	8.57 ± 0.15	15.45 ± 0.04	9.38 ± 0.03	7.26 ± 0.03
Group 2	14.13 ± 0.25	6.91 ± 0.04	12.33 ± 0.21	1.54 ± 0.03	10.77 ± 0.15
Group 3	9.83 ± 0.06	7.25 ± 0.07	13.5 ± 0.30	8.15 ± 0.04	8.4 ± 0.1
Group 4a	9.4 ± 0.20	6.95 ± 0.01	12.23 ± 0.15	7.95 ± 0.03	7.5 ± 0.1
Group 4b	11.67 ± 1.15	7.5 ± 0.05	13.5 ± 0.30	8.74 ± 0.04	9.13 ± 0.25
Group 5a	13.53 ± 0.31	7.94 ± 0.06	13.47 ± 0.35	1.05 ± 0.04	9.47 ± 0.25

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Group 5b	12.77 ± 0.15	8.49 ± 0.05	15.4 ± 0.2	1.23 ± 0.02	9.33 ± 0.15
Group 6a	12.53 ± 0.12	6.95 ± 0.01	12.37 ± 0.15	1.28 ± 0.02	9.57 ± 0.25
Group 6b	16.7 ± 0.20	6.78 ± 0.02	12.37 ± 0.25	1.26 ± 0.02	12.43 ± 0.31
Group 7a	12.4 ± 0.40	7.18 ± 0.04	12.47 ± 0.31	1.65 ± 0.01	9.5 ± 0.3
Group 7b	8.57 ± 0.60	6.61 ± 0.03	11.73 ± 0.21	7.13 ± 0.04	5.27 ± 0.15

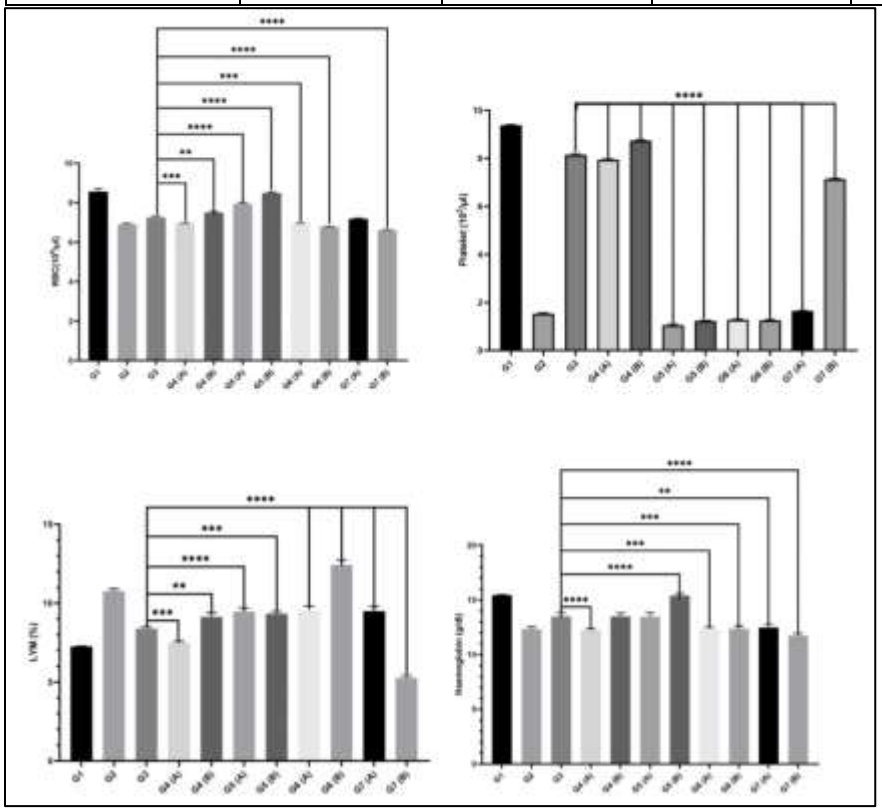

FIG. 2: EFFECT OF STANDARD DRUG AND DRUG CANDIDATES ON RBCS, PLATELETS LYMPHOCYTES, AND HEMOGLOBIN.

