Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2026.079

RESEARCH ARTICLE

Mitigation of Cadmium-Induced Hepatorenal Toxicity in Male Wistar Rats using *Carica papaya* and *Ziziphus mauritiana*

Sajid Hussain[1], Azhar Rafique[1*], Asma Ashraf[1] and Farkhanda Asad[1]

[1]Department of Zoology, Government College University, Faisalabad, 38000 Pakistan
*Corresponding author: azharrafique@gcuf.edu.pk

ARTICLE HISTORY (26-049)

Received: January18, 2026
Revised: April 14, 2026
Accepted: April 17, 2026
Published online: April 29, 2026

Key words:
C. papaya
Cadmium
Comet assay
Hepatorenal
Molecular docking
Z. mauritiana

A B S T R A C T

Plant-derived phytochemicals are promising therapeutic agents against heavy metal–induced toxicity. This study evaluated the protective efficacy of *Carica papaya* (CP) leaf and *Ziziphus mauritiana* (ZM) fruit pulp ethanolic (70%) extracts against cadmium (Cd)-induced hepatorenal toxicity in rats. Fifty-six male Wistar rats (n=7) were divided into eight groups and treated for four weeks. $CdCl_2$ (5 mg/kg/day) was administered orally, whereas CP and ZM extracts were given (separately) (300 mg/kg/day) or in combination (150 mg/kg each). Hepatic, renal, lipid, and hematological parameters, along with antioxidant, oxidative stress, inflammatory, apoptotic, and anti-apoptotic gene expression profiles, were assessed to determine the therapeutic potential of both extracts. Molecular docking, comet assay, and histopathological analyses were also performed. Data were analyzed using one-way ANOVA followed by Dunnett's test (GraphPad Prism, $P<0.05$). Cd exposure caused significant hepatorenal dysfunction, dyslipidemia, and hematological alterations, accompanied by elevated ALT, AST, ALP, GGT, bilirubin, urea, serum creatinine, BUN, NGAL, KIM-1, and Semaphorin-3A. Cd also suppressed antioxidant enzymes (SOD, CAT, GPx, GR, and GSH) and significantly increased oxidative stress, inflammatory mediators, and pro-apoptotic gene expression, while reducing anti-apoptotic markers. Supplementation with CP and ZM extracts, particularly in combination, markedly improved hepatorenal, hematological, and lipid profiles, restored antioxidant defenses and mitigated oxidative damage. Treated groups showed reduced DNA tail length and intensity in comet assay, indicating genoprotection. Histopathology revealed preserved hepatic and renal morphology with minimal necrosis and inflammation. Molecular docking demonstrated strong binding affinities of key phytochemicals with NF-κB and 2AZ5, suggesting potential molecular mechanisms underlying the observed protective effects. Conclusively, CP and ZM showed synergistic therapeutic efficacy against Cd toxicity.

To Cite This Article: Hussain S, Rafique A, Ashraf A and Asad F, 2026. Mitigation of Cadmium-induced hepatorenal toxicity in male Wistar rats using *Carica papaya* and *Ziziphus mauritiana*. Pak Vet J, 46(4): 790-805. http://dx.doi.org/10.29261/pakvetj/2026.079

INTRODUCTION

Heavy metal pollution has become an acute international environmental and public health issue with accelerating industrialization, intensification of agriculture, and urbanization (Pan *et al*., 2021). Among these metals, cadmium (Cd) is a non-essential but highly toxic element known to have significant adverse effects on both humans and animals**.** The International Agency for Research on Cancer (IARC) has classified cadmium as a Group 1 carcinogen, highlighting its strong carcinogenic potential (Moukadiri *et al*., 2024). Exposure to Cd via occupational exposure, contaminated food, water, cigarette smoking, and polluted air. Once ingested, Cd gets accumulated mainly in the liver and kidneys and binds to metallothionein and other intracellular proteins. Due to its long biological half-life (25–30 years) and low rate of excretion, Cd bioaccumulates and thus results in chronic toxicity (Idris et al., 2020; Nordberg and Nordberg, 2022).

