Oxidative Stress, Hematological and Histopathological Alterations Recovery by Methanolic Extract of Celtis Occidentalis L. Leaves in Paracetamol-Induced Hepatic Injury in Rabbit

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ABSTRACT

Background: Paracetamol overdose is a leading cause of hepatotoxicity, resulting in oxidative stress and liver damage. Exploring natural therapeutic agents like Celtis occidentalis for their hepatoprotective effects is essential to mitigate these effects.

Objective: To evaluate the hepatoprotective, antioxidant, and nephroprotective effects of methanolic extract of Celtis occidentalis leaves in paracetamol-induced hepatic injury in rabbits.

Methods: The study involved 40 male rabbits divided into eight groups, including control, paracetamol (2 g/kg), and various doses of Celtis occidentalis extract (100, 200, and 300 mg/kg) with or without silymarin (50 mg/kg). The extract's antioxidant potential was assessed using DPPH and ABTS assays. Hepatic and renal function tests, lipid profiles, and hematological parameters were measured, and histopathological evaluations of liver tissues were conducted.

Results: The extract showed significant antioxidant activity, with 87.7% DPPH and 89.8% ABTS scavenging at 1000 µg/mL. It reduced ALT, AST, ALP, and bilirubin levels significantly ($P<0.05$). High dose extract normalized lipid profiles and improved hematological parameters compared to paracetamol alone.

Conclusion: Celtis occidentalis extract exhibited potent hepatoprotective effects, normalizing liver function and mitigating oxidative stress, suggesting its potential use in human healthcare for liver protection.

INTRODUCTION

Acetaminophen, commonly known as paracetamol (PCM), is widely utilized as a non-prescribed analgesic and antipyretic agent; however, its consumption at high doses leads to significant toxic effects, including hepatic and renal necrosis, positioning it as a critical toxic and poisoning agent (1). The primary mechanism underlying PCM toxicity involves its metabolic breakdown product, N-acetyl-p-benzoquinone imine (NAPQI), which reacts with the sulfhydryl groups of glutathione (GSH), an essential antioxidant enzyme. Excessive PCM ingestion results in an increased formation of NAPQI, depleting GSH levels due to NAPQI's high reactivity with hepatic proteins, which culminates in the necrosis of hepatocytes through oxidative stress pathways (2, 3). The liver, a pivotal organ involved in regulating various biochemical processes including the metabolism of carbohydrates, proteins, and lipids, as well as playing critical roles in disease defense, nutrition, reproduction, and energy production, is particularly susceptible to damage from PCM overdose (4, 5). In experimental animal models, PCM, administered in toxic doses (200 mg/kg or above), induces hepatotoxicity and nephrotoxicity characterized by inflammation and necrosis within hepatic and renal tissues (6). Clinically, PCM overdose can lead to severe liver necrosis in 10-40% of patients, and potentially lethal renal failure in less than 2% of cases. Acute hepatotoxicity ensues when PCM levels exceed therapeutic thresholds, with about 90% of the drug being metabolized into sulfate and glucuronide conjugates, while the remainder is converted into NAPQI through cytochrome P450, a highly toxic intermediate (7). Normally, PCM detoxification occurs via conjugation with hepatic GSH; however, excessive PCM intake results in substantial depletion of GSH, facilitating hepatotoxicity and nephrotoxicity due to elevated NAPQI concentrations (8).

The limitations of modern synthetic drugs designed to mitigate hepatotoxicity often include adverse outcomes such as renal failure and limited therapeutic efficacy, thereby highlighting the need for alternative treatments. Medicinal plants, being inherently compatible with human physiology and typically exhibiting minimal or no side effects, present a promising therapeutic option (9, 10). Numerous plant species have demonstrated hepatoprotective and nephroprotective effects in liver and kidney lesions resulting from various drug-induced toxicities, such as those associated with paracetamol, gentamicin, profenofos, D-galactosamine, and conditions induced by chronic stress, cytotoxic agents, diabetic nephropathy, or chemically induced nephrolithiasis involving inflammatory mediators and oxidative stress (9,

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11). Among these, species from the Celtis genus, belonging to the Cannabaceae family, encompass approximately 60-70 species of trees and shrubs, commonly known as hackberries or nettle trees, which are prevalent across temperate regions of the Northern Hemisphere and parts of Central Africa and South America (12, 14). Despite the historical lack of documentation regarding traditional Celtic medicine, native uses of Celtis occidentalis, a medium-sized deciduous tree native to North America but also growing in Europe, Africa, and Australia, include wood extracts for jaundice and bark decoctions for alleviating menstrual issues and sore throats (15, 16, 17).