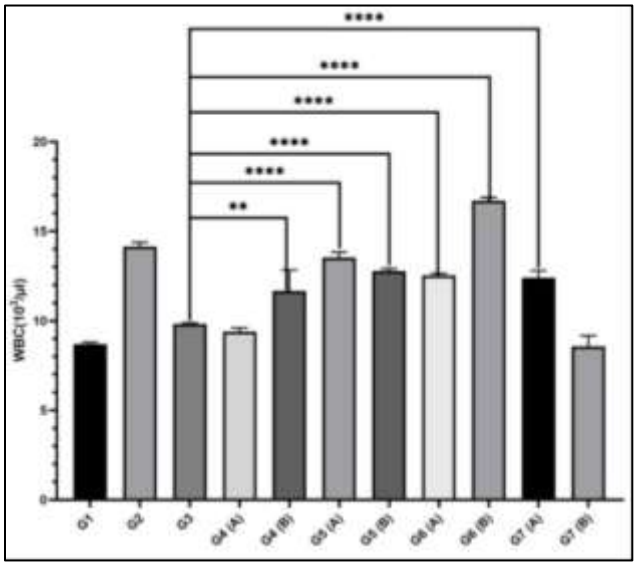

FIG. 3: EFFECT OF STANDARD DRUG AND DRUG CANDIDATES ON WBCS

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Histological Study of Liver: The intoxicated group (Group 2) showed severe architectural distortion, widespread necrosis, hemorrhage, fatty changes, and edema. Treatment with plant extracts resulted in significant, dose-dependent recovery of liver architecture. Plant extracts high-dose (Group 4b & 5b) showed intact liver architecture with mild necrosis and fatty changes. The phytochemicals high-dose (Group 6b & 7b) showed the most profound recovery, with intact liver architecture, and no necrosis, hemorrhage and fatty changes. (Table 4) (Figures 4 and 5)

Table 4: Histological features of CCL_4 induced hepatic tissues in comparison with treatment groups

GROUPS/ PARAMETERS	ARCHITECTURE (INTACT/DISTORTED)	NECROSIS	HEMORRHAGE	FATTY CHANGES	EDEMA
Group 1	intact	no	no	no	no
Group 2	++	++	+++	seen	seen
Group 3	intact	no	no	no	seen
Group 4a	intact	+	no	+	seen
Group 4b	intact	+	+	no	no
Group 5a	intact	+	no	no	seen
Group 5b	intact	no	+	+	no
Group 6a	intact	no	no	no	seen
Group 6b	intact	no	no	no	no
Group 7a	intact	no	no	no	seen
Group 7b	intact	no	no	no	no

Mild (+), Moderate (++), and Severe (+++)