According to recent reports by the World Health Organization, cadmium remains a major global environmental pollutant with significant toxic effects on the kidneys, skeletal system, and other organs (Cañas Portilla *et al*., 2024). The World Health Organization (WHO) has listed cadmium as one of the ten chemicals of utmost concern for major public health issues because it is

very toxic, persistent, and bioaccumulative. A provisional tolerable monthly intake value was established by the Joint FAO/WHO Expert Committee as 25µg/kg body weight, with safe concentrations being 0.003 mg/L in drinking water and 0.5 mg/kg in soils used for agriculture (Satarug *et al*., 2017). But Cd pollution in the environment often surpasses these thresholds. Soil Cd concentrations around the world vary from 0.1 to 0.8 mg/kg but may be up to >3.0 mg/kg in industrial areas (Acosta-Decal *et al*., 2025; Yang *et al*., 2025). In Pakistan, especially Lahore, Kasur, and Faisalabad, soil Cd concentrations reported are between 1.2 and 4.8mg/kg, whereas irrigation water in Punjab holds 0.01–0.07mg/L, which is above WHO standards. Vegetables and rice that are grown in such areas tend to carry 0.5–1.5mg/kg of cadmium far above the acceptable limit of 0.1–0.2mg/kg (Rehman *et al*., 2017). At the molecular level, Cd gets into cells by mimicking essential metals like zinc and calcium, using their transport systems through a process known as the "Trojan horse" pathway (Urani *et al*., 2015). After entering cells, Cd interferes with intracellular metal homeostasis by substituting zinc, iron, and copper in metalloproteins, leading to oxidative stress, DNA damage, mitochondrial dysfunction, and autophagy activation (Pecoraro *et al*., 2021).

Cadmium is highly hepatotoxic and nephrotoxic and primarily accumulates in the liver and kidneys, the main detoxifying and excretory organs (Park *et al*., 2021). It disrupts enzymatic and antioxidant functions, alters lipid metabolism, and enhances reactive oxygen species (ROS) generation, leading to hepatocellular degeneration and necrosis. It also activates NF-κB–mediated inflammatory pathways, upregulating TNF-α and IL-6, and induces caspase-3–dependent apoptosis with Bcl-2 suppression (Souza-Arroyo *et al*., 2022). Cd accumulates within the proximal tubular epithelial cells with ensuing tubular necrosis, damage to the glomeruli, and filtration capacity reduction (Unsal *et al*., 2020). Collectively, these outcomes underscore the critical requirement to develop safe, natural therapeutic interventions having the ability to neutralize Cd-induced oxidative and inflammatory injury, especially within hepatic and renal tissues (M Brzóska *et al*., 2016; Akmal *et al*., 2024) .

Phytochemical-rich medicinal plants have become incredibly important in their potential to counteract heavy metal toxicity by mechanisms involving antioxidant, anti-inflammatory, and detoxication properties (Laib *et al*., 2024). *C. papaya* (Papaya) and *Z. mauritiana* (Indian jujube, ber) are especially prized for their extensive pharmacological properties as well as traditional medicinal applications. *C. papaya* is rich in flavonoids, phenolics, vitamins, and alkaloids which are responsible for its potent antioxidant, hepatoprotective, and nephroprotective activity (Alara *et al*., 2022). Likewise, *Z. mauritiana* abounds in triterpenoids, saponins, and polyphenols, whose free radical–scavenging and membrane-stabilizing effects are highly effective (Hadzhi *et al*., 2024). These phytochemicals counteract redox imbalance, inhibit lipid peroxidation, and inhibit inflammatory and apoptotic pathways initiated by toxicants like cadmium (Dwivedi *et al*., 2022). Despite advances in understanding cadmium-induced toxicity, current therapeutic strategies remain largely supportive and limited in efficacy. Conventional treatments, including chelation therapy, are often associated with adverse effects, limited tissue specificity, and incomplete removal of accumulated cadmium. Therefore, there is a growing need for safer and more effective alternatives, particularly those derived from natural sources with antioxidant, anti-inflammatory, and metal-chelating properties. Based on their reported antioxidant and pharmacological properties, including potential synergistic interactions among phytochemicals such as flavonoids and phenolics, the present study aimed to evaluate the combined therapeutic efficacy of *C. papaya* and *Z. mauritiana* in mitigating cadmium-induced hepatorenal toxicity.