Given the therapeutic potential of Celtis occidentalis, the present study aims to investigate the in-vivo and in-vitro antioxidant and hepatoprotective properties of the methanolic crude extract (met. ext.) of Celtis occidentalis leaves in a paracetamol-induced hepatic injury model in rabbits. Additionally, the study includes the evaluation of total phenolic content (TPC) and total flavonoid content (TFC) to identify possible phenolic and flavonoid compounds, comparing these to standard references, thereby enhancing the understanding of its potential therapeutic benefits.

MATERIALS AND METHODS

Various chemicals were employed in this study, including DPPH, ABTS, ascorbic acid (vitamin C), silymarin (a known synthetic hepatoprotective drug), and normal saline. For the preparation of the methanolic extract, leaves of Celtis occidentalis were collected from Lal Qila, District Dir Lower, Khyber Pakhtunkhwa, Pakistan. The leaves were thoroughly cleaned and placed on paper in a shaded area to dry for 25 days. Once dried, the leaves were ground into a fine powder using a mechanical grinder. Approximately 3 kilograms of this powder were subjected to maceration in 95% methanol for 21 days with occasional shaking to ensure thorough extraction. Filtration was performed using Whatman filter paper, and the resulting filtrate was concentrated into a semisolid mass using a rotary evaporator at the laboratory of the Department of Pharmacy, University of Malakand. The concentrated extract was further dried in a water bath until a completely dried mass of 220 grams was obtained.

Assessment of Total Phenolic and Total Flavonoid Contents (TPC & TFC)

The total phenolic content (TPC) was assessed using the Hillis and Swain method with slight modifications (18). Gallic acid was used as the internal standard for this assay. A mixture containing 0.5 mL of the methanolic extract and 0.1 mL of Folin-Ciocalteu reagent in 8 mL of distilled water was prepared and vortexed for even mixing. After a 5-minute incubation, 1 mL of 20% sodium carbonate (Na_2CO_3) was added. A series of gallic acid solutions at various concentrations (100, 200, 300, 400, and 500 mg/mL) were prepared in the same manner as the methanolic extract. The absorbance of both the extract and the standard solutions was measured against a blank (distilled water) at 725 nm using a UV/Visible spectrophotometer. For the assessment of total flavonoid content (TFC), a previously reported method was utilized (19). Quercetin served as the internal standard. In this procedure, 1 mL of methanolic extract was mixed with 0.3 mL of 5% sodium nitrite ($NaNO_3$) in 4 mL of distilled water. After a 5-minute incubation, 0.3 mL of 10% aluminum chloride ($AlCl_3$) was added, followed by 2 mL of 1.0 M sodium hydroxide (NaOH) after one minute. The solution was diluted to a final volume of 10 mL with distilled water. A series of standard quercetin solutions in concentrations of 100, 200, 300, 400, and 500 mg/mL were prepared using the same procedure as for the methanolic extract. The absorbance of the extract and standard solutions was recorded against a blank reagent (distilled water) at 510 nm using a UV/Visible spectrophotometer.

In Vitro DPPH Free Radical Scavenging Potential

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging potential of the methanolic extract of Celtis occidentalis was determined using the Brand-Williams method (20). A DPPH solution was prepared by dissolving 0.039 g of DPPH in 100 mL of methanol, followed by incubation in the dark for 30 minutes. A stock solution of the methanolic extract (2 mg/mL) was prepared, from which a series of dilutions were made at concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL. Each dilution (0.1 mL) was mixed with 3 mL of the DPPH solution and incubated at 25 °C for 30 minutes. The absorbance of each dilution was measured using a UV spectrophotometer. The percentage of DPPH scavenging potential was calculated using the formula:

$$\%\text{DPPH Scavenging} = \left(\frac{A_0 - A_s}{A_0}\right) \times 100$$

where A_0 represents the absorbance of the control and A_0 represents the absorbance of the sample. Each experiment was conducted in triplicate to ensure accuracy and reproducibility.

In Vitro ABTS Free Radical Scavenging Potential

The ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) free radical scavenging potential of the methanolic extract of Celtis occidentalis was evaluated using a previously reported method (21). The ABTS radical cation solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulfate, and the mixture was incubated overnight at 25 °C. A stock solution of the methanolic extract (2 mg/mL) was prepared, and a series of dilutions were made at concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL. Each dilution (0.1 mL) was mixed with 3 mL of the ABTS solution and incubated for 6 minutes at room temperature. The absorbance of the mixture was measured at 745 nm using a UV spectrophotometer. The percentage of ABTS scavenging potential was calculated using the same formula as described for the DPPH scavenging assay.

