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Original Research Article

Alleviation of Hepatotoxicant-Induced Liver Injury in Rats through the Hepatoprotective Effects of *Lophira lanceolata* Extract and Fractions

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ABSTRACT

Liver disease is a major global health problem. Exposure to hepatotoxic agents, such as carbon tetrachloride (CCl4), paracetamol (PCM), and doxorubicin (DOX), causes significant biochemical and structural liver damage. This study aimed to determine the hepatoprotective effects of Lophira lanceolata leaf extract and its fractions on liver damage induced by CCl4, PCM, and DOX. The methanol extract was prepared and fractionated into n-hexane (HF), ethyl acetate (EF), and methanol (MF) fractions. Liver function was evaluated using total protein (TP) and total bilirubin (T. Bil), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and albumin (ALB); histopathology was also performed. In rat models (n = 5/group) of CCl4-, PCM-, and DOX-induced liver injury, daily oral pretreatment with the methanol extract and fractions (100–400 mg/kg for 7 days) significantly normalised serum biomarkers compared to toxin controls, with effects comparable to silymarin (20 mg/kg). Across models, treatment reduced ALT by 55%, AST by 52%, ALP by 47%, and T. Bil by 50%, while increasing TP by 23% ($p < 0.05$ to $p < 0.001$). ALB and TP were dose-dependently enhanced, and HF and EF outperformed silymarin at 100 mg/kg at selected endpoints. Histopathology corroborated the biochemical findings, showing attenuation of degeneration, inflammation, and necrosis and evidence of hepatocyte regeneration, particularly at 400 mg/kg.These results indicate that *L. lanceolata* leaf extract and its fractions possess hepatoprotective activity against CCl4-, PCM-, and DOX-induced liver injury, supporting their potential for managing liver disorders.

Keywords: Hepatoprotective, *Lophira lanceolata*, Carbon tetrachloride, Paracetamol, Doxorubicin, Liver, Rats

Introduction

Liver diseases are a significant global health burden, with various hepatotoxic agents like carbon tetrachloride (CCl4), paracetamol (PCM), doxorubicin (DOX), and others induce deleterious effects on hepatic function.[1] Despite advancements in medical science, the development of effective hepatoprotective medicines remains a paramount objective. This underscores the significance of exploring botanicals for potential therapeutic remedies. Medicinal plants have attracted significant interest in this regard, emerging as a promising field of study.[2] Many plant species have demonstrated hepatoprotective properties, underscoring their potential to aid in the development of novel and safe therapeutic approaches for liver diseases.[3] One potential source under investigation is the leaf extract of L. lanceolata, a plant known for its rich phytochemical profile, which includes alkaloids, flavonoids, glycosides, steroids, saponins, terpenoids, and other bioactive compounds.[4] *L. lanceolata* has been reported to possess antioxidant,[5] antilipidemic and antidiabetic,[6] antimalarial.[7]

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This study focused on evaluating the hepatoprotective potential of L. lanceolata against liver injuries induced by CCl_4, PCM, and DOX. Hepatotoxicants pose a formidable challenge due to their prevalence and capacity to compromise liver function, leading to a cascade of biochemical and histopathological alterations. Current therapeutic approaches exhibit limitations, necessitating the exploration of alternative and complementary strategies to safeguard liver health.[8]
This research aims to evaluate the effect of the leaf extract of *L. lanceolata*, along with its fractions (n-hexane, ethyl acetate, and methanol), against hepatotoxicity induced by CCl_4, PCM, and DOX in rats. The objective of the study is to thoroughly assess liver function markers, including total protein (TP), total bilirubin (T.Bil), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and albumin (ALB) to understand the protective effect of the plant-based intervention. Although much work has been done using the crude extract of the plant specimen, this present study tends to determine the extent to which the solvent fractions of *L. lanceolata* can alleviate hepatotoxicity in liver damage caused by CCl_4, PCM and DOX. Histopathological examinations will show structural alterations within the liver tissue. The relevant research methods used in this present study include liver function test and histological screening. Liver biomarkers and histological studies are the most important parameters in determining the physiological state of the liver, this is because liver disorders affects the morphological and histological structure of the liver.[9] The results of this study are expected to provide significant insights into the hepatoprotective abilities of *L. lanceolata,* shedding light on its capacity to mitigate hepatotoxicant-induced liver injuries. By elucidating the biochemical and histopathological alterations associated with the protective effects of *L. lanceolata*, this research seeks to pave the way for the development of novel therapeutic strategies for liver disorders.

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Materials and Methods

Plant Material and Extract Preparation

Fresh leaves (10 kg) of *L. lanceolata* were collected from Emene Forest (Latitude 6°31'21.8N Longitude 7°35'07.8E), Enugu East LGA, Enugu state, and Emabu forest (Latitude: 7° 24' 8.96" N Longitude: 7° 37' 55.06" E.), Enjema ward, Ankpa LGA, Kogi state, Nigeria during March and April 2021. A voucher specimen (UNH/38a *Lophira lanceolata*, Ochnaceae) was deposited at the University Herbarium. These leaves were air-dried, ground into a fine powder **(2.00 kg)**, and soaked in 5 litres of methanol for 72 h at 25 ± 2 °C room temperature. The resulting methanol extract (ME) was then concentrated under reduced pressure and stored for further use. Subsequently, the ME (100 g) was fractionated using n-hexane, ethyl acetate, and methanol to obtain the respective fractions (HF, EF, and MF).

Experimental Animals

Adult Swiss albino rats (7–8 weeks old, weighing 100–150 g) were obtained and housed under standard conditions at a controlled temperature of 25 ± 2 °C. The rats were fed standard laboratory chow. All procedures were conducted in accordance with guidelines approved by the University of Nigeria's Animal Care and Use Research Ethics Committee (approval reference number FPSRA/UNN/21/0019). On the last day of the experiment, the animals were fasted overnight (12 hours) prior to blood sample collection.

Chemicals and Reagents

Pure doxorubicin, paracetamol, carbon tetrachloride, and tween 80 were procured from Alpha Pharmacy, Nigeria. Distilled water, Diagnostic kits for ALT, ALP, AST, ALB, TP, and T.Bil were sourced from Randox Laboratories, China. Analytical-grade solvents from Sigma, USA, were used.

Qualitative Phytochemical Analysis

Qualitative phytochemical screening of the methanol extract (ME) and its fractions (HF, EF, and MF) was conducted using the method outlined by Trease and Evans.[10]

Experimental Design

The doses used on previous study by.[11]

Carbon Tetrachloride (CCl_4)-induced Hepatotoxicity

Twenty-five (25) rats of both sexes were randomly divided into five groups, with five animals per group (n = 5). The treatment lasted for 7 days as follows:

- Group I received 3% Tween 80 (5 mL/kg, orally) daily for 7 days, followed by CCl_4 (1 mL/kg, orally) on the 8th day and served as the negative control.
- Groups II–IV received the methanol extract (ME) at doses of 100, 200, and 400 mg/kg (orally) for 7 days, followed by CCl_4 (1 mL/kg, orally) on the 8th day.
- Group V received silymarin (20 mg/kg/day, orally) for 7 days, followed by CCl_4 (1 mL/kg, orally) on the 8th day, serving as the positive control.All animals were fasted for 12 hours prior to CCl_4 administration to enhance acute liver injury induction.[12]

Paracetamol-induced Hepatotoxicity

Twenty-five rats of both sexes were randomly assigned into five groups, each containing five animals (n = 5). Treatments were administered for 7 consecutive days as follows: Paracetamol (PCM) was prepared at a dose of 2 g/kg, diluted with liquid paraffin in a 1:2 ratio.

- Group I received 3% Tween 80 (5 mL/kg, orally) daily for 7 days, followed by a single dose of PCM (2 g/kg, orally) on day 8, acting as the negative control.
- Groups II to IV were given the methanol extract (ME) orally at doses of 100, 200, and 400 mg/kg respectively for 7 days, then PCM (2 g/kg, orally) on the 8th day.
- Group V was treated with silymarin (20 mg/kg/day, orally) for 7 days, followed by PCM (2 g/kg, orally) on day 8, serving as the positive control.

All animals were fasted for 12 hours before PCM administration to promote acute liver injury.[13]

Doxorubicin (DOX)-induced Hepatotoxicity

A total of twenty-five rats of both sexes were assigned into five groups, with five animals in each group (n = 5). Treatments were carried out for 7 days as follows: Doxorubicin (DOX) was prepared at a dose of 10 mg/kg, diluted in 3% Tween 80 (w/v).

- Group I received 3% Tween 80 (5 mL/kg, orally) daily for 7 days, followed by a single dose of DOX (10 mg/kg, orally) on day 8, serving as the negative control.
- Groups II through IV were given the methanol extract (ME) orally at doses of 100, 200, and 400 mg/kg respectively for 7 days, followed by DOX (10 mg/kg, orally) on the 8th day.
- Group V was treated with silymarin (20 mg/kg/day, orally) for 7 days, followed by DOX (10 mg/kg, orally) on day 8, acting as the positive control.

All animals were fasted for 12 hours prior to DOX administration to promote acute liver injury.[13]

The Effect of HF, EF and MF of L. lanceolata on Carbon Tetrachloride (CCL_4)-induced Hepatotoxicity

A total of forty rats, both male and female, were randomly assigned to eight groups with five rats each (n = 5). The animals were treated for 7 days with either the HF, EF, and MF fractions or control treatments. On the eighth day, all groups received CCl_4 to induce liver injury.

- Group I served as the negative control, receiving 3% Tween 80 (5 mL/kg orally) for 7 days, followed by CCl_4 (1 mL/kg orally) on day 8.
- Group II acted as the positive control, treated with silymarin (20 mg/kg/day orally) for 7 days prior to CCl_4 (1 mL/kg orally) administration on day 8.
- Groups III and IV were given HF at 100 and 400 mg/kg orally for 7 days, then CCl_4 (1 mL/kg orally) on the eighth day.
- Groups V and VI received EF orally at doses of 100 and 400 mg/kg for 7 days, followed by CCl_4 (1 mL/kg orally) on day 8.
- Groups VII and VIII were administered MF orally at 100 and 400 mg/kg for 7 days, then given CCl_4 (1 mL/kg orally) on the eighth day.

All animals were fasted for 12 hours before CCl_4 treatment to enhance the induction of liver toxicity.

Effects of HF, EF and MF on Paracetamol (PCM)-induced Hepatotoxicity in L. lanceolata

Forty rats of both sexes were randomly divided into eight groups, with five rats per group (n = 5). After 7 days of treatment with the HF, EF, and MF fractions, paracetamol (PCM) was administered to induce hepatotoxicity, similar to the control groups.

- Group I received 3% Tween 80 (5 mL/kg, p.o.) daily for 7 days, followed by PCM (2 g/kg, p.o.) on the 8th day and served as the negative control.
- Group II received silymarin (20 mg/kg/day, p.o.) for 7 days, followed by PCM (2 g/kg, p.o.) on the 8th day, serving as the standard drug (positive control) group.
- Groups III and IV were treated orally with HF at doses of 100 mg/kg and 400 mg/kg, respectively, for 7 days, followed by PCM on the 8th day.
- Groups V and VI received EF at 100 mg/kg and 400 mg/kg (p.o.) for 7 days, followed by PCM on the 8th day.
- Groups VII and VIII were administered MF at 100 mg/kg and 400 mg/kg (p.o.) for 7 days, followed by PCM (2 g/kg, p.o.) on the 8th day.

All animals were fasted for 12 hours prior to PCM administration to enhance liver injury.

Blood Sample Collection and Biochemical Analysis

Blood samples were collected for ALT, AST, ALP, ALB, TP, and T-Bil analysis using kits from BioMed Diagnostics. Liver tissues were harvested and prepared for histopathological examination using standard procedures.

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Histological studies

A portion of the liver was cut into three pieces of approximately 6 mm^3 size and fixed in 10% neutral buffered formalin. After embedding in paraffin wax, thin sections of 5 µm thickness of liver tissue were cut and stained with haematoxylin-eosin. The thin sections of the liver were made into permanent slides and examined under high-resolution microscope with photographic facility and photomicrographs were taken. These sections were examined photomicrographically for cirrhosis, necrosis, steatosis, fatty changes, or apoptosis of hepatic cells.[14]

Statistical analysis

Data analysis was conducted using one-way ANOVA followed by Dunnett's multiple comparisons post hoc test in GraphPad Prism version 8.4. Results are presented as mean ± SEM, with statistical significance indicated by *p < 0.05, *p < 0.01, **p < 0.001, and ****p < 0.0001.