MATERIALS AND METHODS

Ethical statement approval: Design of experiment, together with the procedures and techniques were done in accordance with the guidance of the Ethics Review Committee of Government College University, Faisalabad (GCUF/ERC/638), Dated 4 August 2025.

Collection and preparation of plant extracts: Fresh leaves of *C. papaya* and mature fruits of *Z. mauritiana* were collected from the Botanical Garden, University of Agriculture Faisalabad, and authenticated by a taxonomist at Government College University Faisalabad. The leaves and the edible fruit pulp were thoroughly washed with distilled water, shade-dried, and ground into fine powder. Each sample was extracted with 70% ethanol using a Soxhlet apparatus for 6–8 hours. The filtrates were concentrated under reduced pressure with a rotary evaporator, and the crude extracts were stored at 4°C in airtight containers for further analysis (Omidiwura, 2017). The percentage yield of *C. papaya* and *Z. mauritiana* extracts was calculated as 3.31% and 3.51%, respectively.

Phytochemical analysis by HPLC: HPLC analysis was performed using a Shimadzu system equipped with a UV detector set at 280 nm. Separation was achieved using a C18 reverse-phase column (250 × 4.6mM, 5µM). The mobile phase consisted of solvent A (water containing 0.1% formic acid) and solvent B (methanol) under isocratic conditions at a flow rate of 1.0mL/min. The injection volume was 20µL, and total runtime was 30 minutes. Samples were filtered through a 0.45µM membrane filter prior to injection (Khadam *et al*., 2019).

Experimental animals: Fifty-six (56) healthy adult male Wistar rats weighing 180–220g were procured from the animal house facility of the Government College University, Faisalabad, Pakistan. Prior to the commencement of the experiment, all animals were acclimatized for two weeks under standard laboratory conditions, maintaining a temperature of 25±2°C**,** relative humidity of 50±10% and a 12-hour light/dark cycle**.** The rats were housed in clean polypropylene cages with sterilized rice husk bedding, which was changed every two days. They were freely supplied with water and food (Benjamin, 2019).

Chemicals and reagents: All chemicals and reagents used in the present study were of analytical grade. Cadmium chloride ($CdCl_2$) was used as a toxicant to induce

hepatorenal damage in experimental animals. All general laboratory chemicals, solvents, and reagents, including ethanol (70%), were obtained from the Department of Zoology, Government College University Faisalabad (GCUF), and Pakistan. Biochemical assay kits were purchased from Sigma-Aldrich, USA (Elbaghdady *et al*., 2018).

Experimental design: The selected doses of *C. papaya* (300 mg/kg) and *Z. mauritiana* (300mg/kg) extracts were based on previous studies demonstrating their safety and efficacy in experimental animal models. The combined dose (150 mg/kg each) was chosen to evaluate potential synergistic effects while maintaining the total administered dose within a therapeutically relevant range. All treatments were administered orally via gastric gavage. The fifty-six male Wistar rats (Wistar strain) were randomly divided into eight groups (n=7 per group) as follows:

- Group I: Normal control (distilled water only)
- Group II: Cadmium (Cd) ($CdCl_2$ at 5 mg/kg body weight/day)
- Group III: *C. papaya* (CP) extract only (300 mg/kg body weight/day)
- Group IV: *Z. mauritiana* (ZM) extract only (300 mg/kg body weight/day)
- Group V: Cd + *C. papaya* extract ($CdCl_2$+300 mg/kg *C. papaya*)
- Group VI: Cd + *Z. mauritiana* extract ($CdCl_2$+300 mg/kg *Z. mauritiana*)
- Group VII: Cd + combined extract of CP and ZM ($CdCl_2$+150 mg/kg each)
- Group VIII: Extract combination only (CP 150 mg/kg+ZM 150 mg/kg)

