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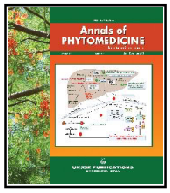

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HPTLC profiling and hepatoprotective effect of *Plumeria obtusa* L. against CCl_4 induced liver damage in rats

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Abstract

The present study aimed to perform the high-performance thin-layer chromatography (HPTLC) profiling and hepatoprotective effect of *Plumeria obtusa* L. (*P. obtusa*) on carbon tetrachloride (CCl_4) induced liver damage in rats. The HPTLC profiling was carried out to determine the phytochemical composition of the alcoholic extract of *P. obtusa* leaves. The obtained chromatograms revealed the presence of various bioactive compounds, including flavonoids, terpenoids, and phenolic compounds. To evaluate the hepatoprotective effect, male Wistar rats were divided into different groups such as control group, CCl_4 group, and *P. obtusa* treated groups. Liver damage was induced in rats by intraperitoneal administration of CCl_4. The treatment groups received the extract of *P. obtusa* at various doses (100, 200 and 400 mg/kg) for seven consecutive days. Biochemical parameters such as serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and antioxidant enzymes were assessed to evaluate liver function. Histopathological examination of liver tissues was also performed. The results demonstrated that treatment with *P. obtusa* extract significantly attenuated the elevated levels of AST, ALT, ALP, and antioxidants enzymes against CCl_4 induced by hepatotoxicity, that indicating its hepatoprotective potential. Histopathological examination revealed reduced hepatic lesions, necrosis, and inflammation in the treated groups compared to the CCl_4 control group. In conclusion, HPTLC profiling of *P. obtusa* revealed the presence of diverse phytochemicals, while the extract exhibited a significant hepatoprotective effect against CCl_4 induced hepatotoxicity in rats. It can be concluded that the potential use of *P. obtusa* as a natural remedy for liver disorders, warranting further exploration of its mechanisms of action andclinical applications.

1. Introduction

The liver is a vital organ located in the upper right portion of the abdomen, under the ribcage. It plays a crucial role in various bodily functions, including metabolism, digestion, detoxification, and the production of essential proteins. It regulates the metabolism of carbohydrates, fats, and proteins, and it helps maintain a steady blood glucose level, detoxifies harmful substances and produces bile, which aids in digestion and the absorption of fats, as well as various proteins necessary for blood clotting, immune function, and transport of nutrients and hormones (Michael *et al*., 2018; Pandey *et al*., 2007). Liver malfunction causes numerous types of liver diseases, some of which include such as hepatitis often caused by viral infections (hepatitis A, B, C, *etc*.) or excessive alcohol consumption, cirrhosis represents to carrying of the liver tissue, usually resulting from long-term liver damage caused by chronic hepatitis, alcoholism, fatty liver disease, or other factors (Delgado-Montemayor *et al*., 2022; Sharma and Dabur, 2015).

Liver diseases can lead to various symptoms and complications, including jaundice (yellowing of the skin and eyes), fatigue, abdominal pain, fluid accumulation, liver failure, and an increased risk of infections. Prompt diagnosis, appropriate medical care, and lifestyle changes (such as avoiding alcohol, maintaining a healthy weight, and following a balanced diet) can help manage or prevent liver disease.The analysis of mortality data is commonly utilized to evaluate the impact of a particular disease, and concerning chronic liver disease (CLD), there has been a significant rise in mortality rates worldwide. Specifically, between 1980 and 2013, there was a notable 46% increase in CLD mortality, highlighting the growing public health significance of this condition. The majority of this rise in CLD-related deaths has been observed in low and low-middle income countries, predominantly located in Asia and Africa (Mukherjee *et al*., 2017).

Carbon tetrachloride (CCl_4) is widely used in experimental models to induce hepatotoxicity (liver damage) in research settings. When CCl_4 is metabolized in the liver, it produces highly reactive free radicals, such as trichloromethyl (CCl_3) and peroxy trichloromethyl ($•OOCCl_3$)

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radicals, which can initiate oxidative stress and cause damage to liver cells. CCl_4 - induced hepatotoxicity cause inflammation, necrosis (cell death), and fibrosis (Insaf *et al*., 2022 ; Kengar *et al*., 2017). The initial injury caused by CCl_4 exposure leads to the activation of inflammatory cells and the release of pro-inflammatory cytokines, which further contribute to liver damage. The hepatotoxic effects of CCl_4 can be observed through various biochemical and histological changes in the liver, including elevated liver enzymes such as alanine transaminase (ALT) and aspartate transaminase (AST) in the bloodstream and damaged liver cells (Chester *et al*., 2017; Kengar *et al*., 2017).

Medicinal plants have played a significant role in traditional medicine for centuries and continue to be a valuable resource in modern healthcare (Gaurav *et al*., 2023b; Mehrotra, 2020). Here are some key roles that medicinal plants have in the management and treatment of diseases. Many medicinal plants contain bioactive compounds such as alkaloids, flavonoids, terpenoids, phenolic compounds, and others. These compounds can have various pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, analgesic, and immunomodulatory effects (Gautam *et al*., 2021; Gaurav *et al*., 2022; Khan *et al*., 2022). Extracts or isolated compounds from medicinal plants serve as the basis for developing pharmaceutical drugs or as natural remedies for specific ailments (Kiran *et al*., 2021; Zahiruddin *et al*., 2020). Medicinal plants form the foundation of traditional systems of medicine, such as ayurveda, traditional chinese medicine (TCM), and indigenous medicine. These systems utilize plants and their preparations to diagnose, prevent, and treat diseases (Zahiruddin *et al*., 2022; Zahiruddin *et al*., 2021). Many people still rely on medicinal plants and herbal remedies as alternative or complementary treatments, either independently or alongside conventional medicine. Medicinal plants are employed for preventive healthcare and promoting general health and wellness. Certain plants possess properties that support immune function, provide antioxidant protection, regulate metabolism, and improve overall vitality. Herbal supplements and botanical preparations are often used as preventive measures to maintain health and prevent the onset of diseases (Gaurav, 2022; Gaurav *et al*., 2023a; Gaurav *et al*., 2022).