Figure 1: Leaf of *L. lanceolata*

Results and Discussion

Phytochemical analysis of the extracts and fractions

Phytochemical analysis of the methanol extract (ME) and its fractions (HF, EF, and MF) from L. lanceolata revealed the presence of diverse bioactive compounds. Positive reactions were observed for a number of phytochemicals, as shown in Table 1. Alkaloids, flavonoids, glycosides, steroids, saponins, terpenoids, carbohydrates, proteins, oils, reducing sugars, and acidic compounds were detected in both the extracts and fractions, which is indicative of the diverse chemical composition of *L. lanceolata*.

Table 1: Phytochemical Composition of *L. lanceolata* Extract and Fractions

Phytochemical	ME	HF	EF	MF
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Glycosides	+	+	+	+
Steroids	+	+	+	+
Saponins	+	+	+	+
Terpenoids	+	+	+	+
Carbohydrates	+	+	+	+
Proteins	+	+	+	+
Oils	+	+	+	+
Reducing Sugar	+	+	+	+
Acidic compounds	+	+	+	+

Key: + = Present
ME: Methanol Extract, HF: n-Hexane Fraction, EF: Ethylacetate fraction, MF: Methanol fraction

Phytochemicals are essential components of medicinal plants and play a crucial role in their diverse biological effects.[15] Plant derivatives have shown significant effectiveness and extensive advantages in treating and controlling illnesses. The existence of phytochemicals in plants hints at their potential medicinal uses. Phytochemical analysis of the methanol extract and its fractions from L. lanceolata revealed the presence of alkaloids, flavonoids, glycosides, steroids, saponins, terpenoids, carbohydrates, proteins, oils, acidic compounds, and reducing sugars in both the extract and fractions.[5] The presence of these phytochemicals in the extract and fractions suggests possible medicinal applications. Therefore, the hepatoprotective activity exhibited by the extract and fractions could be attributed to one or more of the various phytochemical constituents detected from the plant.

Effects of ME on Serum Liver Markers in Rats

CCl_4-induced liver injury

Relative to the negative control group, rats with CCl_4-induced liver injury exhibited significant elevations ($p < 0.05$) in serum levels of ALT, AST, ALP, and total bilirubin, alongside notable reductions ($p < 0.05$) in serum albumin and total protein levels. Treatment with varying doses of the methanol extract (ME) significantly lowered ($p < 0.05$, $p < 0.01$, $p < 0.001$) the increased serum concentrations of ALT, AST, ALP, and total bilirubin (see Fig. 2a, b, c, and f). Furthermore, ME administration significantly elevated ($p < 0.05$, $p < 0.01$, $p < 0.001$) albumin and total protein levels (Fig. 2d and e), with the highest dose (400 mg/kg) demonstrating effectiveness comparable to the reference drug silymarin (20 mg/kg).

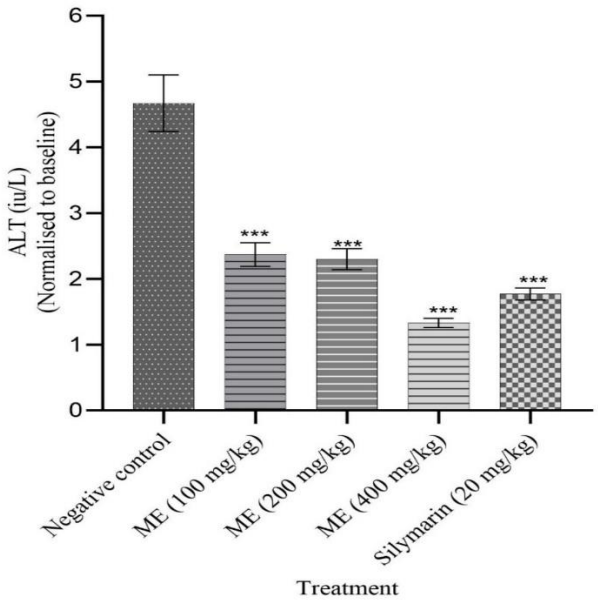

Figure 2a: The effect of ME on CCl4-induced elevation of ALT in rats
***p < 0.001 verses negative control; ALT values are normalized to the baseline enzyme levels for the group treated with ME alone. ME: methanol extract, CCL4 : Carbon tetrachloride, ALT: alanine aminotransferase

PCM-Induced Liver Injury

In the group treated with PCM, administration of the methanol extract (ME) led to a significant reduction in serum levels of ALT, ALP, AST, and total bilirubin ($p < 0.05$, $p < 0.01$, $p < 0.001$; Fig. 3a, b, c, and f). In contrast, ME significantly elevated the serum concentrations of albumin (ALB) and total protein (TP) ($p < 0.05$, $p < 0.01$, $p < 0.001$; Fig. 3d and e). The 400 mg/kg dose of ME demonstrated a therapeutic effect similar to that of the reference drug silymarin (20 mg/kg).

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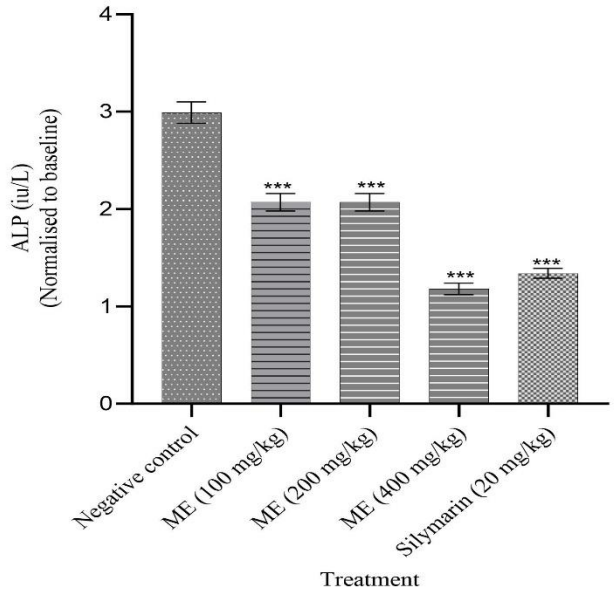

Figure 2b: The effect of ME on CCl4-induced elevation of ALP in rats
***p < 0.001 verses negative control; ALP values are normalized to the baseline enzyme levels for the group treated with ME alone. ME: Methanol extract, ALP: alkaline phosphatase

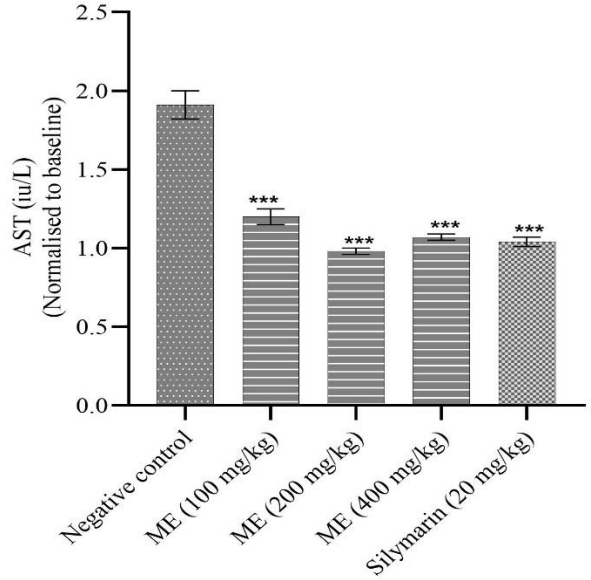

Figure 2c: The effect of ME on CCl4-induced elevation of AST in rats
***p < 0.001 verses negative control; AST values are normalized to the baseline enzyme levels for the group treated with ME alone. AST: aspartate aminotransferase, ME: Methanol extract, CCl4: Carbon tetrachloride

DOX-Induced Liver Injury
In DOX-induced liver injury models, treatment with ME at all tested doses significantly lowered the elevated serum concentrations of ALT, ALP, AST, and total bilirubin ($p < 0.05$, $p < 0.01$, $p < 0.001$; Fig. 4a, b, c, and f). In contrast, ME administration significantly restored the reduced serum levels of albumin and total protein ($p < 0.05$, $p < 0.01$, $p < 0.001$; Fig. 4d and e).

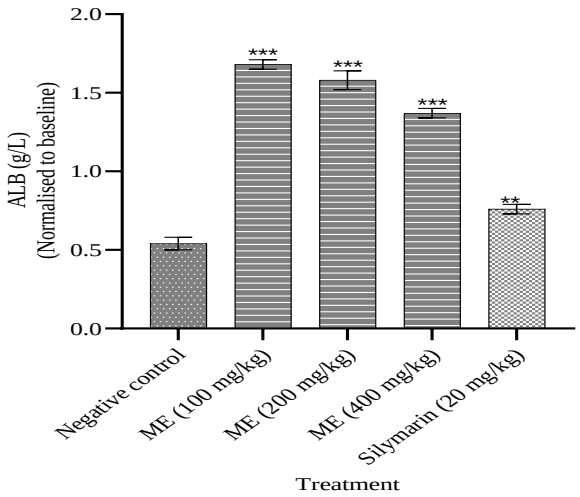

Figure 2d: The effect of ME on CCl4-induced elevation of ALB in rats
**p < 0.01, ***p < 0.001 verses negative control; ALB values are normalized to the baseline enzyme levels for the group treated with ME alone. ME: Methanol extract, ALB: albumin

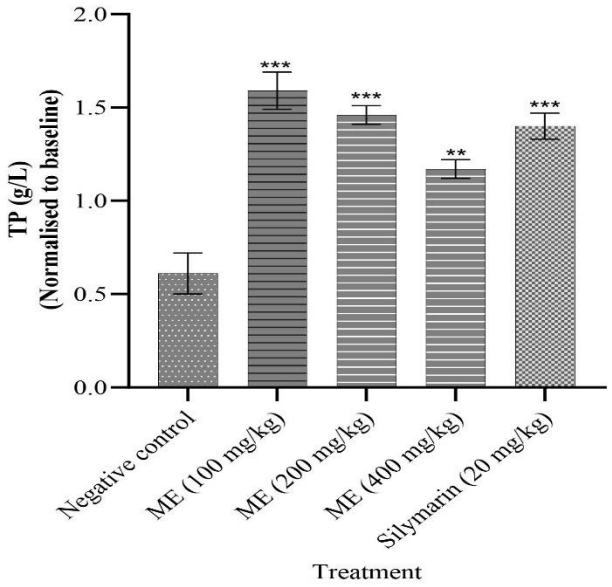

Figure 2e: The effect of ME on CCl4-induced elevation of TP in rats
**p < 0.01, ***p < 0.001 verses negative control; TP values are normalized to the baseline enzyme levels for the group treated with ME alone. TP: Total protein

Notably, the 400 mg/kg dose of ME exhibited a hepatoprotective effect comparable to that of the standard drug silymarin (20 mg/kg).

HF, EF, and MF in CCl_4-Induced Liver Injury
Administration of HF, EF, or MF significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.0001$) ameliorated the elevated serum levels of ALT, ALP, AST, and T. Bil while increasing the levels of ALB and TP (Fig. 5a–f). At the lowest dose (100 mg/kg), HF and EF demonstrated significant ($p < 0.001$) efficacy comparable to that of the standard drug silymarin (20 mg/kg).

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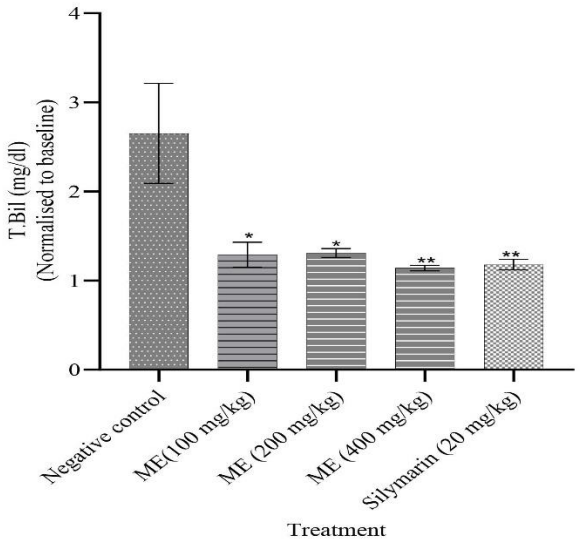

Figure 2f: The effect of ME on CCl4-induced elevation of T.Bil in rats
*p < 0.05, **p < 0.01 verses negative control; Bil values are normalized to the baseline enzyme levels for the group treated with ME alone.