Collection of blood samples: Rats were anesthetized using ketamine (80mg/kg) and xylazine (10mg/kg) prior to blood collection. The samples were placed in EDTA tubes and plain tubes, respectively to be subjected to hematological tests and serum biochemistry tests respectively. Serum was centrifuged at 3000rpm for 10 min and then kept at -20°C until analysis. The experiment manipulation followed the institutional ethical principles of animal treatment and care (El-Boshy *et al*., 2015).

Estimation of liver function markers: The assessment of markers of hepatic function, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), bilirubin, and albumin, was done following standardized protocols included in the diagnostic kit (Randox Laboratories, UK; Cat. No: 42455/A).

Analysis of renal function markers: The assessment of renal function biomarkers, such as serum creatinine, blood urea nitrogen (BUN), serum urea, uric acid, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and semaphorin-3A, was carried out utilizing standardized protocols included in the diagnostic kits from Abcam (Cambridge, UK) and MyBioSource (California, USA).

Assessment of lipid metabolism: The evaluation of serum lipid profile (total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL) were estimated according to the method described by Chen *et al*. (2023).

Assessment of antioxidant enzymatic activities: The activities of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), total antioxidant capacity (TAC), and glutathione (GSH) were measured by following protocol reported by (El-Bahr, 2015).

Evaluation of inflammatory markers: The protein levels of nuclear factor kappa B (NF-kB), tumor necrosis factor-alpha (TNF-a), interleukin-6 (IL-6), interleukin-8 (IL-8), and cyclooxygenase-2 (COX-2) were measured using conventional enzyme-linked immunosorbent assay (ELISA) kits from Cusabio (Wuhan, China; Cat. No: CSB-E13148r, CSB-E11987r, CSB-E04640r, CSB-E08055r, CSB-E13399r).

Determination of oxidative stress indicators: Blood samples were centrifuged at 3000 rpm for 10 min at 4°C, and the resulting serum was utilized for biochemistry analysis. The reactive oxygen species (ROS), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), total oxidant status (TOS), nitric oxide (NO), protein carbonyls, and thiobarbituric acid reactive substances (TBARS) were measured by standard spectrophotometric assays (Sinzato *et al*., 2023).

Assessment of apoptotic markers: Apoptotic markers such as caspase-3 (cysteinyl aspartate-specific protease-3), caspase-8, caspase-9, p53 (tumor protein p53), FAS (tumor necrosis factor receptor superfamily member 6), BAK (Bcl-2 homologous antagonist/killer), and BAX (Bcl-2-associated X protein) were measured with the help of standardized enzyme-linked immunosorbent assay kits (RayBiotech, USA) method used by Eissa *et al*. (2018).

Assessment of anti-apoptotic markers: Anti-apoptotic markers, including B-cell lymphoma 2 (Bcl-2), myeloid cell leukemia-1 (Mcl-1), B-cell lymphoma-extra-large (Bcl-xL), X-linked inhibitor of apoptosis protein (XIAP), and Survivin, were analyzed using standardized enzyme-linked immunosorbent assay (ELISA) kits (CUSABIO, China) according to the manufacturer's instructions.

Hematological studies: Rats were anesthetized with chloroform vapor in a closed chamber. Blood was collected via cardiac puncture using sterile syringes, and about 1 mL was transferred into EDTA tubes for hematological analysis. Parameters including RBC, WBC, Hb, Hct, RDW, platelets count, MPV, Neutrophils and Lymphocytes were measured using an Automated Hematology Analyzer (Jacob Filho *et al*., 2018).