Acute Toxicity Analysis of Celtis occidentalis Methanolic Extract

The acute toxicity of the methanolic extract of Celtis occidentalis was assessed by orally administering the extract to experimental animals. Two rabbits were treated with a single dose of 1500 mg/kg body weight (b.w.), while two control animals received normal saline (5 mL/kg b.w.). The animals were observed continuously for 2 hours for any signs of toxicity, such as loss of motor activity, salivation, diarrhea, or muscle spasms. The animals were

subsequently monitored for up to 10 days, during which no indications of toxicity or mortality were observed. The methanolic extract was found to be non-toxic at doses up to 3000 mg/kg b.w., confirming its safety for further experimental studies (22).

Experimental Design

For the in-vivo study, a 21-day experiment was conducted using forty healthy male rabbits (Oryctolagus cuniculus), initially weighing between 0.5 and 1.2 kg. The animals were purchased from the National Institute of Health (NIH) Islamabad and housed in the animal facility at the University of Malakand. They were kept under standard conditions with a 12-hour light/dark cycle at a constant temperature of 25 ± 2.5 °C and relative humidity of 56 ± 10%. The animals were allowed to acclimatize for one week prior to the start of the experiment and were fed a regular diet of fresh green vegetables along with chaw pellets and clean water. The study protocol was approved by the ethical committee (E-SA-11-2010) under the "Animal Bye-Laws 2009" of the Department of Zoology, University of Malakand. The animals were divided into eight groups with five rabbits in each group (n = 5). A solution of Celtis occidentalis methanolic extract and paracetamol was prepared in normal saline according to the body weight of the animals and administered orally.

The group assignments were as follows:

Group I: Received normal saline.

Group II: Received 2 g/kg paracetamol (PCM).

Group III: Received 200 mg/kg methanolic extract of Celtis occidentalis.

Group IV: Received 2 g/kg paracetamol plus 50 mg/kg silymarin.

Group V: Received 50 mg/kg silymarin only.

Group VI: Received 100 mg/kg methanolic extract plus 2 g/kg paracetamol.

Group VII: Received 200 mg/kg methanolic extract plus 2 g/kg paracetamol.

Group VIII: Received 300 mg/kg methanolic extract plus 2 g/kg paracetamol.

Assessment of Blood and Biochemical Parameters

At the conclusion of the experiment on day 21, all animals were anesthetized using chloroform via inhalation. Blood samples (5 mL) were collected via cardiac puncture; 3 mL of the blood was transferred to non-EDTA tubes for serum separation by centrifugation at 4000 rpm for 10 minutes, and the serum was stored at 4 °C for subsequent biochemical analysis. Liver function tests (LFTs) including alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) were measured using a Cobas 111 analyzer (USA). Additional parameters such as triglycerides (TG), cholesterol, low-density lipoproteins (LDL), high-density lipoproteins (HDL), and serum bilirubin were also assessed. The remaining 2 mL of blood was stored in EDTA-containing tubes and analyzed for a complete blood count (CBC) using standard hematological procedures.

Analysis of Lipid Peroxidation and Antioxidant Biomarkers

To assess lipid peroxidation and antioxidant biomarkers, three lobes of liver tissue were collected from each group, immediately perfused with cold saline, and cleaned. The tissues were homogenized in cold phosphate-buffered saline (PBS, pH 7.4, 0.1 M). The homogenate was filtered and centrifuged at 3000 g for 20 minutes. The supernatant was stored at -80 °C until further analysis. The oxidative status was evaluated by measuring enzymatic antioxidant biomarkers, including catalase (CAT) and superoxide dismutase (SOD), according to previously published methods (23, 24). Non-enzymatic antioxidant markers, such as reduced glutathione (GSH) and the activity of cellular glutathione peroxidase (GPx), were measured using the Bioxytech GPx-340 Assay Kit, following established protocols (25).

Histopathology

For histopathological evaluation, a lobe of liver tissue from each animal was collected, washed thoroughly with normal saline to remove blood, and processed following standard protocols (6). The liver tissues were fixed in 10% formalin, dehydrated using a series of ethanol and xylene solutions, and embedded in paraffin wax. Tissue sections were prepared using a microtome with a thickness of 4.5 to 6 µm. The sections were stained with hematoxylin and eosin, cleaned, and observed under a microscope to assess alterations caused by paracetamol and the regenerative potential of Celtis occidentalis extract and silymarin.

Data Analysis

All in-vitro and in-vivo results were expressed as means ± standard deviation (SD). Data were analyzed for significance using the Student's t-test and one-way ANOVA (analysis of variance) followed by post hoc Tukey's multiple comparison test to determine differences among treatment groups. Statistical significance was considered at $p < 0.05$.