P. obtusa, commonly known as the Singapore Plumeria or Frangipani, is a species of flowering plant in the Apocynaceae family. It is native to various regions of Southeast Asia, including Singapore, Malaysia, Thailand, and Indonesia. It is a small to medium-sized deciduous tree that can reach heights of up to 8 meters (26 feet). It has a spreading and rounded crown with thick branches. The trunk is grayish and smooth, while the branches are thick and fleshy. The leaves are leathery, glossy, and oblong in shape. They are arranged spirally and clustered at the branch tips. The leaves are typically green and can reach a length of about 15-30 cm (6-12 inches).The most notable feature of *P. obtusa*is its fragrant and showy flowers. The flowers are large and have a distinctive shape with overlapping petals (Salar *et al*., 2022). They are usually white or creamy-yellow, sometimes with a hint of pink at the center. The flowers bloom in clusters at the branch tips, creating an eye-catching display. They are highly fragrant, emitting a sweet and captivating scent, especially in the evenings. *P. obtusa* is popular in tropical and subtropical regions as an ornamental tree. It is often grown in gardens, parks, and as a street tree. It prefers well-draining soil and full sun exposure to thrive. The tree is drought-tolerant and relatively low-maintenance once established. Propagation can be done through seeds, stem cuttings, or grafting (Bihani *et al*., 2021).

P. obtusa has been used in traditional medicine in some regions. Different parts of the plant, including the leaves, bark, and flowers, are believed to possess medicinal properties. They have been used to treat various ailments, such as skin infections, inflammation, gastrointestinal issues, and as a general tonic. *P. obtusa* contains various chemical constituents that contribute to its pharmacological properties. Several chemical constituents such as contains alkaloids such as plumericin, obtusin, and isoplumericinhave been reported to possess anti-inflammatory, analgesic, and antimicrobial activities. It hasseveral terpenoids, including triterpenes and sesquiterpenes. These compounds have shown potential antioxidant, anti-inflammatory, and antitumor properties.The flavonoids such as rutin, quercetin, and kaempferol are known for their antioxidant and anti-inflammatory activities. It also have potential of cardioprotective effects (Salar *et al*., 2022; Bihani *et al*., 2021).

Pharmacological studies on *P. obtusa* have revealed several potential therapeutic effects, although further research is needed to fully understand its pharmacological actions. The plant extracts and isolated compounds from *P. obtusa* have shown anti-inflammatory effects, potentially mediated through the inhibition of inflammatory mediators and pathways. *P. obtusa* extracts have exhibited analgesic effects in animal models, suggesting its potential use for pain management. Several studies have reported the antimicrobial properties of *P. obtusa* against various bacteria and fungi. The plant extracts and isolated compounds have shown inhibitory effects against pathogenic microorganisms.The presence of phenolic compounds and flavonoids in *P. obtusa* contributes to its antioxidant properties, which help in reducing oxidative stress and protecting cells from oxidative damage. Some studies have suggested that *P. obtusa* extracts may promote wound healing by accelerating the formation of granulation tissue and enhancing collagen synthesis (Bihani *et al*., 2021). However, it is important to note that the effectiveness and safety of these traditional uses have not been extensively scientifically validated. Based on the present study, the study is associated to explore the hepatoprotective activity of *P. obtusa* against CCl_4 induced hepatotoxicity.

2. Materials and Methods

2.1 Chemical, reagents and software's

HPTLC system (CAMAG, Muttenz, Switzerland), TLC Silica gel 60 F254 (Merck KGaA, 64271 Darmstadt, Germany), Swiss ADME tool, Cytoscape (Version 3.8.2), Autodock Vina (Version 1.5.7), Network Analyst (https://www.networkanalyst.ca/) Metascape. The solvents used in the experimental process wasof analytical grade and purchased from SRL pvt. ltdand SD Fine-Chem Limited, Mumbai.

2.2 Collection and authentication of plant material

The plant matter was acquired from the garden area of Saini Enclave society in Delhi or through purchase. To ensure the reliability of the raw material, it was authenticated by Dr. Sunita Garg, an expert Botanist affiliated with CSIR NIScPR. The voucher with specimen number (NIScPR/RHMD/Consult/2022/4100-01) was submitted to the laboratory for future reference.

2.3 Preparation of extracts

The dried material (250 g) was powdered using a grinder and soaked with ethanol (2.5 l) for overnight. After the day, the extraction method

proceeded using the Soxhlet method for 10 h till complete extraction at 60°C. The prepared extract was filtered and concentrated on a water bath at 60°C temperature. The percentage yield of the obtained extracts was calculated and stored in a dried and air-tight container (Gaurav *et al*., 2020; Khan *et al*., 2021).