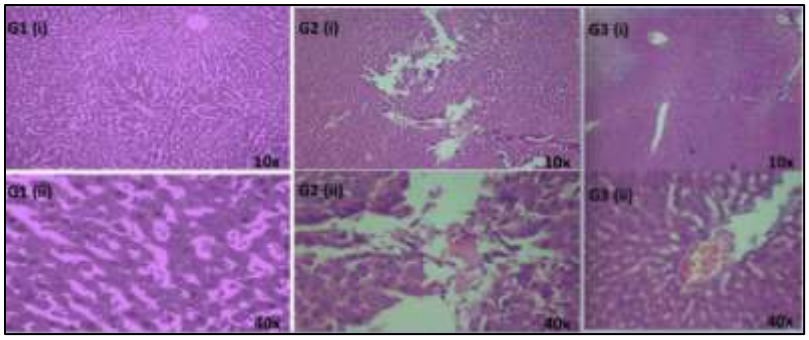

FIG. 4: PHOTOMICROGRAPH OF RAT LIVER TISSUE OF HEALTHY, INTOXICATED, AND STANDARD DRUG GROUPS AT 10X AND 40X

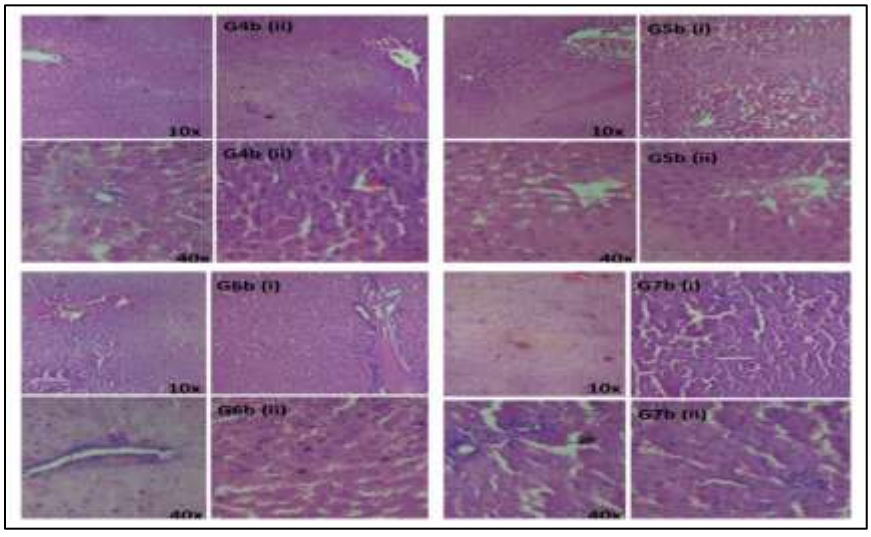

FIG. 5: PHOTOMICROGRAPH OF RAT LIVER TISSUE OF TREATMENT GROUPS AT 10X AND 40X

DISCUSSION

Liver fibrosis, characterized by the deposition of collagen and ECM by replacing hepatic parenchyma, was believed to be an incurable condition that may lead to liver failure. However, recent advancements have proven that liver fibrosis is a curable disease. Although it cannot be reversed, different treatment methods can help stop the progression of fibrosis. Since the development of a better understanding of the mechanism of liver fibrosis, novel therapeutic approaches are now being developed to treat it (Glass et al., 2015; Li et al., 2017; Yuan et al., 2019). This study chose Euphorbia hirta and Lepidium sativum because of their antioxidant, anti-inflammatory, anti-bacterial, and cytotoxic properties. On the other hand, the overlapping and the most involved genes in liver cancer, IL-6, AKT1, EGFR, and Caspase-3, were selected to be treated using the selective treatment. Wang et al. reported that Oroxylin-A, an active ingredient of Scutellaria baicalenseis, inhibits HSC proliferation and reduces pro-inflammatory factors, thereby aiding in achieving anti-fibrotic effects (Wang et al., 2024). An insilico study conducted in 2023 used IL-6, AKT1, JUN, CASP3, and other genes that regulate liver fibrosis as target proteins for molecular docking studies (Liu et al., 2024). PyRx Virtual Screening software was used for ligand formation, and the Discovery Studio software was used to visualize interactions between the receptor proteins and key active compounds. Two of the seven phytochemicals, Kaempferol and Coumestrol, were selected based on high binding energy and maximum interaction with the target proteins at the binding site. The molecular docking results showed that Kaempferol and Coumestrol exhibited strong affinities with the target proteins, IL-6, AKT1, EGFR, and CASP3. Coumestrol showed higher binding affinities than Kaempferol, signifying that it may have a stronger inhibitory effect on cancer-promoting pathways known for metastasis. Coumestrol exhibited higher binding affinities for three of the target proteins, AKT1 (-6.4 kcal/mol), EGFR (-7.1 kcal/mol), and CASP3 (-7.1 kcal/mol).