RESULTS

In Vitro DPPH and ABTS Free Radical Scavenging Potential

The methanolic extract of Celtis occidentalis leaves demonstrated a concentration-dependent inhibition of DPPH free radicals, as shown in Table 1. The highest percentage inhibition was observed at a concentration of 1000 µg/mL, reaching 87.7 ± 0.030%, whereas at the lowest concentration of 31.25 µg/mL, the inhibition was 67.9 ± 0.040%. Comparatively, the standard antioxidant, ascorbic acid, exhibited 92.7 ± 0.003% inhibition at 1000 µg/mL and 71.9 ± 0.030% at 31.25 µg/mL. Similarly, the ABTS free radical scavenging potential of the methanolic extract was evaluated, showing maximum inhibition of 89.8 ± 0.040% at 1000 µg/mL and 70.3 ± 0.065% at 31.25 µg/ml *Values are expressed as mean ± standard deviation. Significance compared to control: *P<0.05, **P<0.01, ***P<0.001. Paracetamol intoxication is associated with elevated levels of liver function markers, including ALT, AST, ALP, and bilirubin, indicating hepatic injury. As shown in Table 2, rabbits treated with only paracetamol displayed significant increases in these biomarkers. In contrast, administration of the methanolic extract of Celtis occidentalis resulted in a dose-dependent reduction of these elevated markers, with the highest dose (300 mg/kg) nearly normalizing the levels, comparable to the effects observed with the standard hepatoprotective drug, silymarin.

Table 1: In-Vitro DPPH and ABTS Scavenging Potential of Methanolic Extract and Ascorbic Acid (Standard)

Concentration (µg/mL)	%DPPH Scavenging (Met. Ext.)	%ABTS Scavenging (Met. Ext.)	Concentration (µg/mL)	%DPPH & %ABTS Scavenging
1000	87.7 ± 0.030***	89.8 ± 0.040***	1000	92.7 ± 0.003
500	83.3 ± 0.033***	87.4 ± 0.030**	500	88.2 ± 0.023
250	80.1 ± 0.042**	82.4 ± 0.060**	250	85.3 ± 0.033
125	76.1 ± 0.043*	78.5 ± 0.040**	125	80.3 ± 0.034
62.5	72.2 ± 0.046*	73.8 ± 0.030*	62.5	76.1 ± 0.022
31.25	67.9 ± 0.040	70.3 ± 0.065	31.25	71.9 ± 0.030

Table 2: Effects of Methanolic Extract on Liver Function Tests in Rabbits

Experimental Groups	ALT (µ/L)	ALP (µ/L)	AST (µ/L)	Bilirubin (mg/dL)
Control Group	32.6 ± 2.1***	68.3 ± 2.1***	72.2 ± 1.2***	0.4 ± 0.6***
PCM (2 g/kg)	122.0 ± 2.6	123.6 ± 2.5	124.1 ± 2.3	1.4 ± 0.1
Met. Ext. (200 mg/kg)	33.3 ± 1.8***	67.6 ± 1.5***	74.3 ± 0.9**	0.4 ± 0.1***
PCM + Silymarin (50 mg/kg)	66.3 ± 1.5*	89.6 ± 2.1*	96.2 ± 2.7ns	1.1 ± 0.2ns
Silymarin (50 mg/kg)	35.2 ± 1.3**	68.1 ± 1.7***	73.2 ± 1.2**	0.5 ± 0.3***
Met. Ext. (100 mg/kg) + PCM (2 g/kg)	47.9 ± 1.7*	72.5 ± 1.5**	84.3 ± 2.1**	0.7 ± 0.2**
Met. Ext. (200 mg/kg) + PCM (2 g/kg)	36.8 ± 1.8**	67.5 ± 1.5**	77.7 ± 2.3***	0.4 ± 0.2**
Met. Ext. (300 mg/kg) + PCM (2 g/kg)	30.9 ± 1.7***	63.9 ± 0.9***	66.9 ± 2.5***	0.3 ± 0.2***

*Values are expressed as mean ± standard deviation. Significance compared to control: *P<0.05, **P<0.01, ***P<0.001. The serum lipid profile of the different treatment groups is summarized in Table 3. Paracetamol-treated rabbits exhibited elevated levels of total cholesterol, triglycerides, HDL, and LDL, indicating hepatic dysfunction. The administration of the methanolic extract of Celtis occidentalis, particularly at higher doses, significantly reduced these lipid parameters to levels comparable to those of the control group, demonstrating its lipid-lowering effects.