2.4 HPTLC quantitative analysis

HPTLC quantitative analysis was conducted for determination of major constituents, successfully. Briefly, a stock solution extract (30 mg/ml) and reference compounds (ferulic acid quercetin) in methanol was prepared. The solvent system in form of toluene, ethyl acetate and formic acid (6: 4: 1, v/v/v) was used for development of TLC. The TLC plate was developed up to the height 80 mm in a pre-saturated TLC development chamber. Thereafter, the developed TLC plate was air dried and visualized under 254 as well as 366 nm of UV light and scanned at 371 nm using an HPTLC scanned. The quantitative analysis was performed as the method validation analysis guidelines of International Conference Harmonization (ICH) (Gaurav *et al*., 2023b; Ibrahim *et al*., 2021).

2.5 *In vivo* hepatoprotective analysis

2.5.1 Experimental animals

The certain Wistar albino rats were used for conducting the successful *in vivo* experimental studies. These rats, weighing between 200-250 g, were accommodated in polypropylene cages and acclimated to the standard laboratory conditions, which included a 12:12 h light-dark cycle, a temperature of 23 ± 2°C, and a relative humidity of 55 ± 5% RH. During the experimentation phase, the rats were provided with a standard pellet diet and had unrestricted access to normal saline (*ad libitum*). The entire study followed the strict guidelines and principles set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Institutional Animal Ethical Committee (IAEC) in India to ensure ethical and responsible treatment of the animals (Eid *et al*., 2015).

2.5.2 Experimental group

Thirty-six rats were used for the study and were randomly divided into 6 groups, each containing 6 rats. Group 1, serving as the control group, received a daily oral dose of 0.5% carboxy methyl cellulose (CMC) solution (0.1 ml) for 7 consecutive days. Group 2 (CCl_4) was given a daily oral dose of 0.5% CMC solution (0.1 ml) for 7 days. Group 3, the standard control group, received 25 mg/kg of silymarin orally once daily for 7 days.

Groups 4 to 6 were administered different doses (high, medium, and low) of the drug (PAHE + CCl_4) orally for 7 days at 400, 200, and 100 mg/kg body weight, respectively. On the 7th day of the study, Groups 2 to 6 were also injected with a single dose of CCl_4(0.5 ml/kg i.p.), whereas, Group 1 received an equal volume of normal saline or olive oil.

On the 8th day, blood samples were collected from the rats after an overnight fast, using retro-orbital sinus puncture under mild ether anesthesia. Plasma was separated from the blood samples for further biochemical analysis. Subsequently, the rats were sacrificed by cervical dislocation under mild ether anesthesia, and their livers were excised and stored at -80 °C for subsequent estimations (Jain *et al*., 2012).

*Full form of PAHE: *P. obtusa* hydroalcoholic extract

2.5.3 Serum biochemical analysis

Biochemical analysis was performed on the serum obtained from the blood of rats in different experimental groups to measure AST, ALT, ALP, total bilirubin, and total protein levels. The analysis followed the standard protocol provided by the commercial kit manufacturer, Reckon Diagnostics, Baroda, India (Ekbbal *et al*., 2022; Sadeghi *et al*., 2006).

2.5.4 Hepatic antioxidants and lipid peroxidation

The livers of both the control and treated animals were excised, weighed, and then homogenized in chilled tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). After homogenization, the samples were centrifuged at 10,000 × g and 0°C for 20 min using a high-speed cooling centrifuge. The resulting supernatants were used for the assay of SOD, CAT, GSH, total protein, and LPO in the liver homogenates. The analysis protocol used for these assessments followed the standard procedure provided by the kit manufacturer (Gaurav *et al*., 2022; Nain *et al*., 2012).

2.5 Histopathological examination

The liver samples were first fixed in a 4% buffered paraformaldehyde solution. After fixation, they underwent dehydration using a graded alcohol series and were then embedded in paraffin wax. Subsequently, 5 µm thick sections were cut from the paraffin-embedded liver tissue. These sections were stained with hematoxylin and eosin to facilitate the examination of any gross structural changes. Under a microscope, the samples were carefully observed, and relevant images were captured using a digital camera set at 200X magnification (Gaurav *et al*., 2022).

2.6 Statistical analysis

The data was subjected to statistical analysis to determine its significance using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. The results were presented as Mean ± SD (n=3/6) using Graph Pad Prism version 5.0 for Windows.

3. Results

Extraction process of the plant material was done successfully using one of the most conventional methods that is Soxhlet extraction method and the extractive yield of the material was obtained 13.267 ± 0.342 % (w/w). After extraction, the HPTLC profiling of the sample was performed as per the standard protocol.