In-Silico molecular docking: Molecular docking was performed using PyRx 0.8 with AutoDock Vina algorithms

to predict the inhibitory binding modes of selected bioactive compounds with target proteins. Ligands and receptors were energy-minimized before docking, and binding affinities were evaluated based on the lowest binding energy (kcal/mol). The top-ranked poses were visualized and analyzed for hydrogen bonding, hydrophobic, π–π, and van der Waals interactions using Discovery Studio Visualizer 3.5 to elucidate potential protective mechanisms against cadmium-induced toxicity (De Ruyck *et al*., 2016).

Assessment of DNA damage by Comet-assay: DNA damage in liver and kidney tissues was assessed using the alkaline comet assay following the method of (Ganapathy *et al*., 2016) with minor modifications. Single-cell suspensions were embedded in low-melting-point agarose, lysed, and subjected to alkaline electrophoresis. Slides were then stained with a working solution of Vista Green DNA dye prepared according to the Abcam Comet Assay Kit (Abcam, Cambridge, UK) instructions. Comet parameters, including tail length and tail DNA percentage, were analyzed manually using fluorescence microscopy. to evaluate genotoxic damage.

Histopathological examination: Histopathological changes in the liver and kidney were determined in this study. The liver and kidney tissues were immediately removed from the rats after blood collection. The tissues were then weighed, fixed in 10% neutral buffered formalin, and embedded in paraffin. After that, sections (5µm thickness) of paraffin-embedded tissues were stained with hematoxylin and eosin. The histological slides and representative photomicrographs were examined and captured using a light microscope, respectively (Mossa *et al*., 2015).

Statistical analysis: Data were analyzed using GraphPad Prism version 10. Normality was assessed using the Shapiro–Wilk test. One-way ANOVA followed by Dunnett's post hoc test was applied to compare treated groups with the control group. Values were expressed as mean ± SEM, and P<0.05 was considered statistically significant.

RESULTS

Phytochemical analysis of *C. papaya* and *Z. mauritiana*: Fig. 1(A) indicates the HPLC profiling of ethanolic extracts peaks for *C. papaya* at quercetin (3.270min), gallic acid (4.128min), vanillic acid (13.288min), chlorogenic acid (15.333min), syringic acid (16.729min), cinnamic acid (25.190min) and Fig. 1(B) for *Z. mauritiana* at quercetin (3.400min), caffeic acid (12.840min), vanillic acid (13.360min), syringic acid (16.090min), *p*-coumaric acid (17.430min) and sinapic acid (26.425min).

Hepatic effects of *C. papaya* and *Z. mauritiana* in cadmium-treated rats: *C. papaya* and *Z. mauritiana* extracts on hepatic biomarkers in Cd-exposed rats. Cd intoxication significantly (P<0.05) elevated serum ALT, AST, ALP, GGT, and bilirubin levels while reducing albumin concentration compared to the control group, indicating hepatic dysfunction (Table 1). Co-administration of *C. papaya* (Cd + CP), *Z. mauritiana* (Cd+ZM) and their combined treatment (Cd+CP+ZM) markedly (P<0.05) ameliorated these alterations, restoring enzyme activities and protein levels toward normal values. The combined extract treatment (Cd+CP+ZM) exhibited a more pronounced hepatoprotective effect compared to individual treatments, suggesting synergistic activity of both plant extracts against Cd-induced hepatic damage.