The current study was planned to evaluate the effect of the extracts of Euphorbia hirta and Lepidium sativum, and the most efficient phytochemicals of both the plants, Kaempferol and Coumestrol respectively, in CCL_4 induced hepatic fibrosis. The drug candidates, Kaempferol and Coumestrol were nomintaed through the in silico studies, out of the sixty-nine phytochemicals of both the plants. For evaluation of the hepatoprotective and anti-fibrotic potential of these drug candidates, sixty-four male albino rats, weighing about 120-150g, were used for an animal trial of twelve weeks. It included the induction of hepatic fibrosis in to the experimental animals using CCL_4, and then treatment of the animals using low dose (50 mg/kg) and high dose (100 mg/kg) of plant extracts and the phytochemicals. The experimental animals were divided into eleven groups and subgroups, and the weight of each rat was measured weekly to maintain accurate doses. When fibrosis was induced, the animals were treated with Silymarin, the standard drug, and the low dose and high dose of the drug candidates', including the plant extracts.

A research study conducted in 2016 used CCL_4 to induce hepatic fibrosis, and revealed that the progression of fibrosis is directly related to the proliferation of connective tissues of the liver. The experiment used male Wistar rats, administered CCL_4 to the rats for about nine weeks, and as a result the liver tissues of the experimental animals started to proliferate, leading to the development of liver fibrosis. Moreover, these were followed by changes in body weight and the liver/body ratio of the animals, and ALT and AST levels dropped significantly. The liver tissues were proliferated with the development of collagen, and the liver organs developed fibrotic hyperplasia (Dong et al., 2016). Another research study reported that oral administration of CCL_4 damages liver cells, and this leads to the escape of liver enzymes into blood, indicating abnormality in liver function (Bencheikh et al., 2019; Dineshkumar et al., 2013). The critical biomarkers that help in assessing the function of the liver are alkaline phosphatase (ALP), alanine aminotransferase (ALT), alanine aspartate (AST), and bilirubin. High levels of ALT indicate injury to liver tissues, while elevated AST levels may indicate muscle injury; therefore, both enzymes are specific in indicating liver injury (Kausar et al., 2010). The liver enzymes escape into the blood after liver tissue injury; the presence of these enzymes in the serum confirms damage to liver tissue (Motto et al., 2021)**.** The current study revealed that rats of the intoxicated group (Group 2) had very high levels of ALT, AST, and ALP, and total bilirubin was decreased in comparison to the healthy group (Group 1), which clearly indicated liver injury. Group 3, the group of rats treated with the standard drug, Silymarin, a well-known hepatoprotective drug, showed a marked reduction in liver enzymes.

This study demonstrated that treating rats with CCL4-induced hepatic fibrosis with plant extracts and their phytochemicals showed significant hepatoprotective activity. The protective effect was evaluated with serum analysis for liver enzymes, hematological profiles, and histopathological examination of experimental animals. Kaempferol showed the strongest hepatoprotective effect, followed by Coumestrol, and the extracts of both plants showed dose-dependent hepatoprotective activity. Euphorbia hirta, at low and high doses, effectively improved liver architecture and reduced liver enzymes. These results align with experimental studies that concluded Euphorbia hirta restores normal histology of liver tissues (Akinboboye et al., 2025; Balasubramanian et al., 2022) . Lepidium sativum extracts also reduced ALP, ALT, and AST levels, with the high-dose group showing more marked results. This supported a previous study conducted by Ali et al. in 2019, which concluded that the presence of flavonoids and phenolic compounds in the seeds of Lepidium sativum helps to enhance antioxidant activity and keep the membranes of liver cells intact (Ali & Rajab et al., 2019).