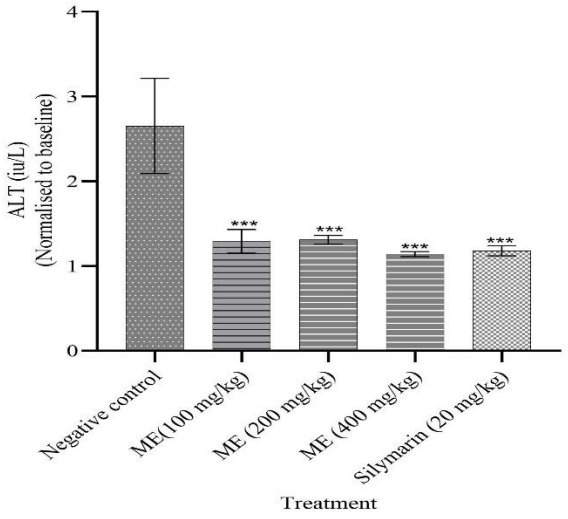

Figure 3a: The effect of ME on PCM-induced elevation of ALT in rats
***p < 0.001 verses negative control; ALT values are normalized to the baseline enzyme levels for the group treated with ME alone

HF, EF, and MF in PCM-Induced Liver Injury
HF, EF, and MF at different doses significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$) decreased the elevated serum levels of ALT, ALP, AST, and T. Bil in PCM-treated rats (Fig. 6a, b, c, and f) but markedly increased ($p < 0.05$, $p < 0.01$, $p < 0.001$) the serum ALB and TP levels (Fig. 6d and e). HF and EF at 100 mg/kg exhibited significantly greater efficacy than the standard drug silymarin (20 mg/kg).
The levels of ALT, ALP, AST, ALB, TP, and T. Bil were used as are markers of liver injury (necrosis) induced by selected hepatotoxicants: CCl_4, PCM, and DOX. These hepatotoxic agents are known to generate free radicals, which impact the cellular permeability of hepatocytes, resulting in elevated levels of serum biochemical parameters such as ALT, ALP, AST, and T. Bil.[15]

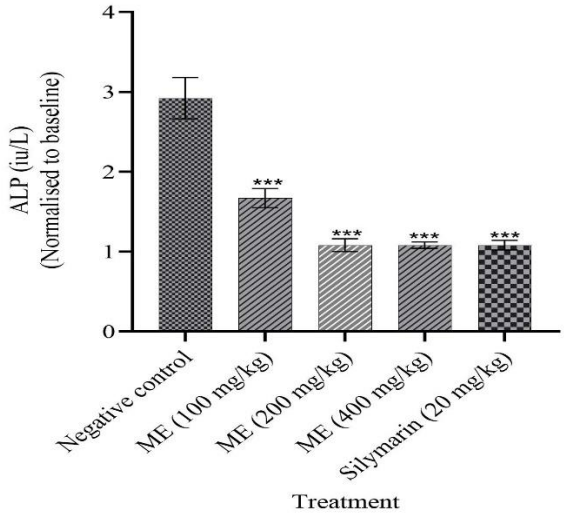

Figure 3b: The effect of ME on PCM-induced elevation of ALP in rat
***p < 0.001 verses negative control; ALP values are normalized to the baseline enzyme levels for the group treated with ME alone

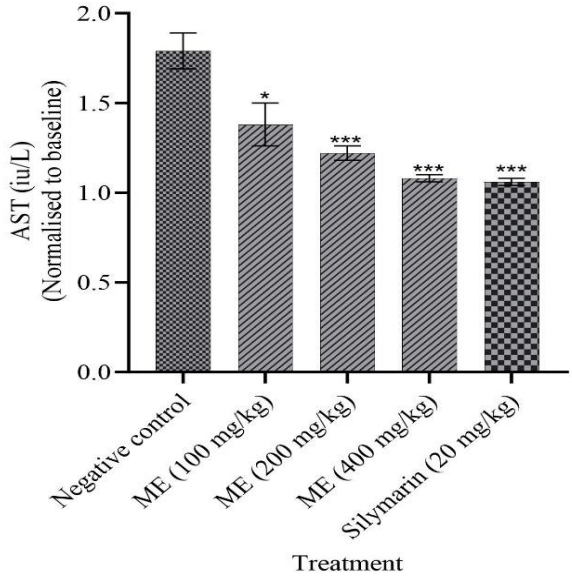

Figure 3c: The effect of ME on PCM-induced elevation of AST in rats
*p < 0.05, ***p <0.001 verses negative control; AST values are normalized to the baseline enzyme levels for the group treated with ME alone.

Methanol extract and its fractions effectively mitigated liver damage caused by CCl_4, PCM, and DOX in rats, indicating strong hepatoprotective potential. Results revealed that daily oral dosing with ME, HF, EF, and MF significantly lowered the elevated serum levels of ALT, ALP, AST, and total bilirubin. Notably, treatment with the extract and its fractions at a dose of 400 mg/kg produced greater reductions in these liver markers compared to the standard drug, silymarin, although both exhibited similar levels of statistical significance. The observed reduction in this study suggested the protective effect of *L. lanceolata* on the structural integrity of hepatocyte cells against damage induced by CCl_4, PCM, and DOX.

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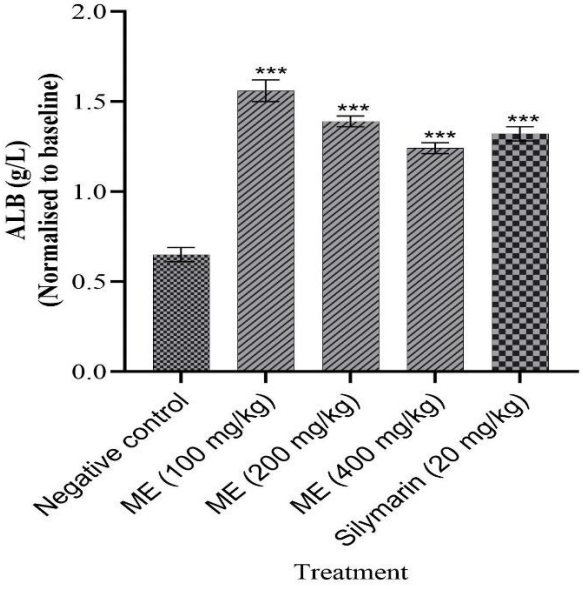

Figure 3d: The effect of ME on PCM-induced elevation of ALB in rats
***p < 0.001 verses negative control; ALB values are normalized to the baseline enzyme levels for the group treated with ME alone.

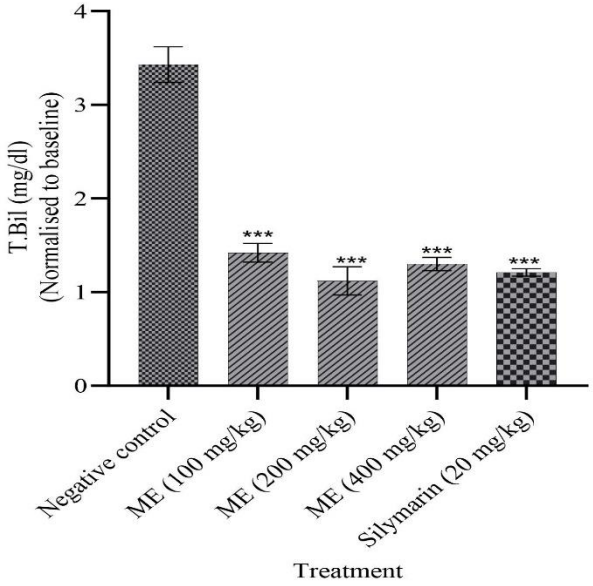

Figure 3f: The effect of ME on PCM-induced elevation of T.Bil in rats
***P < 0.001 verses negative control; T.Bil values are normalized to the baseline enzyme levels for the group treated with ME alone.

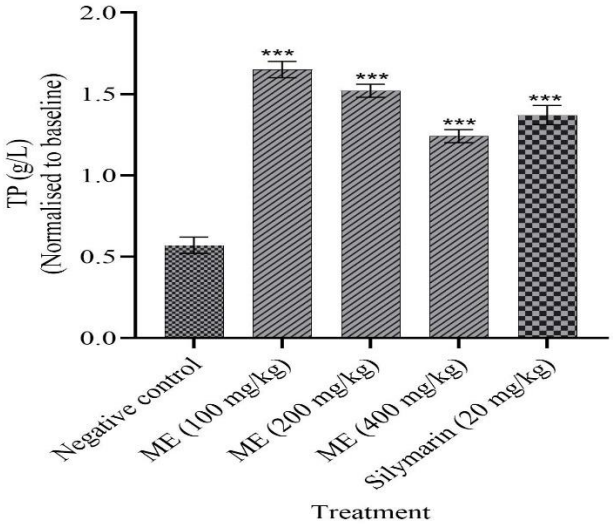

Figure 3e: The effect of ME on PCM-induced elevation of TP in rat
***p < 0.001 verses negative control; TP values are normalized to the baseline enzyme levels for the group treated with ME alone.

This is supported by the fact that ALT, ALP, and AST serve as reliable indicators of liver injury[16] and the reduction in elevated bilirubin levels and the suppression of increased ALP activity suggest the stabilization of biliary dysfunction in the liver of rats during hepatic injury induced by CCl_4.[17] The ME reduced the serum levels of T. Bil, although not as significantly as ALT, ALP, or AST. The effect of silymarin, the standard drug, was more pronounced than that of 100 mg/kg ME.

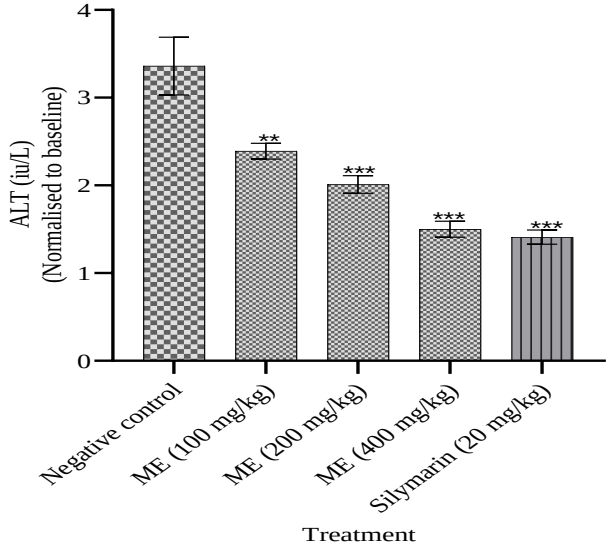

Figure 4a: The effect of ME on DOX-induced elevation of ALT in rats
**p < 0.01, ***p < 0.001 verses negative control; ALT values are normalized to the baseline enzyme levels for the group treated with ME alone.

Overall, the gradual decrease in the serum liver enzyme levels suggested that the plant extracts and fractions effectively scavenged free radicals generated by CCl_4, PCM, and DOX toxicity after seven days of pretreatment, indicating early improvement in hepatic cell membrane integrity.
The reduction in serum ALT, ALP, AST, and T. Bil levels induced by HF and EF at a lower dose (100 mg/kg) exceeded that achieved by silymarin at 20 mg/kg. Liver diseases are known to elevate conjugated and unconjugated bilirubin concentrations, and the extracts and fractions decreased T. Bil concentrations relative to those in the control groups.