Table 3: Effects of Methanolic Extract on Serum Lipid Profile of Rabbits

Experimental Groups	Cholesterol (mg/kg)	Triglyceride (mg/kg)	HDL (mg/kg)	LDL (mg/kg)
Control Group	36.2 ± 1.6***	54.1 ± 2.3***	18.7 ± 1.9**	12.9 ± 1.7***
PCM (2 g/kg)	48.9 ± 1.5	77.4 ± 2.8	27.1 ± 2.1	18.8 ± 1.7
Met. Ext. (200 mg/kg)	32.5 ± 1.1***	51.8 ± 1.8***	19.1 ± 1.5**	12.9 ± 1.2***
PCM + Silymarin (50 mg/kg)	43.1 ± 1.2*	65.6 ± 2.9*	24.8 ± 2.3**	16.8 ± 1.4**
Silymarin (50 mg/kg)	33.2 ± 1.4***	52.1 ± 1.3**	20.7 ± 1.8**	13.2 ± 1.1**
Met. Ext. (100 mg/kg) + PCM (2 g/kg)	40.7 ± 1.4**	53.3 ± 2.9***	22.6 ± 2.1**	14.9 ± 2.3*
Met. Ext. (200 mg/kg) + PCM (2 g/kg)	36.7 ± 1.9*	52.8 ± 2.8**	19.9 ± 1.8*	11.3 ± 2.8**
Met. Ext. (300 mg/kg) + PCM (2 g/kg)	31.9 ± 1.1***	52.2 ± 2.1***	15.8 ± 1.8**	9.8 ± 2.3***

*Values are expressed as mean ± standard deviation. Significance compared to control: *P<0.05, **P<0.01, ***P<0.001. The hematological parameters of the different treatment groups are presented in Tables 4 and 5. Rabbits treated with paracetamol showed significant increases in WBC counts, indicative of an inflammatory response. However, co-administration of the methanolic extract of Celtis occidentalis normalized these parameters, as shown in Table 5. A significant reduction in WBC counts was observed, highlighting the extract's potential in mitigating inflammation.

The total phenolic content (TPC) and total flavonoid content (TFC) of the methanolic extract of Celtis occidentalis leaves were evaluated, as illustrated in Figure 1. The TPC was determined using gallic acid as the standard, while TFC was assessed using quercetin as the standard. A linear correlation was observed between TPC and TFC with the DPPH and ABTS scavenging activities, indicating strong antioxidant properties

Body weight changes across different treatment groups are shown in Table 6. Rabbits treated with paracetamol showed an average weight loss of 20 g by the end of the experiment. Conversely, the control group and those treated with the methanolic extract displayed significant weight gain, indicating a protective effect of the extract against PCM-induced weight loss. *Values are expressed as means ± SD. Significance differences compared to control: ns = non-significant, *P<0.05, **P<0.01, ***P<0.001. The effects of the methanolic extract on hepatic lipid peroxidation and antioxidant status are summarized in Table 7. Paracetamol treatment resulted in a significant reduction in antioxidant markers, including GSH, SOD, CAT, and GPx. Co-administration of silymarin and the methanolic extract significantly elevated these markers, restoring them to near-normal levels, demonstrating the extract's potent hepatoprotective and antioxidant effects. Correlation Between TPC and TFC with DPPH and ABTS Inhibition Figures 1(b) and 1(d) demonstrate the correlation between

TPC and TFC against the DPPH and ABTS free radicals. High regression coefficients (R^2 = 0.981 and R^2 = 0.987) were obtained when TPC was plotted against %DPPH and %ABTS inhibition, while the coefficients for TFC against %DPPH and %ABTS were R^2 = 0.99 and R^2 = 0.98, respectively.

Table 4: Effects of Methanolic Extract on Hematological Parameters of Rabbits

Experimental Groups	RBCs (M/µL)	Hb (g/dL)	MCH (pg)	MCV (fL)	MCHC (g/dL)
Control Group	12.24 ± 0.1**	6.16 ± 0.8**	29.8 ± 1.3***	67.4 ± 2.9**	42.3 ± 0.7***
PCM (2 g/kg)	7.08 ± 0.9	4.06 ± 0.3	17.2 ± 2.2	51.2 ± 2.3	28.3 ± 1.7
Met. Ext. (200 mg/kg)	12.96 ± 0.4***	6.21 ± 0.6***	27.2 ± 0.3**	64.3 ± 1.7**	41.5 ± 2.5**
PCM + Silymarin (50 mg/kg)	9.61 ± 0.7ns	5.07 ± 0.9*	20.3 ± 1.5*	55.4 ± 1.6ns	33.3 ± 2.1*
Silymarin (50 mg/kg)	11.58 ± 0.4*	6.03 ± 0.3**	27.9 ± 0.6**	65.9 ± 2.5**	41.8 ± 1.1**
Met. Ext. (100 mg/kg) + PCM (2 g/kg)	6.44 ± 0.9ns	4.84 ± 0.5ns	18.8 ± 1.2*	53.6 ± 3.1ns	29.5 ± 2.6ns
Met. Ext. (200 mg/kg) + PCM (2 g/kg)	8.26 ± 0.4*	5.83 ± 0.7*	23.1 ± 1.9**	57.2 ± 2.8ns	35.9 ± 2.1*
Met. Ext. (300 mg/kg) + PCM (2 g/kg)	12.26 ± 0.6**	6.74 ± 0.5**	27.1 ± 0.7***	63.7 ± 3.3**	41.2 ± 1.9***