3.1 HPTLC quantitative analysis

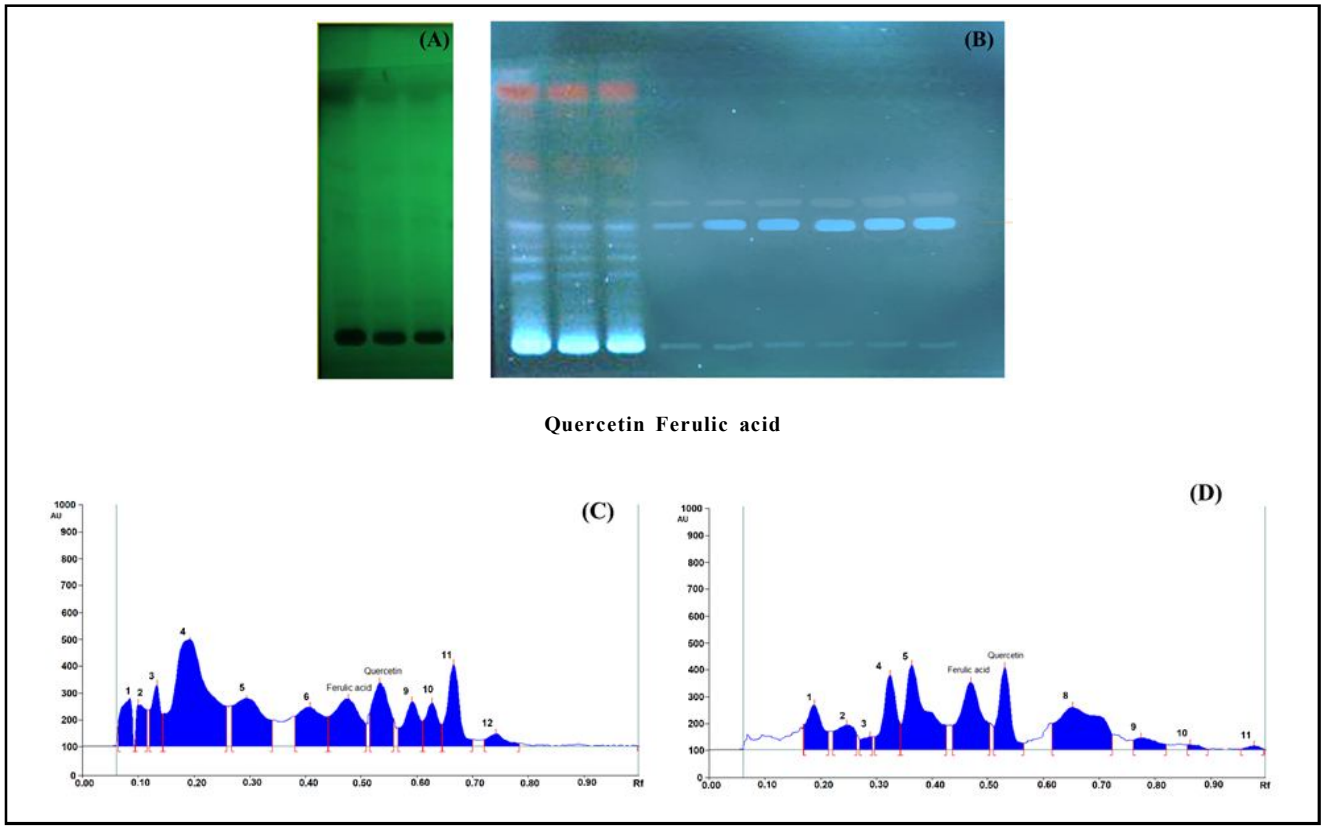
HPTLC analysis for quantification of ferulic acid and quercetin in *P. obtusa* was determined as per the reference protocol, successfully. The outcome of the study showed that the developed HPTLC method was found good linear, accurate and precise showed good specificity, as evident from the distinct separation of ferulic acid and quercetin from potential interfering substances, resulting in well-defined peaks.

Calibration curves for ferulic acid and quercetin exhibited excellent linearity over the tested concentration ranges, with correlation coefficients (r^2) exceeding 0.99 with the calibration curve for ferulic acid and quercetin found as y = 1.3978x + 17.082 and y = 1.2119x + 25.676, respectively. The limit of detection and the limit of quantification was found as 0.0105, 9.113 for ferulic acid while 6.774, 20.512 µg spot was found for quercetin.

The mean recovery of ferulic acid and quercetin, determined by spiking known amounts of standards into test samples, ranged from 102.59 to 102.77% for ferulic acid and 102.07-102.39% for quercetin that indicating high accuracy.The intra-day and inter-day precision of the method, expressed as the relative standard deviation (RSD), was found to be less than 2%, demonstrating excellent precision.The HPTLC method showed robustness with minimal impact on the results when slight changes were introduced in critical parameters, such as mobile phase composition and development time. The validation profiling of phytochemicals such as ferulic acid and quercetin using HPTLC method has been described in Table 1.

Table 1: Validation profiling of phytochemicals such as ferulic acid and quercetin using HPTLC method

Parameters	Biomarkers	
	Ferulic acid	**Quercetin**
Rf value	0.55	0.86
Scanning wavelength	289 nm	289 nm
Linearity range (ng/spot)	100 – 4000 ng/spot	100-4000 ng/spot
Regression equation	y = 1.3978x + 17.082	y = 1.2119x + 25.676
Regression coefficient ± SD	R^2 = 0.9934	R^2 = 0.9965
Slop ± SD	1.3978	1.2119
LOD ± SD (ng/spot)	0.0105 ± 0.0001	6.774 ± 0.038
LOQ ± SD (ng/spot)	9.113 ± 0.053	20.512 ± 0.284
Precision (%RSD range)		
Intraday	0.510 – 1.578	0.313 – 1.741
Interday	0.064 – 1.312	0.654 – 1.578
Accuracy (% drug recovered)	102.596 – 102.779	102.077 – 102.395
	Drug content (µg/mg, w/w)	
Content of drug	1.679 ± 0.026 µg/mg	1.345 ± 0.031 µg/mg