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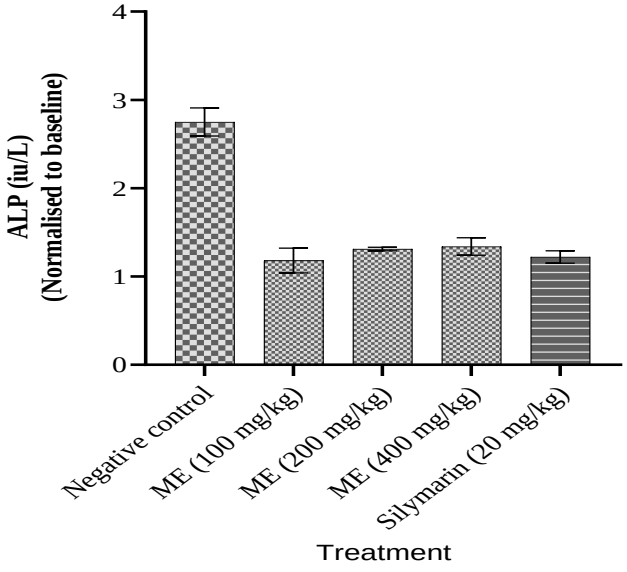

Figure 4b: The effect of ME on DOX-induced elevation of ALP in rats
***p < 0.001 verses negative control; ALP values are normalized to the baseline enzyme levels for the group treated with ME alone.

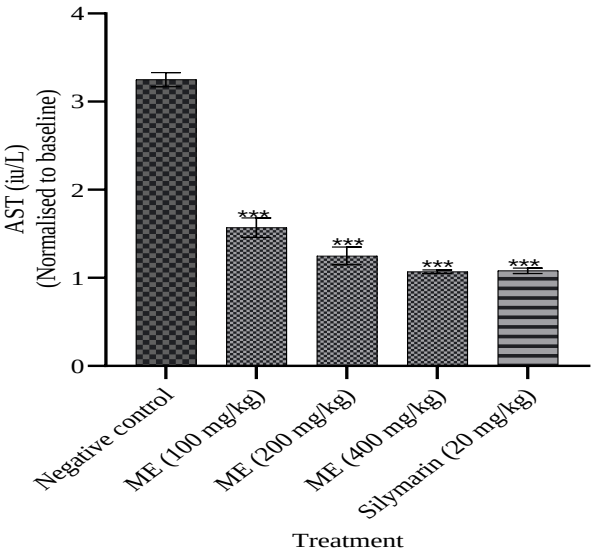

Figure 4c: The effect of ME on DOX-induced elevation of AST in rats
***p < 0.001 verses negative control; AST values are normalized to the baseline enzyme levels for the group treated with ME alone.

A reduction in ALP activity corresponds with a decrease in T. Bil levels, indicating the stabilization of biliary dysfunction in the liver of rats by *L. lanceolata* during hepatic injury. In liver toxicity, impairment of total protein (TP), albumin (ALB), and globulin synthesis leads to decreased serum concentrations of these proteins.[18] The effectiveness of any hepatoprotective medication relies on its ability to either mitigate the adverse effects or restore the disrupted normal physiological function caused by hepatotoxic substances.[19,20]

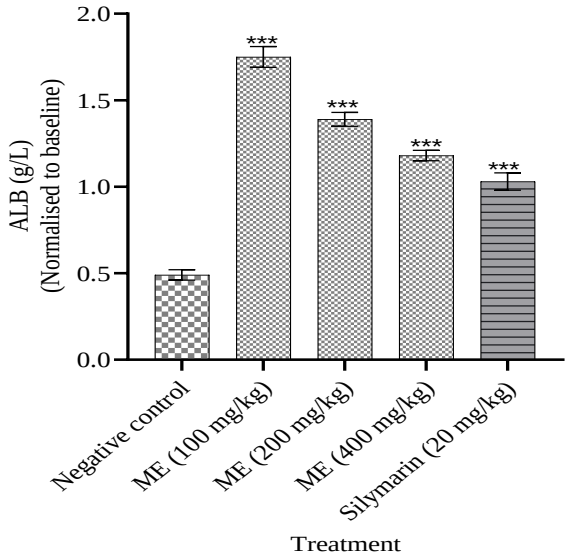

Figure 4d: The effect of ME on DOX-induced elevation of ALB in rats
***p < 0.001 verses negative control; ALB values are normalized to the baseline enzyme levels for the group treated with ME alone.

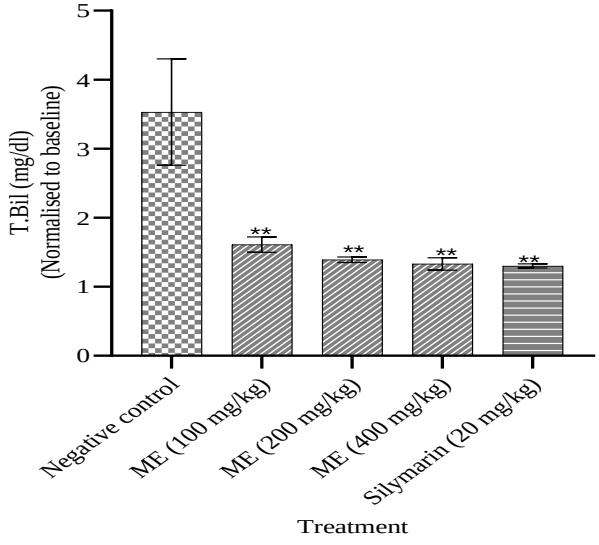

Figure 4f: The effect of ME on DOX-induced elevation of T.Bil in rats
**p < 0.01 verses negative control; T.Bil values are normalized to the baseline enzyme levels for the group treated with ME alone.

The mechanisms of hepatotoxic agents such as CCl_4, PCM, and DOX include cytotoxic injury, steatosis (fatty liver), cholestasis, mixed cytotoxicity/hepatitis, cirrhosis, subacute necrosis, liver tumour phospholipidosis, and nonspecific changes. Carbon tetrachloride is known to generate free radicals, which disrupt the cellular permeability of hepatocytes, resulting in increased levels of serum biochemical parameters such as ALT, ALP, AST, and T. Bil.[21] One of the liver's primary functions is the synthesis and metabolism of serum proteins. Hepatotoxicity can impair this function, leading to a reduced capacity for protein synthesis.[22]

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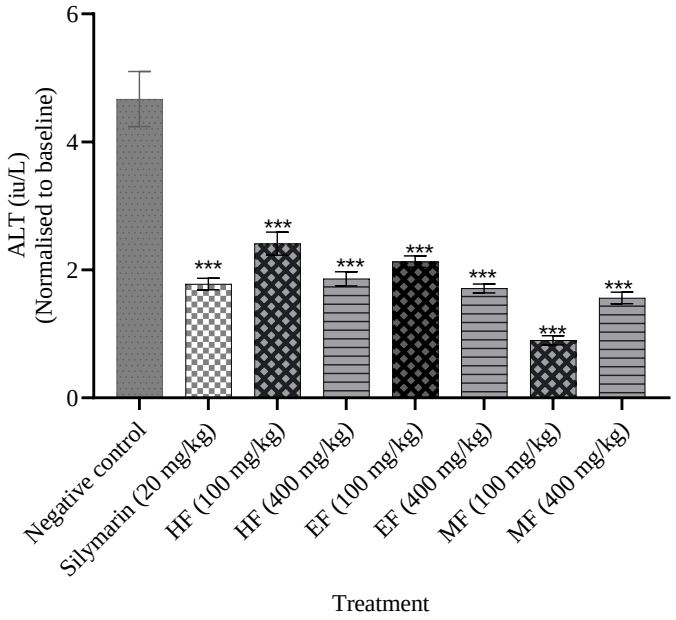

Figure 5a: The effect of HF, EF and MF on CCl4-induced elevation of ALT in rats
***p < 0.001 verses negative control; ALT values are normalized to the baseline enzyme levels for the group treated with fraction alone.

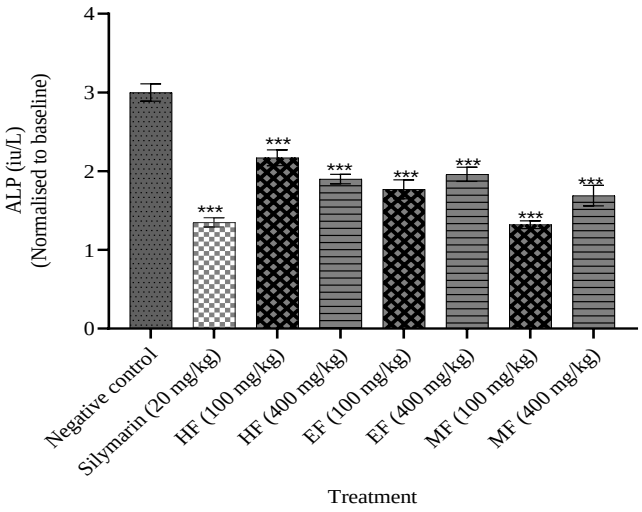

Figure 5b: The effect of HF, EF and MF on CCl4-induced elevation of ALP in rats
***p < 0.001 verses negative control; ALP values are normalized to the baseline enzyme levels for the group treated with fraction alone.

The extract resulted in an increase in serum TP and ALB concentrations compared to both the negative and positive control groups. Notably, the increase in ALB levels was more pronounced in the 100 mg/kg group than in the positive control group. These findings suggest that the extract effectively reverses liver damage induced by CCl_4, PCM, and DOX at various doses and supports its potential as a marker of improved liver function. PCM is a widely used analgesic and antipyretic agent. At hepatotoxic doses, it elevates hepatic markers such as ALT, AST, ALP, and T. Bil, while reducing TP and ALB levels.

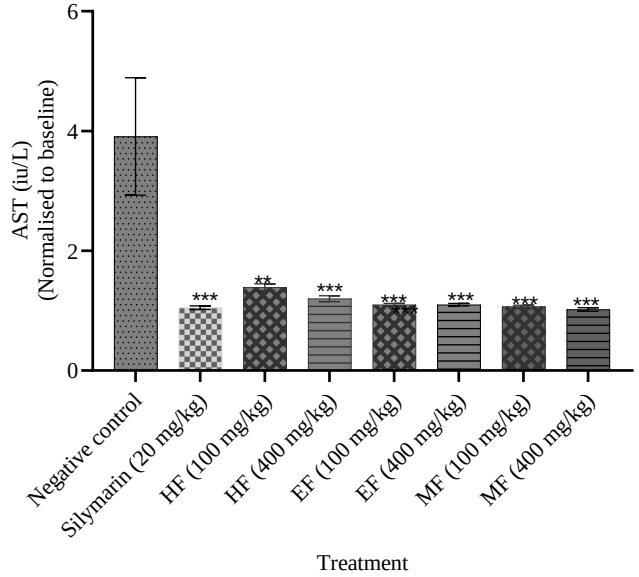

Figure 5c: The effect of HF, EF and MF on CCl4-induced elevation of AST in rats
**p < 0.01, ***p <0.001 verses negative control; AST values are normalized to the baseline enzyme levels for the group treated with fraction alone.

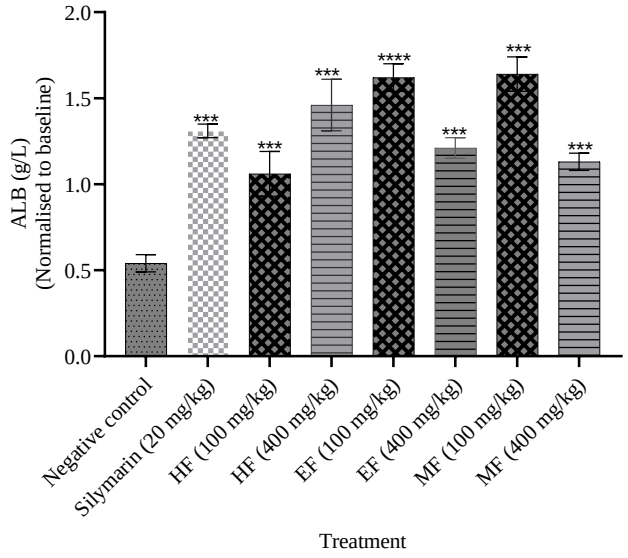

Figure 5d: The effect of HF, EF and MF on CCl4-induced elevation of ALB in rats
***p < 0.001, * ***p < 0.0001 verses negative control; ALB values are normalized to the baseline enzyme levels for the group treated with fraction alone.