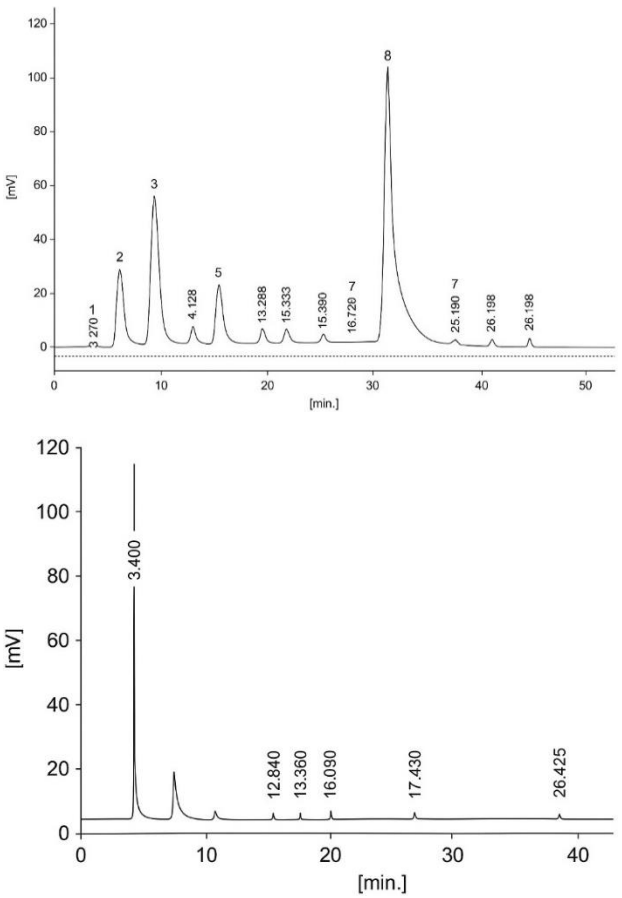

Fig. 1: A: HPLC chromatograms of *C. papaya,* **B**: *Z. mauritiana* extracts recorded at 280 nm.

Table 1: Modulatory effects of *C. papaya* (CP) and *Z. mauritiana* (ZM) extracts on cadmium-induced alterations in liver function test of male Wistar rats

Parameter	Control	Only Cd	Only CP	Only ZM	Cd + CP	Cd + ZM	Cd + CP + ZM	CP + ZM
ALT (U/L)	35.20±0.17	98.40±0.35 ***	33.10±0.15 ***	34.00±0.12 **	55.60±0.23 ***	52.80±0.17 ***	47.30±0.19 ***	34.20±0.13 *
AST (U/L)	42.50±0.17	106.20±0.14***	40.80±0.12 ***	41.50±0.15 **	64.70±0.19 ***	59.60±0.13 ***	55.10±0.17 ***	44.90±0.18 ***
ALP (U/L)	85.60±0.23	178.30±0.21***	82.30±0.22 ***	84.10±0.23 **	110.30±0.22***	114.90±0.21***	105.50±0.23***	90.70±0.22 ***
GGT (U/L)	5.80±0.06	9.50±0.05 ***	5.50±0.07 *	5.60±0.06 ns	6.80±0.08***	7.10±0.06 ***	6.90±0.08 ***	6.10±0.09 *
Albumin (g/dL)	4.20±0.06	2.80±0.04 ***	4.30±0.06 ns	4.20±0.05 ns	3.30±0.07 ***	3.70±0.06 ***	3.90±0.05 *	4.00±0.06 ns
Bilirubin (mg/dL)	0.50±0.06	1.90±0.05 ***	0.40±0.07 ns	0.50±0.05 ns	1.10±0.06 ***	1.30±0.07***	0.90±0.08 ***	0.70±0.06 ns

The values are depicted as the mean±SE (n=7). The significance at the level of P<0.05.S.E: Standard error, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, ALP: Alkaline Phosphatase, GGT: Gamma-Glutamyl Transferase. * significant difference with P<0.05, ** more significant, *** highly significant, ns → not statistically significant (P>0.05), meaning no meaningful difference from control.

Renal effects of *C. papaya* and *Z. mauritiana* in cadmium-treated rats: *C. papaya* and *Z. mauritiana* extracts on renal function biomarkers in Cd-exposed rats. Cd intoxication caused a significant (P<0.05) elevation in serum creatinine, urea, BUN, uric acid, NGAL, KIM-1, and Semaphorin-3A levels compared with the control group, indicating severe renal dysfunction (Table 2). Co-treatment with *C. papaya*, *Z. mauritiana*, or their combinations markedly (P<0.05) reduced these elevated parameters, reflecting improved kidney function. Among all treated groups, the combined extract (Cd+CP+ZM) showed the most pronounced improvement in renal biomarkers potential against Cd-induced nephrotoxicity.