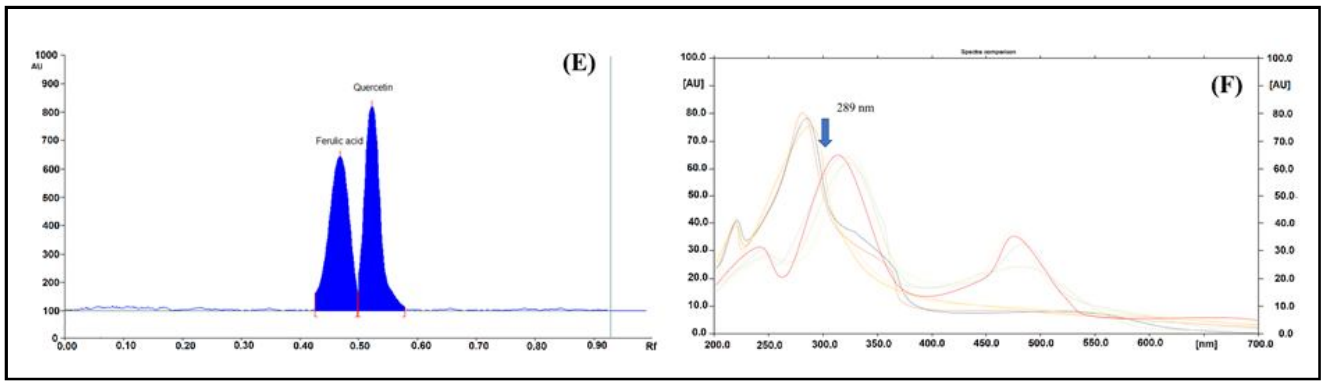


Figure 1: HPTLC quantitative analysis of the *P. obtusa* for simultaneous estimation of ferulic acid and quercetin. Figures (A and B) represents the TLC plate at the wave length of 254 and 366 nm. While the quantitative assessment was done to determine the quantity of ferulic acid and quercetin in the sample. Figures (C and D) represents the HPTLC chromatogram of sample at 254 and 366 nm while Figures (E and F) represents standards chromatogram and UV spectrum of the standard components.

3.2 *In vivo* hepatoprotective analysis

The objective of the *in vivo* hepatoprotective analysis in rats and biochemical estimation is to assess the potential hepatoprotective effects of a specific intervention or substance. The study aims to evaluate the impact of the intervention on liver function and oxidative stress markers in rats. The desired outcome of this study is to determine the hepatoprotective effects of the intervention by analyzing various biochemical markers related to liver function and oxidative stress. The outcome of the study showed that the levels of liver enzymes, such as ALT, AST, and ALP, which are indicators of liver damage and found ameliorated in the tested group against CCl_4-induced hepatotoxicity. In the high dose of the drug significantly ameliorated the effect of the toxicant *via* ameliorating the hepatoprotection and normalizing the cellular function of hepatocytes. A significant difference between the low dose and high dose found to be more significant than the dose of the drug used in high amount. Nevertheless, despite the test drug exhibiting higher activity, no significant difference was observed between the standard drug and the test drug. The results of this study will offer valuable insights into the intervention's potential to either protect the liver from injury or restore its function.

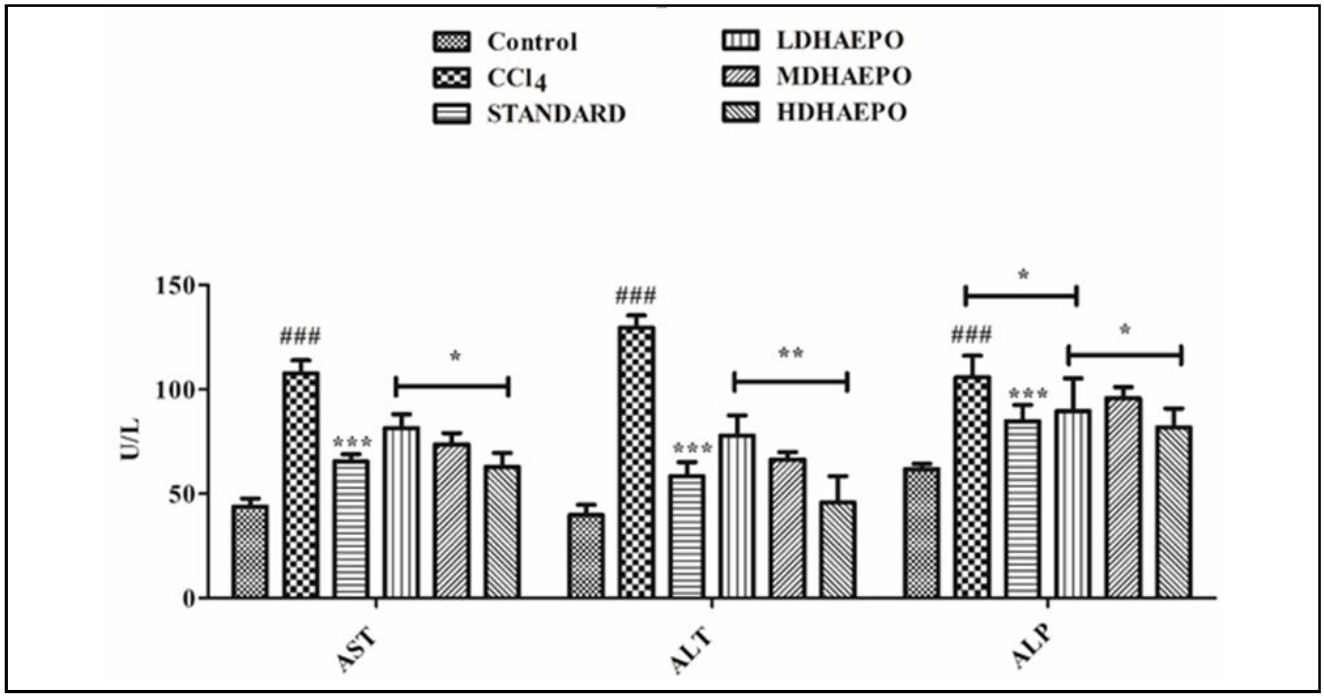


Figure 2: Estimation of hepatic biomarkers against CCl_4-induced liver toxicity and its amelioration by *P. obtusa*.