The liver is highly sensitive to toxic agents, making it a key organ for evaluating toxicity in biological systems. Assessing the activities of enzymes like ALT, AST, ALP, and levels of ALB, TP, and T. Bil is crucial for diagnosing and monitoring both clinical and experimental liver damage.[23-25] In this study, animals treated with CCl_4, PCM, and DOX exhibited significant hepatic damage, as evidenced by elevated levels of serum markers. However, pretreatment with ME, HF, EF, or MF significantly mitigated the elevated levels of these serum markers.

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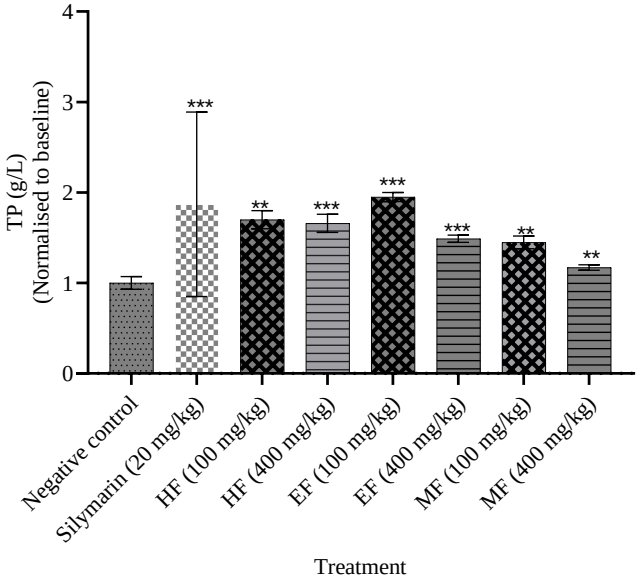

Figure 5e: The effect of HF, EF and MF on CCl4-induced elevation of TP in rats
**p < 0.01, ***p < 0.001 verses negative control; TP values are normalized to the baseline enzyme levels for the group treated with fraction alone

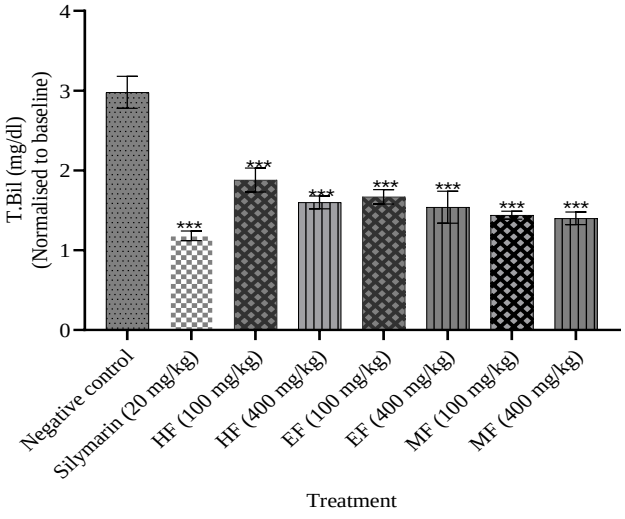

Figure 5f: The effect of HF, EF and MF on CCl4-induced elevation of T.Bil in rats
***p < 0.001 verses negative control; T.Bil values are normalized to the baseline enzyme levels for the group treated with fraction alone.

The normalization of serum markers by ME, HF, EF, and MF suggested protection of hepatocytes, thereby safeguarding cellular integrity against CCl_4-, PCM-, and DOX-induced leakage of marker enzymes into circulation. This protection may be attributed to increased synthesis in increasing biliary pressure. PCM induces liver injury through the action of toxic metabolites, such as N-acetyl-p-benzoquinone imine (NAPQI), produced by cytochrome P450.[26]

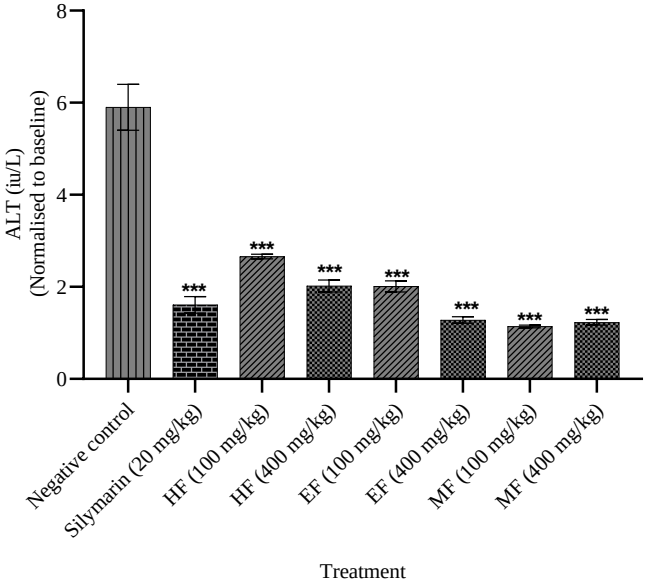

Figure 6a: The effect of HF, EF and MF on PCM-induced elevation of ALT in rat
***p < 0.001 verses negative control; ALT values are normalized to the baseline enzyme levels for the group treated with fraction alone.

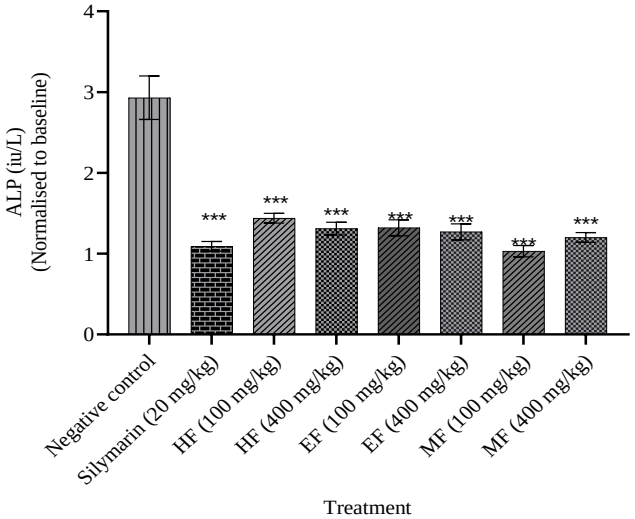

Figure 6b: The effect of HF, EF and MF on PCM-induced elevation of ALP in rats
***p < 0.001 verses negative control; ALP values are normalized to the baseline enzyme levels for the group treated with fraction alone.

Histological Study
Histological observations of the ME of the control animals (negative group) subjected to CCl_4-induced liver injury revealed massive hepatocellular degeneration and necrosis with mild infiltration of mononuclear inflammatory cells in the periportal area (PA). In animals treated with ME (100, 200 and 400 mg/kg), liver sections showed mild random hepatocellular necrosis (asterisks) and mild infiltration of mononuclear cells around the central vein (CV) in the 100 mg/kg group; the 200 mg/kg group showed mild hepatocellular degeneration and necrosis and areas of hepatocyte regeneration (R), while the 400 mg/kg group showed normal hepatocytes and proliferation of bile ducts (BD)

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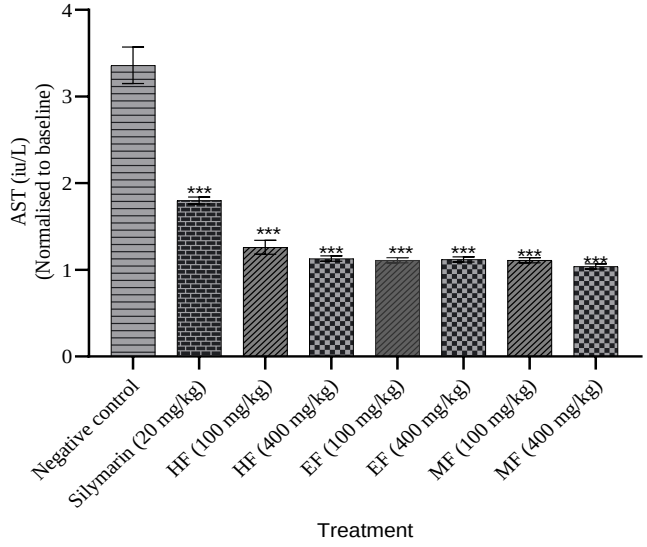

Figure 6c: The effect of HF, EF and MF on PCM-induced elevation of ALP in rats
***p < 0.001 verses negative control; ALP values are normalized to the baseline enzyme levels for the group treated with fraction alone.

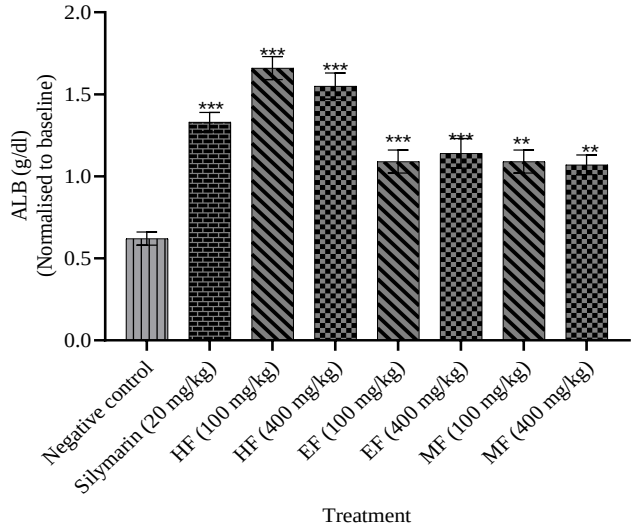

Figure 6d: The effect of HF, EF and MF on PCM-induced elevation of ALB in rats
**p < 0.01, ***p < 0.001 verses negative control; ALB values are normalized to the baseline enzyme levels for the group treated with fraction alone.

with moderate infiltration of mononuclear inflammatory cells in the periportal area (arrow) (Figure 7).
Figure 8 shows a photomicrograph of liver sections from PCM-treated rats. Group I showed normal cords of hepatocytes around the portal area. However, there is peribiliary fibrosis and proliferation of bile ducts (arrows) and a congested portal vein (PV) with perivascular oedemas (asterisk). HA-hepatic artery. Group II showed moderate coagulative necrosis of periportal hepatocytes (asterisks), congestions of the portal vein (PV) and sinusoids (s) around the portal area and proliferation of bile ducts with peribiliary fibrosis (arrows) with hemosiderin pigments deposited in the periportal area (arrowheads). HA= hepatic artery. Group III exhibited normal hepatocyte cords and proliferation of bile ducts (BD) with moderate infiltration of

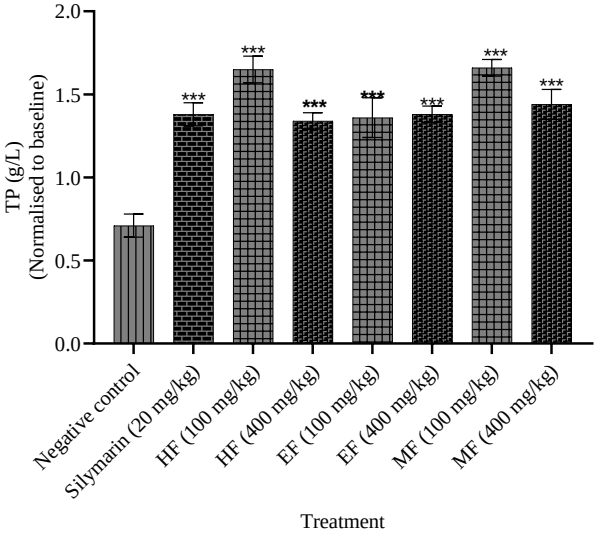

Figure 6e: The effect of HF, EF and MF on PCM-induced elevation of TP in rats
***p < 0.001 verses negative control; TP values are normalized to the baseline enzyme levels for the group treated with fraction alone

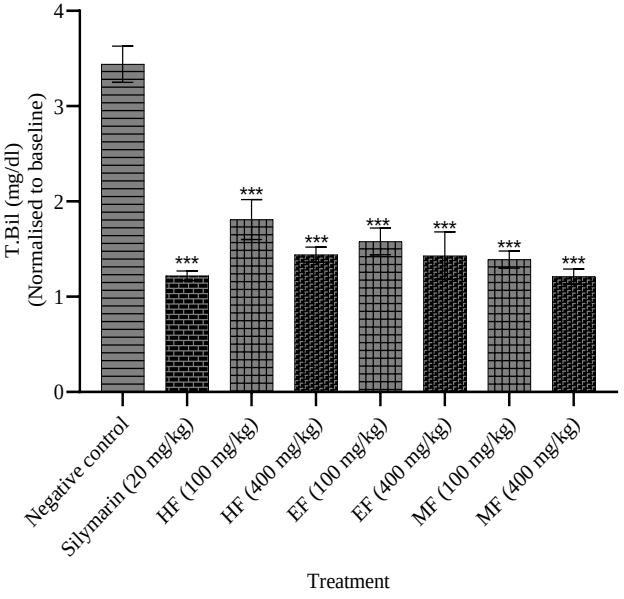

Figure 6f: The effect of HF, EF and MF on PCM-induced elevation of total T.Bil in rats.
***p < 0.001 verses negative control; T.Bil values are normalized to the baseline enzyme levels for the group treated with fraction alone

mononuclear inflammatory cells (arrows). The portal vein (PV) and hepatic artery (HA) were observed. In Group IV, hepatocyte cords were evident. Attention was given to the central veins (CV) and portal area (PA). In Group V, liver sections displayed typical cords of hepatocytes around the central vein (CV) and portal area (PA).
Figure 9 shows a photomicrograph of liver sections from PCM-treated rats; group I exhibited severe periportal hepatocellular degeneration and necrosis (asterisks) and mild infiltration of mononuclear inflammatory cells around the bile duct (BD).