Effects of *C. papaya* and *Z. mauritiana* on lipid metabolism in Cadmium-intoxicated rats: Cadmium intoxication elicited significant (P<0.05) elevations in serum total cholesterol, triglycerides, VLDL, and LDL levels, accompanied by a marked decline in HDL concentration relative to the control group (Fig. 2). Conversely, administration of *C. papaya* and *Z. mauritiana* extracts, either individually or in combination, produced significant (P<0.05) improvements in these lipid parameters. Notably, the combined treatment group (*C. papaya* + *Z. mauritiana*) exhibited the most pronounced normalization of lipid profiles, indicating a synergistic hypolipidemic and protective effect against Cd-induced dyslipidemia.

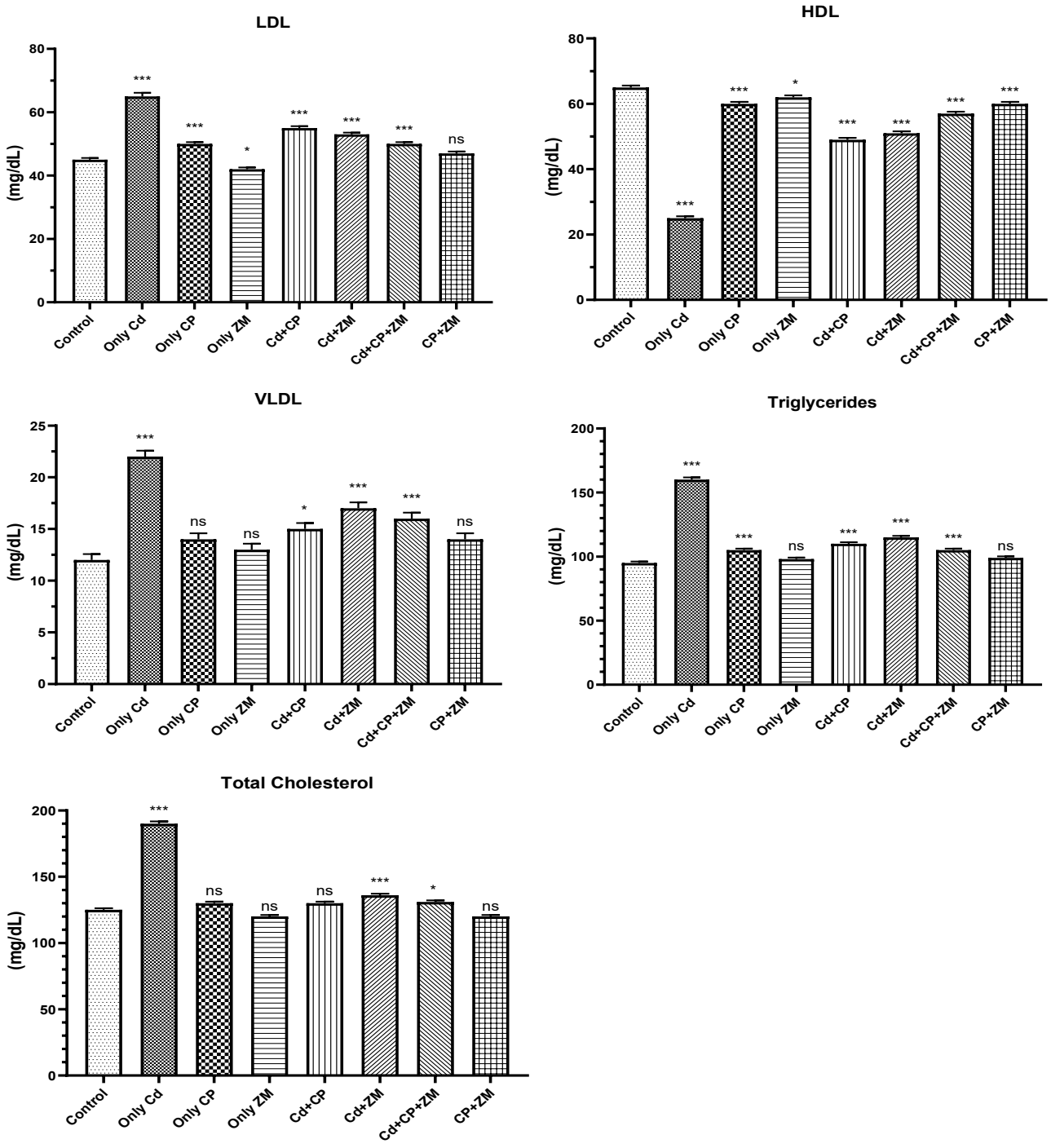

Fig. 2: Effects of *C. papaya* and *Z. mauritiana* extracts on lipid profile parameters in Cd-intoxicated rats. Values represent mean±SE (*n*=7). Significant differences were considered at P<0.05. LDL: low-density lipoprotein; HDL: high-density lipoprotein; VLDL: very-low-density lipoprotein; TG: triglycerides; TC: total cholesterol.

Effects of *C. papaya* and *Z. mauritiana* on antioxidant markers in Cadmium-intoxicated rats: *C. papaya* and *Z. mauritiana* extracts on antioxidant enzyme activities in Cd-intoxicated rats. Cd exposure significantly (P<0.05) decreased SOD, CAT, GSH, GPx, GR, GSR, GST, and TAC levels, reflecting a compromised antioxidant defense system (Table 3). Administration of either extract, particularly in combination, markedly (P<0.05) restored these activities toward normal levels, demonstrating effective mitigation of cadmium-induced oxidative imbalance.