***LDHAEPO:** Low dose of hydroalcoholic extract of *P. obtusa*.

***MDHAEPO:** Medium dose of hydroalcoholic extract of *P. obtusa*.

***HDHAEPO:** High dose of hydroalcoholic extract of *P. obtusa*.

3.3 Hepatic antioxidants and lipid peroxidation

The study was analyzing the levels of various oxidative stress markers, including superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), total protein, and lipid peroxidation (LPO). These markers reflect the oxidative stress status in the liver and provide information about the intervention's impact on oxidative damage and antioxidant defense mechanisms.The study includes a control group that does not receive the intervention to provide a baseline for comparison. By comparing the biochemical markers between the intervention group and the control group, determines the intervention's efficacy in preventing liver damage and modulating oxidative stress. The appropriate statistical methods such as one-way ANOVA test, followed by Tukey test to compare each column significance were performed to analyze the data and determine the significance of the findings. The outcomes of this study contribute to the existing body of scientific knowledge regarding hepatoprotective interventions. The results supportthe research, developed therapeutic strategies, or provide insights for the development of new drugs or interventions aimed at protecting liver health.

In the control group, hepatic antioxidants such as superoxide dismutase SOD, CAT, GSH and LPO are present at normal levels. Lipid peroxidation refers to the oxidative degradation of lipids, which is typically maintained at a balanced level in the control group.

In the toxicant-treated group, the levels of hepatic antioxidants are altered. The activity of antioxidant enzymes like SOD and CAT may decrease, leading to reduced antioxidant capacity. Reduced glutathione (GSH) levels are also found to be depleted against CCl_4 induced hepatotoxicity. Consequently, lipid peroxidation (LPO) levels found to be increased that indicating an imbalance between oxidant and antioxidant defenses. Increased LPO signifies oxidative stress and damage to lipids in the liver.

In the drug-treated group, the impact on hepatic antioxidants and lipid peroxidation can vary depending on the specific drug and its mechanism of action. It enhances antioxidant enzyme activity and increase GSH levels, leading to reduced lipid peroxidation and oxidative stress. Conversely, certain it affects the hepatic antioxidant defense system and result in altered levels of antioxidants and increased lipid peroxidation.