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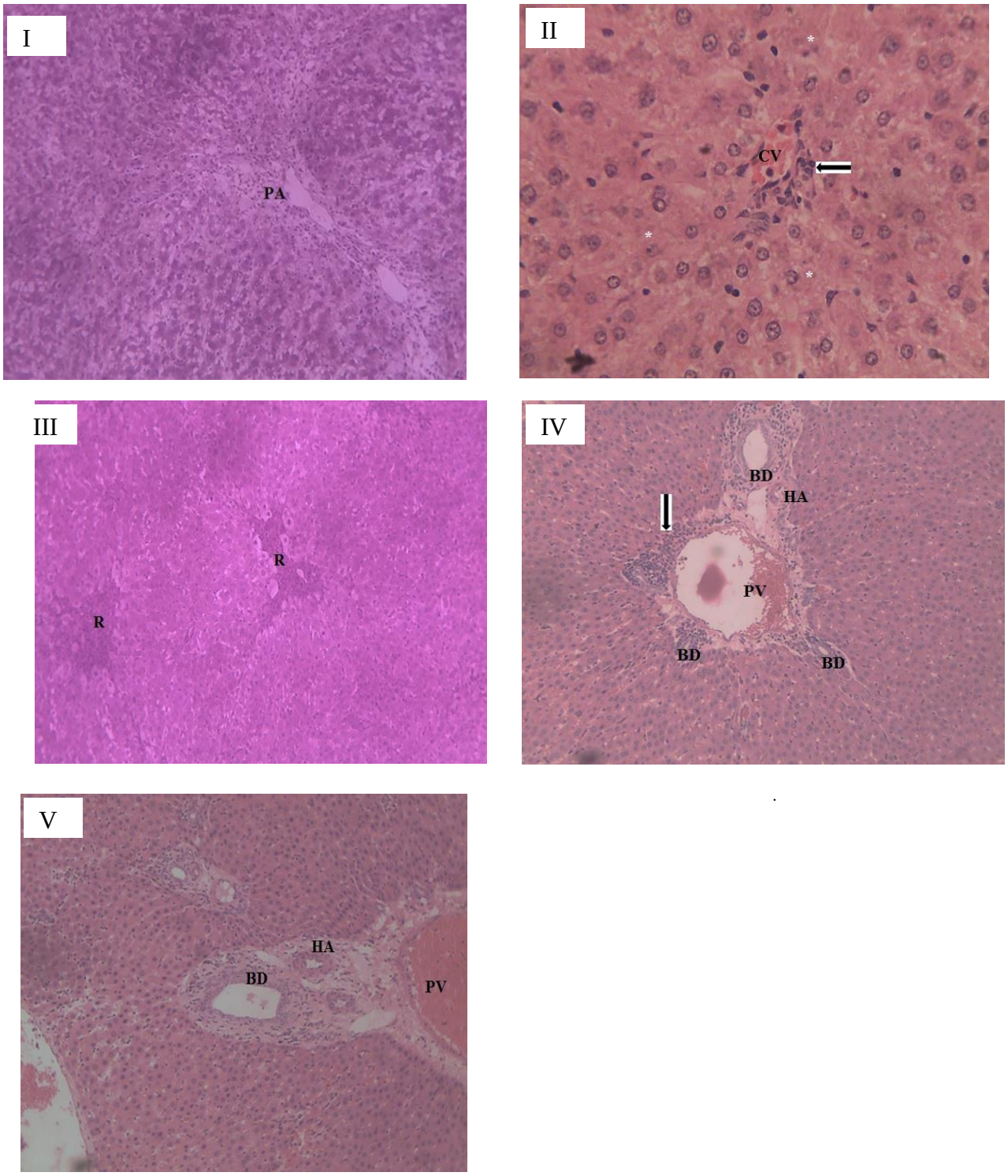

Figure 7: Photomicrograph of the sections of the liver of CCl_4 treated rats
I represent negative control [Tween 80 (5 ml/kg) + CCl_4 (1 ml/kg)] p.o, II, III and IV represent extract at 100, 200 and 400 mg/kg + CCl_4 (1 ml/kg) p.o, V represents positive control [Silymarin (20 mg/kg) + CCl_4 (1 ml/kg)] p.o respectively. H & E. Magnification x 100 (II, III, IV and V) x 400 (I).

Group II exhibited a congested central vein (CV), mild dilatation of the sinusoids (S) and Kupffer cell hyperplasia (arrows). Group III showed normal hepatocytes around the periportal area, with a congested portal vein (PV), bile duct (BD) and hepatic artery (HA). Group IV exhibited normal hepatocytes in the centrilobular, mid-zonal, and periportal regions, with slight infiltration of mononuclear cells around the central vein (CV) and in the periportal area (arrow). Group V displayed typical hepatocytes and mild infiltration of mononuclear inflammatory cells in the portal area (arrow).

Figure 10a presents photomicrographs of liver sections from CCl_4-treated rats administered various doses of the fractions. Group I exhibited extensive periportal hepatocellular degeneration and necrosis, with slight infiltration of mononuclear inflammatory cells in the periportal area (PA).

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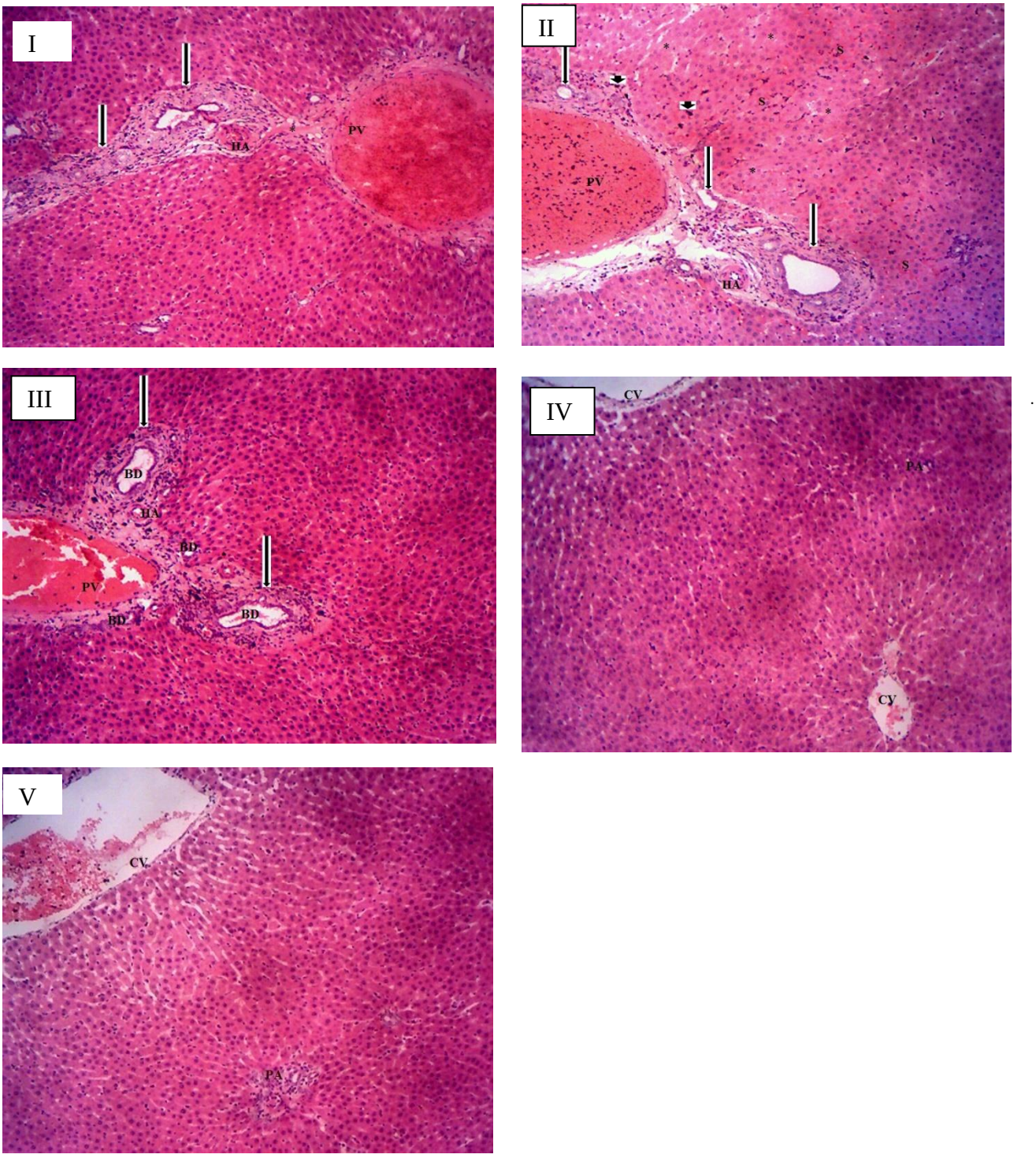

Figure 8: Photomicrograph of the sections of the liver of PCM treated rats
I represent negative control [Tween 80 (5 ml/kg) + PCM (2000 mg/kg)] p.o, II, III and IV represent extract at 100, 200 and 400 mg/kg + PCM (2000 mg/kg) p.o, V represents positive control [Silymarin (20 mg/kg) +PCM (2000 mg/kg)] p.o respectively. H & Magnification X 100 (II, III, IV and V) x 400 (I).

Group II exhibited normal hepatocytes in the periportal area, with mild fibrosis of the bile duct (BD), hepatic artery (HA), and portal vein (PV). Group III exhibited moderate centrilobular degeneration and necrosis of hepatocytes with slight infiltration of mononuclear cells. CV = Central vein. Group IV showed normal cords of hepatocytes around the central vein (CV) and mild infiltration of mononuclear cells in the centrilobular area (arrow).

Figure 10b shows photomicrographs of liver sections from CCl_4-treated rats administered different doses of the fractions. Group V exhibited massive hepatocellular necrosis and hepatocyte regeneration in the periportal area (arrows) via the portal vein (PV). Group VI included normal hepatocytes in the periportal area with fragmentation of the hepatic cords. PV = portal vein, HA = hepatic artery and BD = bile duct. Group VII showed massive hepatocellular degeneration and necrosis, with hepatic regeneration in the periportal area (arrow); CV = central vein. Group VIII showed normal cords of hepatocytes around the central vein (CV).