Table 5: Effects of Methanolic Extract on Blood Profile of Rabbits

Experimental Groups	WBCs (x10³/µL)	Lymphocytes	Neutrophils	Monocytes
Control Group	8.3 ± 0.4**	55.4 ± 1.1**	47.1 ± 2.7*	23.8 ± 1.3***
PCM (2 g/kg)	21.2 ± 0.7	38.6 ± 2.3	63.5 ± 1.8	15.4 ± 1.5
Met. Ext. (200 mg/kg)	9.6 ± 0.9***	50.2 ± 3.1**	49.4 ± 2.4**	21.2 ± 1.3***
PCM + Silymarin (50 mg/kg)	17.1 ± 0.7*	44.2 ± 3.1ns	55.4 ± 3.4*	18.6 ± 3.0ns
Silymarin (50 mg/kg)	10.2 ± 1.2***	47.3 ± 2.5**	50.8 ± 4.4**	20.8 ± 1.9***
Met. Ext. (100 mg/kg) + PCM (2 g/kg)	15.6 ± 1.1*	41.2 ± 1.9ns	58.4 ± 2.7ns	13.2 ± 0.8**
Met. Ext. (200 mg/kg) + PCM (2 g/kg)	12.4 ± 1.2**	46.5 ± 1.4**	56.2 ± 3.4ns	17.7 ± 1.6**
Met. Ext. (300 mg/kg) + PCM (2 g/kg)	9.2 ± 1.2***	52.8 ± 1.9***	49.8 ± 2.9**	22.4 ± 1.9***

Table 6: Changes in Average Body Weight of Different Treatment Groups

Experimental Groups	Day 1	Day 14	Day 21
Control Group	810.5 ± 1.5**	828 ± 1.7***	845 ± 1.2***
PCM (2 g/kg)	1000 ± 2.1	980 ± 2.5	960 ± 1.9
Met. Ext. (200 mg/kg)	1019 ± 2.3**	1030 ± 2.0**	1041 ± 2.4**
PCM + Silymarin (50 mg/kg)	660 ± 2.1*	630 ± 1.6*	689 ± 2.1***
Silymarin (50 mg/kg)	584 ± 1.5**	570 ± 1.8*	611 ± 1.8***
Met. Ext. (100 mg/kg) + PCM (2 g/kg)	620 ± 2.3*	605 ± 2.5*	628 ± 1.8**
Met. Ext. (200 mg/kg) + PCM (2 g/kg)	570 ± 1.7*	577 ± 1.9*	586 ± 1.4*
Met. Ext. (300 mg/kg) + PCM (2 g/kg)	715 ± 1.5**	728 ± 1.8***	745 ± 1.2**

Table 7: Effects of Methanolic Extract on Hepatic Antioxidant Markers

Experimental Groups	GSH (nm/mg Protein)	SOD (U/mg Protein)	CAT (U/mg Protein)	GPx (U/mg Protein)
Control Group	5.24 ± 0.5***	8.23 ± 1.02***	110.3 ± 1.12***	30.54 ± 2.21***
PCM (2 g/kg)	2.95 ± 0.3	2.24 ± 1.1	72.5 ± 1.5	4.21 ± 1.78
Met. Ext. (200 mg/kg)	5.53 ± 0.23***	8.12 ± 1.0***	109 ± 1.04***	28.98 ± 1.64***
PCM + Silymarin (50 mg/kg)	3.02 ± 0.7*	4.96 ± 1.2**	94.1 ± 1.2**	12.32 ± 1.87*
Silymarin (50 mg/kg)	4.42 ± 0.22***	7.98 ± 1.12***	107 ± 1.10**	26.35 ± 1.54**
Met. Ext. (100 mg/kg) + PCM (2 g/kg)	3.21 ± 0.1**	6.76 ± 0.9**	90.2 ± 1.4*	21.12 ± 2.01*
Met. Ext. (200 mg/kg) + PCM (2 g/kg)	4.13 ± 0.2***	7.06 ± 1.1**	97.1 ± 1.3**	25.21 ± 1.76**
Met. Ext. (300 mg/kg) + PCM (2 g/kg)	5.21 ± 0.3***	8.11 ± 1.0***	108.2 ± 1.2***	29.23 ± 1.89***