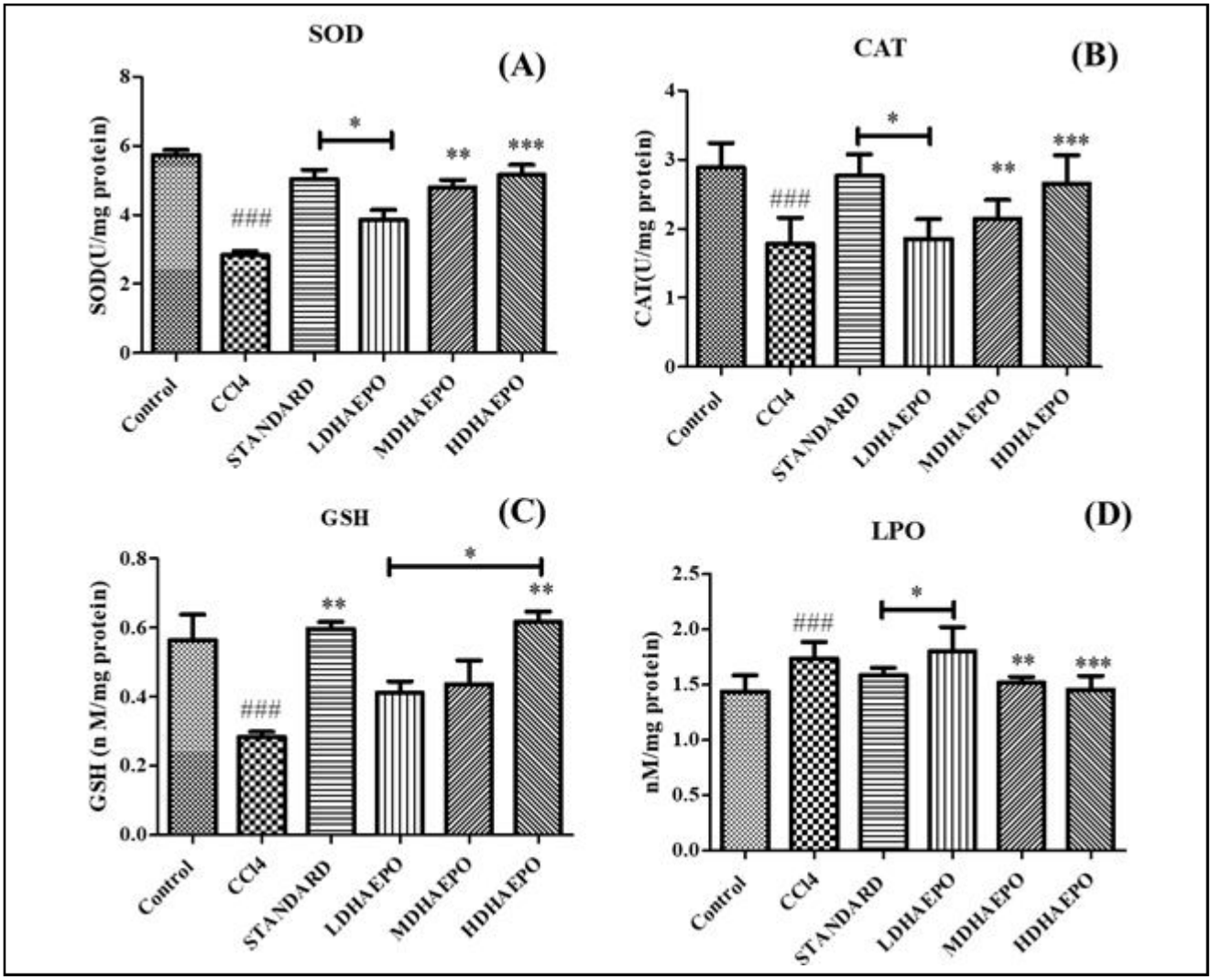


Figure 3: Antioxidant potential of *P. obtusa* against CCl_4-induced hepatotoxicity. Figures (A, B and C) showed SOD, CAT, and GSH activity of *P. obtuse* against CCl_4 induced hepatotoxicity while Figure (D) represents the LPO activity.

3.4 Histopathological examination

Histopathological examination of the normal control group reveals a well-preserved architecture with distinct lobular organization, a polygonal shape with centrally located nuclei, uniformly distributed within the hepatocytes, well-defined and separated by sinusoidal spaces, observed in the center of each lobule, no signs of inflammation, necrosis, fibrosis, or other pathological changes are evident. In the toxicant-treated group, such as the CCl_4 treated group, histopathological examination reveals characteristic changes associated with liver injury. Hepatocellular necrosis leading to the loss of hepatocytes in affected areas, cellular dropout or empty spaces within the liver tissue. Inflammatory infiltrates response triggered by the toxic insult. Ballooning degeneration refers to the swelling and distortion of hepatocytes due to the accumulation of lipids or other cellular components, deposition of extracellular matrix components, resulting in the formation of fibrous tissue within the liver and observed as an increased number of Kupffer cells in the histopathological examination. These are general histopathological findings associated with liver injury caused by toxicants. However, it is important to note that the specific histopathological changes can vary depending on the nature of the toxicant and the individual response to it. A comprehensive histopathological evaluation provides valuable information about the extent of liver damage, the progression of pathological changes, and potential therapeutic interventions.

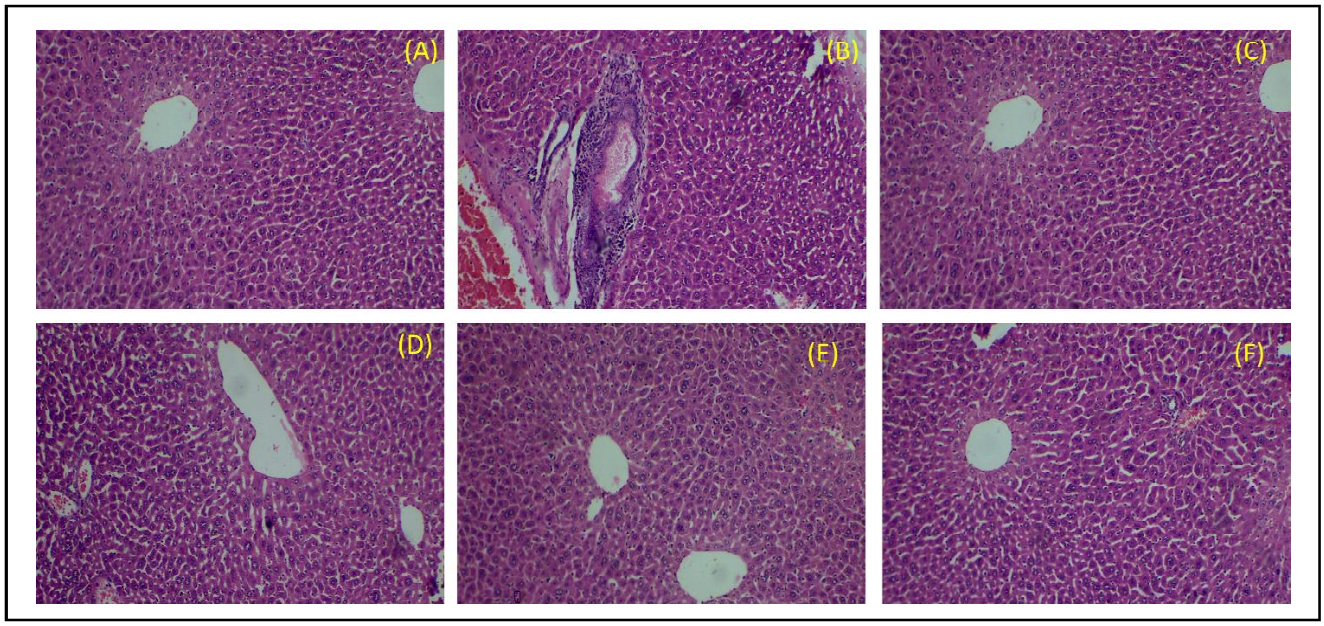


Figure 4: Histopathological analysis of the liver against CCl_4-induced hepatotoxicity. Figure (A) represents the control group and the normal architect of the liver cells. Figure (B) represents toxicant group that reveals characteristic changes associated with liver injury with the loss of hepatocytes in affected areas, cellular dropout or empty spaces within the liver tissue and inflammatory infiltrates. Figure (C) represents the standard control group that ameliorates the deleterious effect of CCl_4 induced hepatotoxicity while different dose of drug sample (Figure D: Low dose, E: Medium dose, F: High dose) significantly cure the hepatotoxicity. However, low dose of the drug sample exhibited comparatively less effect than its high dose.