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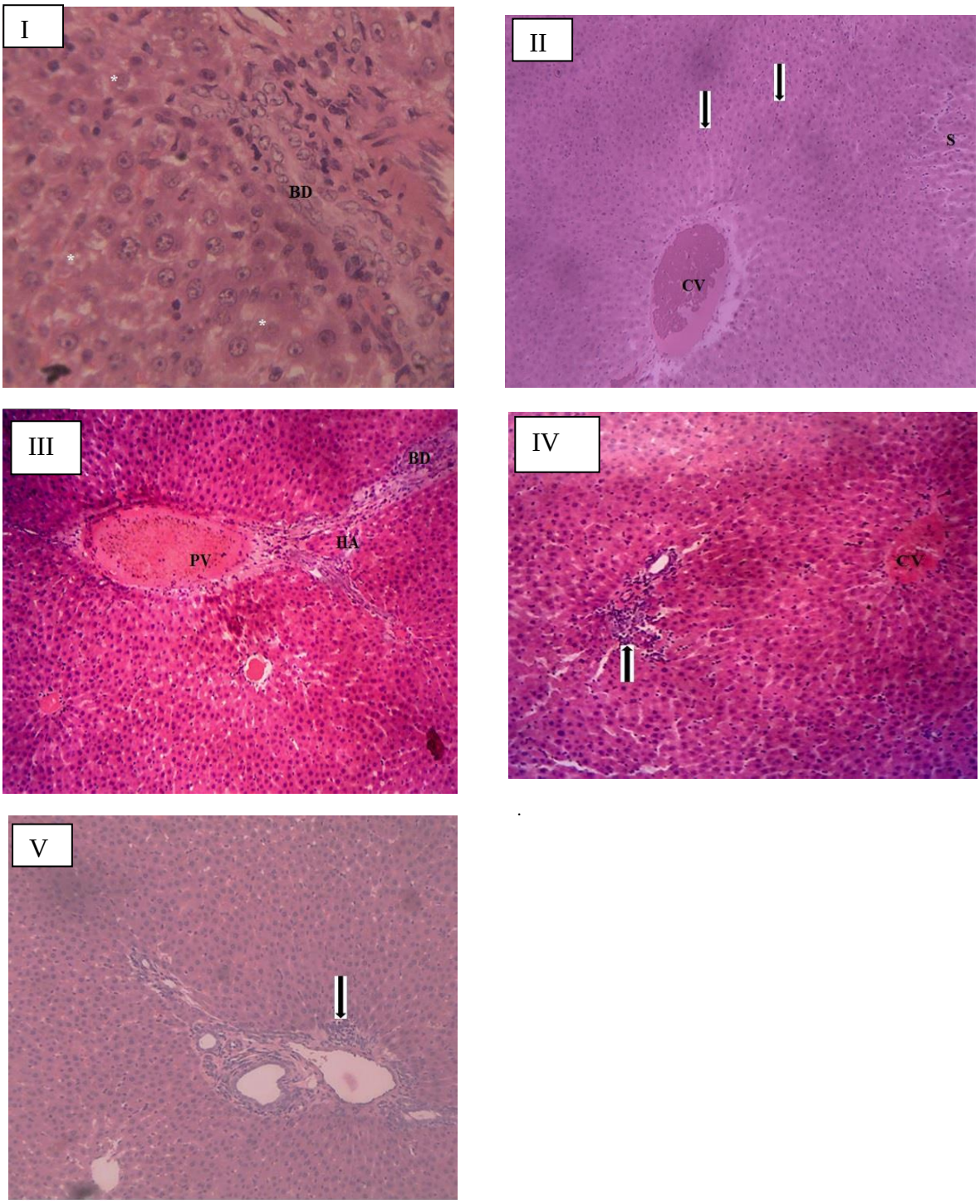

Figure 9: Photomicrograph of the sections of the liver of DOX treated rats
I represent negative control [Tween 80 (5 ml/kg) + DOX (10 mg/kg)] p.o, II, III and IV represent extract at 100, 200 and 400 mg/kg + DOX (10 mg/kg) p.o, V represents positive control [Silymarin (20 mg/kg) + DOX (10 mg/kg)] p.o respectively. H & E. Magnification x 100 (II, III, IV and V) x 400 (I).

Figure 11a shows photomicrographs of liver sections from PCM-treated rats administered various fractions. Group I exhibited extensive periportal hepatocellular degeneration and necrosis, with slight infiltration of mononuclear inflammatory cells in the periportal area (PA). Group II exhibited normal hepatocytes in the periportal area, with mild fibrosis of the bile duct (BD), hepatic artery (HA), and portal vein (PV). Group III exhibited extensive hepatocellular degeneration and necrosis, along with focal areas of mononuclear cell infiltration (arrows) and portal vein (PV) congestion. Group IV showed normal cords of hepatocytes around the central vein (CV), with slight congestion of the central vein (asterisks), sinusoidal dilation (S), and infiltration of mononuclear cells in the centrilobular and periportal areas (arrows). Figure 11b displays photomicrographs of liver sections from PCM-treated rats administered various fractions. Group V exhibited extensive hepatocellular degeneration and necrosis with early periportal fibrosis (arrows) and moderate infiltration of mononuclear cells around the portal vein (PV). Group VI showed normal cords of hepatocytes with mild periportal infiltration of mononuclear cells (arrows). PV = portal vein, HA = hepatic artery, and BD = bile duct.

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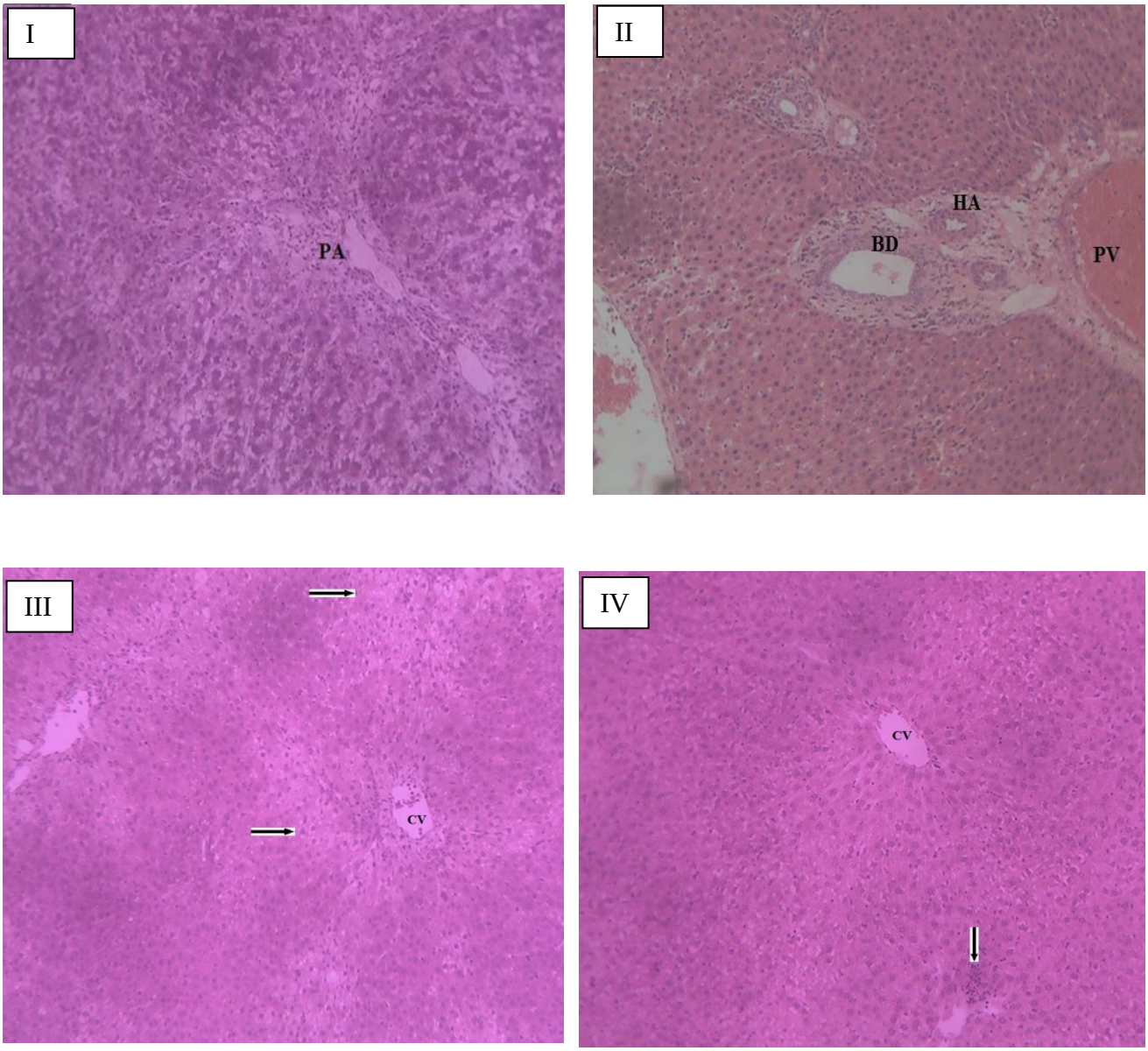

Figure 10a: Photomicrograph of the sections of the liver of CCl_4 treated rats
1 represents negative control [3% Tween 80 (5 ml/kg) + CCl_4 (1 ml/kg)] p.o
II represent positive control with [Silymarin (20 mg/kg) + CCl_4 (1 ml/kg)] p.o
III and IV represent n-Hexane fraction of *Lophira lanceolata* at 100 and 400 mg/kg + CCl_4 (1 mg/kg) p.o. H & E. Magnification x 400 (I), x100 (II, III and IV).

Group VII displayed extensive hepatocellular necrosis with slight focal and periportal infiltration of mononuclear inflammatory cells (arrows) along with bile duct proliferation (BD). PV = portal vein. HA = hepatic artery. Group VIII exhibited typical cords of hepatocytes in the periportal area, with bile duct proliferation (BD) and sinusoidal dilation (S).

Histological examinations offer valuable insights to reinforce the findings observed in biochemical and haematological parameters.[27-28] Histological examination of liver tissues from animals treated with CCl_4, PCM, and DOX revealed signs of inflammation, congestion, and degeneration, particularly within the sinusoids, as well as extensive fibrosis and necrosis in the periportal region, indicating the hepatotoxic effects of these substances. This could be attributed to generating of highly reactive radicals from oxidative stress induced by CCl_4, PCM, and DOX. However, these effects on hepatic tissue morphology were reversed in rats treated with ME, HF, EF, or MF. Pretreatment of animals with the standard drug silymarin was not significantly different from the extracts and fractions, which significantly reduced inflammation, minimized degenerative changes, and decreased cell necrosis. The early regeneration and appearance of normal liver hepatocyte cords at 400 mg/kg of extract and fractions may be attributed to the higher concentrations. These histological findings corroborate previous literature indicating that impaired liver hepatocyte function and viability are crucial indicators of hepatotoxicity according to histopathological results.[29] The recovery of hepatic cells from conditions such as steatosis (fatty liver), cholestasis (bile flow blockage), fibrosis (connective tissue thickening), and necrosis or apoptosis (cell or tissue death) following the administration of the extract and fractions validates the traditional use of *L. lanceolata* for treating liver disorders by herbalists. Thus, ME, HF, EF, and MF may have therapeutic potential in managing liver disorders in humans, particularly those with hepatitis.