These correlations suggest a strong relationship between phenolic and flavonoid contents and their antioxidant potential. Histopathological analysis, depicted in Figure 2, revealed the protective effects of the methanolic extract on liver tissues. The paracetamol-treated group exhibited significant hepatic damage, including necrosis, inflammatory infiltration, and mucus accumulation. In contrast, treatment with the methanolic extract at various doses showed dose-dependent restoration of liver architecture, with the highest dose demonstrating nearly normal hepatocytes with no signs of inflammation or necrosis, comparable to the silymarin-treated group.

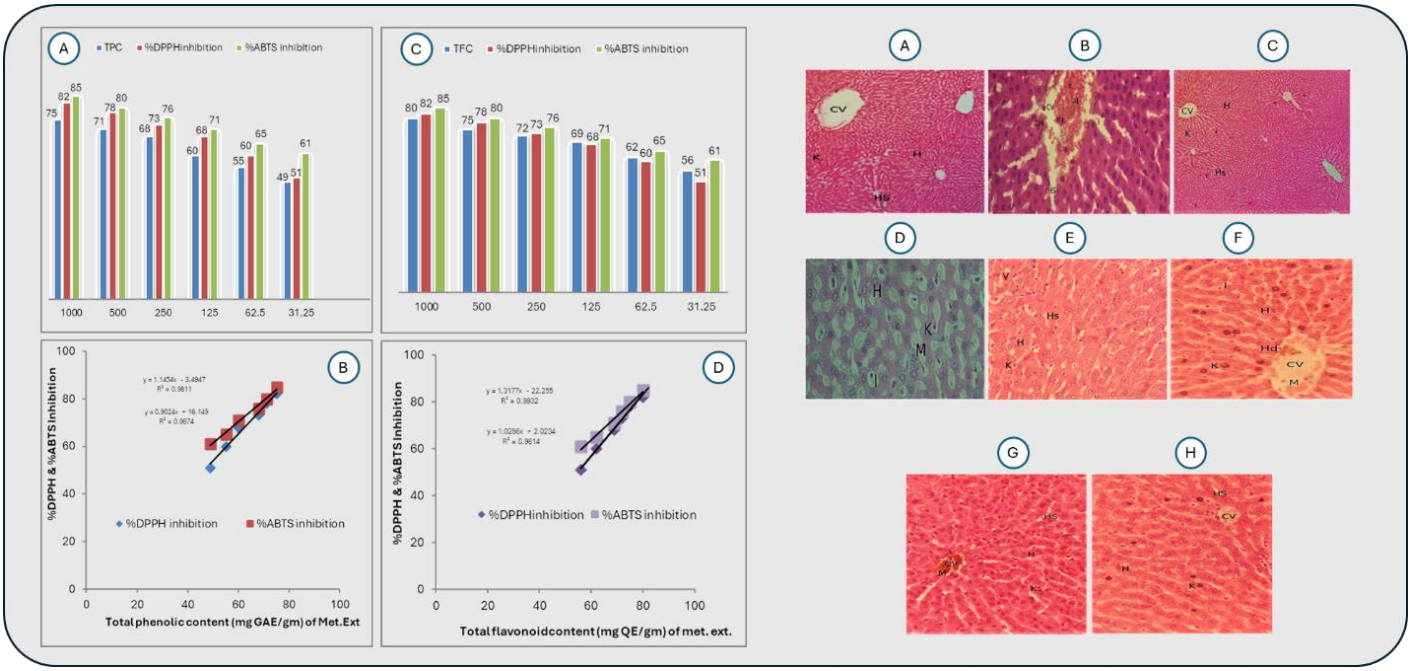

Figure 1 and 2: Antioxidant Activity and Histopathological Analysis of Celtis Occidentalis Methanolic Extract in Paracetamol-Induced Hepatic Injury

These findings collectively highlight the potent hepatoprotective, antioxidant, and restorative properties of the methanolic extract of Celtis occidentalis, making it a promising candidate for further therapeutic development against paracetamol-induced hepatic injury.