Kaempferol exhibited marked improvement in liver biomarker enzymes among the groups treated with phytochemicals. ALT and AST levels were significantly reduced, and ALP and total bilirubin dropped near-normal levels in high-dose Kaempferol group (Group 6b). These results were consistent with the studies, which showed that Kaempferol lowers oxidative stress on liver cells, and reduces inflammation in CCL4-induced liver injury (Zhou et al., 2022). Kaempferol also reduces inflammation, through different mechanisms like NF-κB and MAPK pathways, which reduce oxidative stress as well (Zhou et al., 2022; Zhu et al., 2025). Coumestrol also improved liver biomarker enzymes, though less significantly than the results of Kaempferol. High-dose group of Coumestrol (Group 7b) raised the level of liver enzymes to normal, showing that Coumestrol is a dose-dependent hepatoprotective agent. Different studies show that Coumestrol can regulate lipid metabolism and also show antioxidant and anti-inflammatory properties (Bae et al., 2021). Altogether, improvement in the levels of liver enzymes shows that both the plant extracts and phytochemicals protect liver tissues from damage induced by CCL_4.

Histopathological examination of liver tissues was performed to evaluate the level of damage done to the tissues by CCL_4, and the protective effects of plant extracts and their respective phytochemicals. The intoxicated group (Group 2) showed severe necrosis, fatty changes, hemorrhage, and distorted architecture of liver tissues upon microscopic examination. On the other hand, the treatment groups showed significant signs of recovery of the liver tissues. Standard drug group (Group 3) showed intact architecture of liver tissues, with no necrosis or hemorrhages, coherent with its role as an established hepatoprotective agent. Some previous studies also report that Kaempferol reduces collagen deposition and restores normal liver architecture in CCL_4-induced hepatic fibrosis (Zhu et al., 2025). Coumestrol treatment groups (Group 7a&b) also exhibited marked hepatoprotection., and the high-dose group showed intact liver architecture with no signs of necrosis and no fatty changes**.** Although Coumestrol is less studied in hepatic fibrosis models, its antioxidant and anti-inflammatory activities are in coherence with the histological improvements studied by Frenzel et al. in 2019 (Frenzel et al., 2019).

The results of this research study support the paradigm shift towards natural therapeutic agents for the treatment of liver fibrosis. The dose-dependent hepatoprotective and the anti-fibrotic effects of Kaempferol and Coumestrol suggest that both the phytochemicals can be used as strong candidates for future clinical trials. More importantly, this study bridges the gap between traditional and modern pharmacology, and contributes to the development of cost-effective, less toxic, and evidence-based treatment options for liver fibrosis. Future investigations should emphasize on molecular approach, long term safety evaluations, and practice-oriented research to evaluate the clinical potential of these drug candidates in human population.

CONCLUSION

This study demonstrated that the extracts of Euphorbia hirta and Lepidium sativum, and their best suitable phytochemicals, Kaempferol and Coumestrol have marked anti-fibrotic and hepatoprotective properties. The in silico study identified Kaempferol and Coumestro as the leading drug candidates among sixty-four phytochemicals of both the plants, on the basis of strong binding affinities with key fibrotic protein targets. The in vivo assay in CCL_4-induced animal model gave the most promising evidence that treatment with these compounds resulted in return of liver enzyme and total bilirubin, and hematological profile to normal levels. Restoration of near-normal liver architecture was also observed, especially in high-dose groups. In general, Coumestrol showed the highest hepatoprotective effect, matching those of Silymarin-treated groups, followed by Kaempferol and they both had higher hepatoprotective effect than plant extracts. The findings of this study not only endorse the traditional use of these medicinal plants, but also bring innovation in identification and validation of specific phytochemicals helpful in obtaining these results. This paves the way for development and formulization of potent natural therapeutic agents for the treatment of liver fibrosis. Further research needs to focus on the toxicological and pharmacokinetic studies of the isolated compounds, and clinical trials to take these promising pre-clinical findings into human therapeutics.

DECLARATIONS

Data availability statement: All data generated or analyzed during the study are included in this article.

Authors' contribution

Areeba Imtiaz: Conceptualization, data collection, methodology, writing, and original draft preparation. **Syed**

Muhammad Ali Shah: Formal analysis and supervision. **Dr Sultan Ayaz:** Review.

Ethics Approval Statement: This study was approved by the Institutional Ethics Committee of GC University, Faisalabad, with approval number GCUF/ERC/IRB/2116.

Conflict of interest disclosure: The author declares that this research has no conflict of interest.

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