4. Discussion

Medicinal plants have played a significant role in traditional medicine systems for centuries, including the treatment and management of liver diseases. Several medicinal plants possess hepatoprotective properties, which means they can help protect the liver from damage caused by toxins, medications, or various diseases (Basist *et al*., 2022). These plants often contain bioactive compounds like flavonoids, polyphenols, terpenoids, and antioxidants that exhibit hepatoprotective effects. Liver fibrosis, characterized by the excessive accumulation of scar tissue, is a common outcome of chronic liver diseases (Ahmad *et al*., 2021; Dhama *et al*., 2022; Meenakshi *et al*., 2018). Certain medicinal plants possess antifibrotic properties that can help inhibit or reduce the progression of fibrosis. Medicinal plants support liver detoxification processes and enhance liver cell regeneration, while medicinal plants can offer potential benefits in hepatoprotection and liver disease, their use should be guided by scientific evidence, proper dosage, and consultation with healthcare professionals. Additionally, the effectiveness of medicinal plants may vary depending on individual factors and the specific liver condition. Taking these facts into consideration, the present aim of the study is associated to explore the hepatoprotective effect against CCl_4 induced hepatotoxicity. In this study, HPTLC analysis was performed for qualitative and quantitative analysis. The outcome of the study showed that there are several major and minor constituents belong to several category such as phenols, flavonoids, terpene, *etc.,* that are responsible for the therapeutic action as hepatoprotective activity against CCl_4- induced hepatotoxicity. However, ferulic acid and quercetin were quantified in the extract and found 1.679 ± 0.026 µg/mg and 1.345 ± 0.031µg/mg, respectively.

Furthermore, hepatoprotective analysis was conducted in *W*istar albino rats against CCl_4- induced toxicity. Three different dosages (high, medium and low) were given to the rats and hepatoprotective effect was determined against CCl_4- induced toxicity. The outcomes of the study showed that high dose of the extract ameliorates the

hepatotoxicity significantly against toxicity induced by CCl_4 -Histopathological examination allows for the microscopic evaluation of liver tissue, providing insights into the structural changes and abnormalities associated with normal and toxicant-treated groups. Here is a comparison of the histopathological findings in the liver tissue of the normal control group and the toxicant-treated group:

In the liver tissue of the normal control group, histopathological examination reveals a well-preserved architecture with distinct lobular organization. The hepatocytes exhibit a polygonal shape with centrally located nuclei. The cytoplasm appears eosinophilic and is uniformly distributed within the hepatocytes. The hepatocyte cords are well-defined and separated by sinusoidal spaces, which contain red blood cells. The central vein can be observed in the center of each lobule. No significant signs of inflammation, necrosis, fibrosis, or other pathological changes are evident. The hepatic architecture appears intact and shows no abnormalities. In the toxicant-treated group, such as the CCl_4-treated group, histopathological examination reveals characteristic changes associated with liver injury. These changes may vary depending on the specific toxicant, dosage, and duration of exposure (Kavitha *et al*., 2011).

The toxicant may induce hepatocellular necrosis, leading to the loss of hepatocytes in affected areas. This can be observed as areas of cellular dropout or empty spaces within the liver tissue. Inflammatory infiltrates, primarily composed of immune cells like lymphocytes, may be present around necrotic areas or within the portal tracts (Takami *et al*., 2020). This indicates an inflammatory response triggered by the toxic insult. Ballooning degeneration refers to the swelling and distortion of hepatocytes due to the accumulation of lipids or other cellular components. This can be observed as enlarged hepatocytes with clear cytoplasm under microscopic examination. Prolonged toxicant exposure can lead to the deposition of extracellular matrix components, resulting in the formation of fibrous tissue within the liver (Zhang *et al*., 2017). Kupffer cells, which are specialized macrophages located within the sinusoidal spaces of the liver, may show increased activation or proliferation in response to the toxicant. This can be observed as an increased number of Kupffer cells in the histopathological examination.These are general histopathological findings associated with liver injury caused by toxicants. However, it is important to note that the specific histopathological changes can vary depending on the nature of the toxicant and the individual response to it. A comprehensive histopathological evaluation provided valuable information about the extent of liver damage, the progression of pathological changes, and potential therapeutic interventions and the ameliorative effect after administration of drug.

5. Conclusion

In conclusion, the present study demonstrated that the methanolic extract of *P. obtusa* possesses significant hepatoprotective effects against CCl_4-induced liver damage in rats. The HPTLC profiling of the extract revealed the presence of bioactive compounds, including flavonoids, terpenoids, and phenolic compounds, which likely contribute to its therapeutic properties. Treatment with *P. obtusa* extract resulted in the restoration of liver function markers and attenuated hepatic lesions, necrosis, and inflammation. These findings suggest that *P. obtusa* could be a valuable natural remedy for liver disorders. Further research is necessary to identify the specific active components responsible for the observed effects and to elucidate the underlying mechanisms of action. The study contributes to the understanding of *P. obtusa* potential as a hepatoprotective agent and encourages further exploration of its clinical applications.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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