DISCUSSION

Paracetamol is a commonly used analgesic and antipyretic medication, frequently administered in various infections; however, its overdose is associated with hepatotoxicity, as it undergoes metabolism primarily in the liver, a critical organ responsible for maintaining homeostasis (26). The metabolism of paracetamol through the cytochrome P-450 enzyme system results in the formation of the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI), which depletes hepatic glutathione (GSH), leading to oxidative stress, lipid peroxidation, and hepatocellular damage (27). The depletion of GSH is a key factor in the pathogenesis of paracetamol-induced hepatic injury, as NAPQI binds to cellular macromolecules, including enzymes and nucleic acids, exacerbating oxidative damage (28). Elevated liver function tests (LFTs), including ALT, AST, ALP, and bilirubin, serve as reliable biomarkers of liver injury, reflecting hepatocellular necrosis and loss of membrane integrity due to excessive paracetamol exposure (22).

The findings of the current study align with existing literature, demonstrating significant increases in serum ALT, AST, ALP, and bilirubin levels in paracetamol-treated rabbits, indicative of severe hepatic injury (26, 22). The co-administration of methanolic extract of Celtis occidentalis significantly reduced these elevated serum markers in a dose-dependent manner, suggesting the extract's hepatoprotective properties, likely attributable to its antioxidant constituents. These results corroborate previous studies showing that silymarin, a known hepatoprotective agent, similarly normalizes serum biomarkers through its free radical scavenging and antioxidant activities (29). The comparative efficacy of Celtis occidentalis extract with silymarin in mitigating paracetamol-induced hepatotoxicity supports the hypothesis that the extract contains bioactive compounds with potent antioxidant and hepatoprotective effects, potentially comparable to those in silymarin (30).

The liver's endogenous antioxidant defense, comprising enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), plays a crucial role in protecting against oxidative damage induced by hepatotoxic agents like NAPQI (31). These enzymes neutralize reactive oxygen species (ROS) generated during metabolic processes, thereby preventing oxidative stress and maintaining cellular integrity. In the current study, the methanolic extract of Celtis occidentalis not only restored the levels of GSH but also significantly increased the activities of SOD, CAT, and GPx in paracetamol-intoxicated rabbits, demonstrating its antioxidative potential. The observed antioxidative effects may be attributed to the phenolic and flavonoid compounds present in the extract, as evidenced by the total phenolic content (TPC) and total flavonoid content (TFC) analysis, which correlated strongly with the extract's free radical scavenging activity against DPPH and ABTS radicals (32, 33).

The study also evaluated the impact of the extract on lipid profiles and hematological parameters, which are often disrupted in hepatic injury. Paracetamol administration resulted in elevated levels of cholesterol, triglycerides, HDL, and LDL, reflecting hepatic dysfunction and disrupted lipid metabolism. Treatment with the extract restored these lipid parameters to near-normal levels, suggesting that the extract aids in normalizing lipid metabolism, potentially through the modulation of cytochrome P-450 activity and reduction of oxidative stress (22). Additionally, significant

improvements in hematological parameters, including RBC count, hemoglobin, and indices such as MCV, MCH, and MCHC, were observed in extract-treated groups, indicating a protective effect on erythropoiesis and overall hematopoietic function. This effect may be due to the presence of bioactive compounds such as polyphenolics, flavonoids, alkaloids, and vitamins in the extract, which are known to support hematopoiesis and bone marrow function (34).

While the findings demonstrate the potent hepatoprotective and antioxidative effects of the methanolic extract of Celtis occidentalis, the study's strengths include the use of a well-established animal model and comprehensive evaluation of both biochemical and histopathological parameters, providing robust evidence of the extract's efficacy. However, the study is not without limitations. The exact bioactive compounds responsible for the observed effects were not isolated or characterized, warranting further research to identify and evaluate these compounds. Additionally, while the results in rabbits are promising, translational studies in humans are needed to confirm the extract's safety and efficacy in clinical settings. The study also did not explore the long-term effects of the extract, which is critical for understanding its potential as a therapeutic agent in chronic conditions.

CONCLUSION

In conclusion, the methanolic extract of Celtis occidentalis leaves exhibited significant in-vitro and in-vivo antioxidant and hepatoprotective properties against paracetamol-induced hepatic injury in rabbits. The extract effectively reduced elevated biochemical markers, normalized lipid profiles, improved hematological parameters, and mitigated histopathological alterations in liver tissues, suggesting a robust protective effect against oxidative stress and hepatic damage. These findings highlight the potential of Celtis occidentalis as a natural therapeutic agent for managing hepatic toxicity, though further studies are necessary to isolate its active constituents and validate its efficacy in clinical trials. Future research should also explore the mechanistic pathways involved in the extract's protective effects, with a focus on its interaction with hepatic metabolism and antioxidant defense systems.

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