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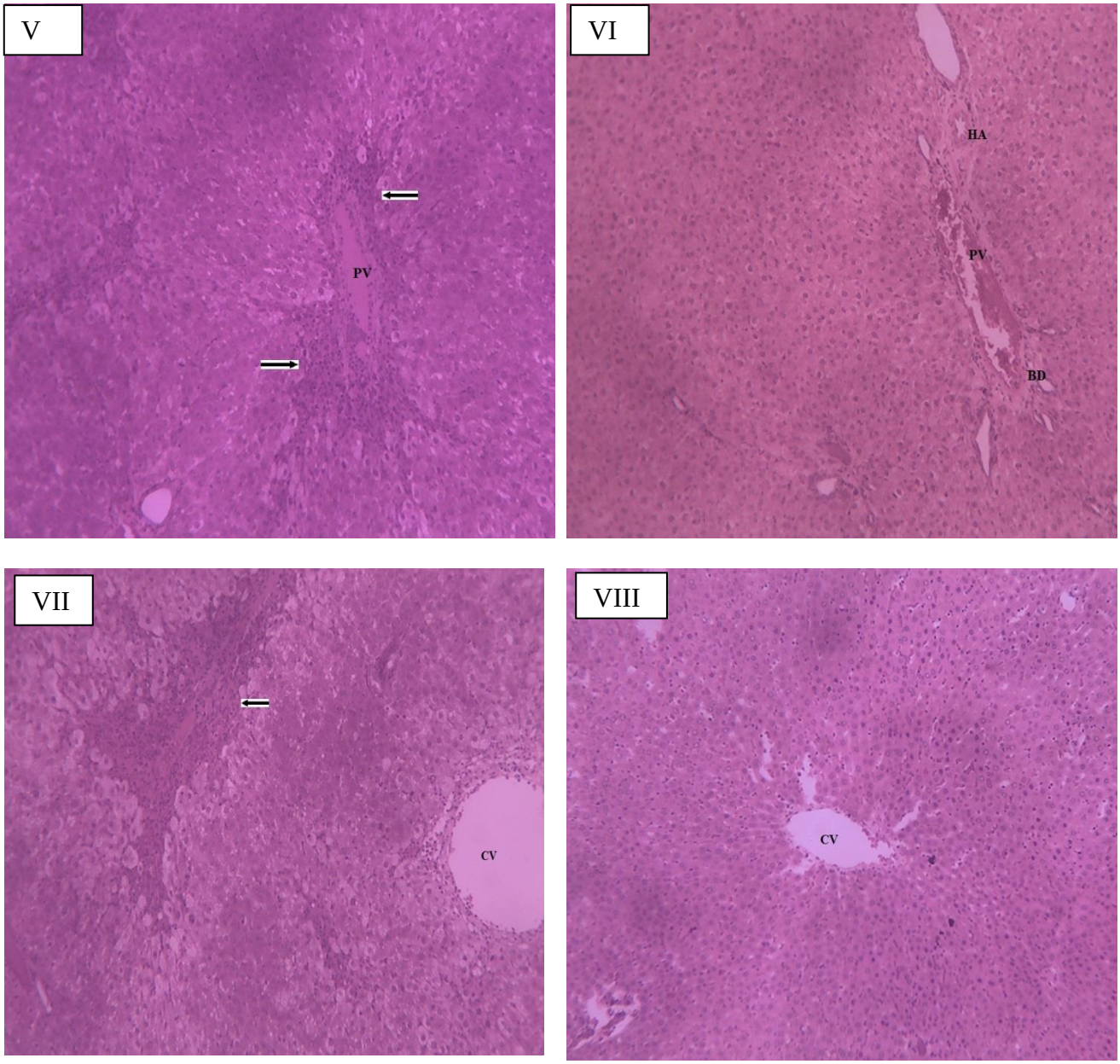

Figure 10b: Photomicrograph of the sections of the liver of CCl_4 treated rats
V and VI represent ethyl-acetate fraction of *Lophira lanceolata* at 100 and 400 mg/kg + CCl_4 (1 ml/kg) p.o VII and VIII represent methanol fraction of *Lophira lanceolata* at 100 and 400 mg/kg + CCl_4 (1 ml/kg) p.o. respectively. H & E. Magnification x 100.

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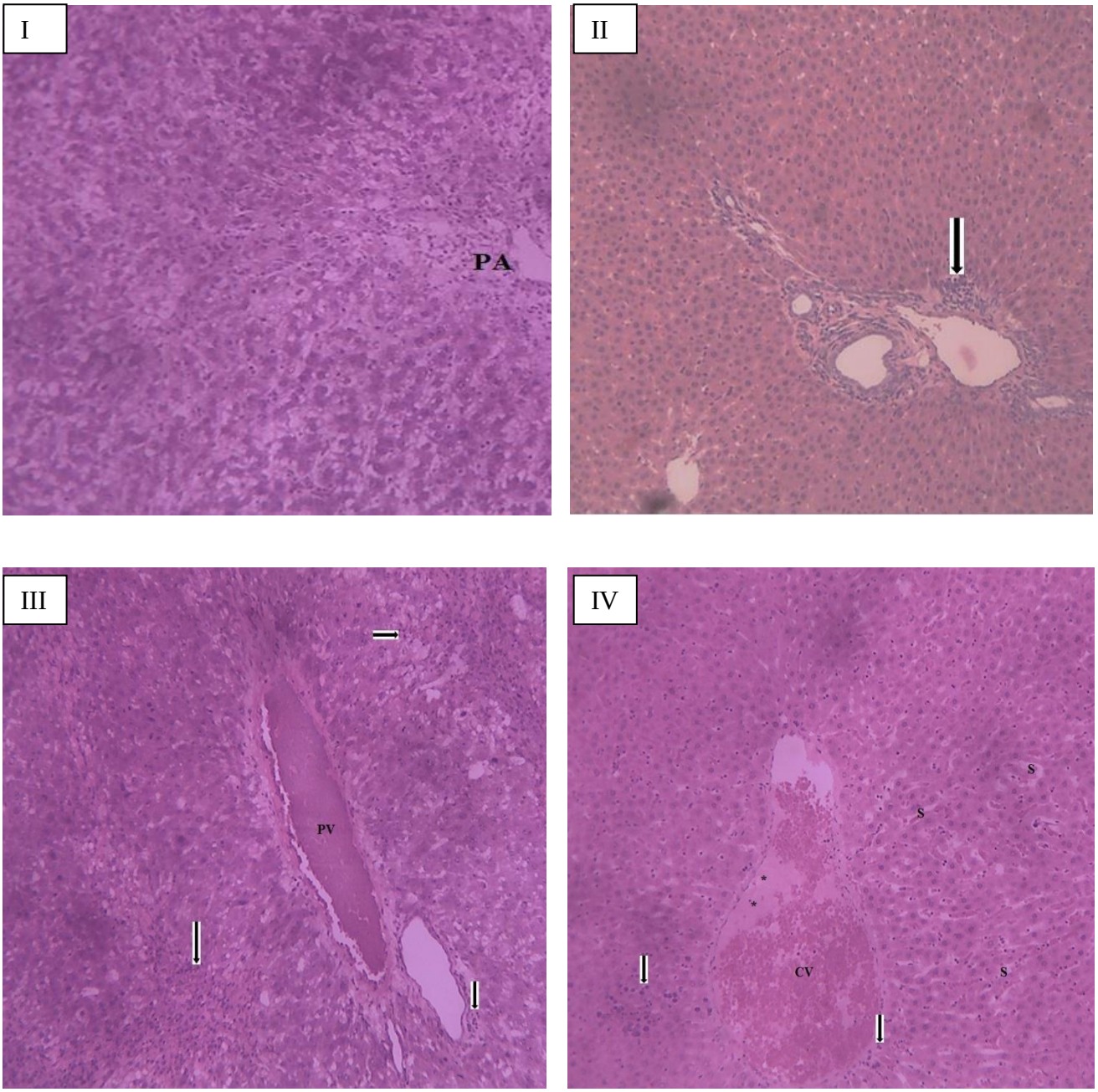

Figure 11a: Photomicrograph of the sections of the liver of PCM treated rats
1 represents negative control with [3% Tween 80 (5 ml/kg) + PCM (2000 mg/kg)] p.o
II represent positive control with silymarin (20 mg/kg) + PCM (2000 mg/kg) p.o
III and IV represent n-hexane fractions at 100 and 400 mg/kg + PCM (2000 mg/kg) p.o
H & E. Magnification x 100.

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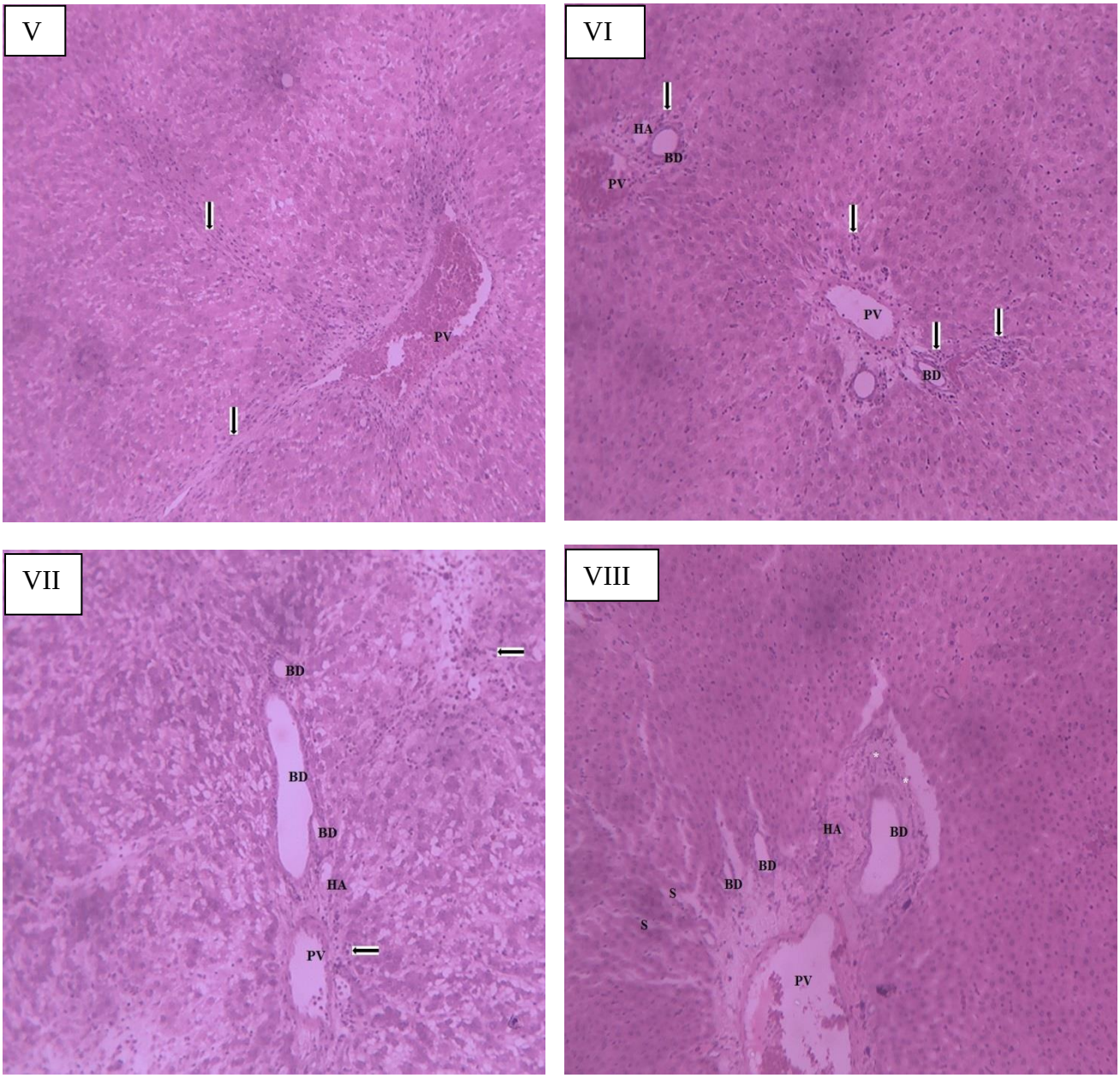

Figure 11b: Photomicrograph of the sections of the liver of PCM treated rats
V and VI represent ethyl-acetate fraction of *Lophira lanceolata* at 100 and 400 mg/kg + PCM (2000 mg/kg) p.o. VII and VIII represent methanol fraction of *Lophira lanceolata* at 100 and 400 mg/kg + PCM (2000 mg/kg) p.o. respectively
H & E. Magnification x 100.

Conclusions

The results suggests that the leaf extract and fractions of *L. lanceolata* have hepatoprotective effects against hepatotoxicity induced by CCl_4, PCM, and DOX. This study supports the traditional practice of using *L. lanceolata* for liver disorder management, suggesting potential therapeutic advantages. This study was limited by its sample size and the fact that inflammatory mediators and lipid profiles were not assessed. Furthermore, this research provides valuable insights into the pharmacological potential of *L. lanceolata*, stressing the importance of additional investigations, such as detailed mechanistic studies and clinical trials, to confirm its therapeutic effectiveness and safety for liver-related conditions.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgement

Authentication was carried out by Mr. Alfred Ozioko, a taxonomist affiliated with the International Centre for Ethnomedicine and Drug Development (InterCEDD).

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