Effects of *C. papaya* and *Z. mauritiana* on inflammatory markers in Cadmium-intoxicated rats: *C. papaya* (CP) and *Z. mauritiana* (ZM) extracts on Cd-induced inflammatory mediators in male Wistar rats. Cadmium intoxication markedly elevated COX-2, TNF-α, NF-κB, IL-6, and IL-8 levels, reflecting pronounced oxidative and inflammatory stress (Fig. 3). Treatment with CP or ZM notably downregulated these biomarkers, while the combined extract (Cd + CP + ZM) exerted superior ameliorative efficacy, restoring values closer to the control levels (Ijaz *et al*., 2024). These findings highlight the potent synergistic anti-inflammatory action of CP and ZM against Cd-induced hepatic inflammation.

Effects of *C. papaya* and *Z. mauritiana* on oxidative stress markers in Cadmium-intoxicated rats: *C. papaya* (CP) and *Z. mauritiana* (ZM) extracts on oxidative stress biomarkers in Cd-exposed rats (Table 4). Cd administration significantly (P<0.05) elevated ROS, TOS, MDA, H_2O_2, NO, protein carbonyls, and TBARS levels compared to the control group, indicating marked oxidative disturbance. Treatment with CP or ZM alone partially mitigated these alterations, while their combined administration (Cd+CP+ZM) produced a more substantial (P<0.05) restoration of oxidative parameters toward normal levels. The CP+ZM group without Cd exposure showed no significant deviation from control values, highlighting the potent antioxidant potential of the combined extracts. The combined treatment group (Cd+CP+ZM) showed significantly greater improvement compared to individual treatment groups (P<0.05), indicating enhanced efficacy.

Effects of *C. papaya* and *Z. mauritiana* on apoptotic markers in Cadmium-intoxicated rats: Table 5 highlights the effects of *C. papaya* (CP) and *Z. mauritiana* (ZM) extracts on apoptosis-associated biomarkers in Cd-intoxicated rats. Cd exposure caused a pronounced upregulation (P<0.05) of pro-apoptotic markers including BAX, BAK, caspase-3, caspase-8, caspase-9, p53, and FAS compared with control rats, reflecting enhanced apoptotic signaling. Treatment with CP or ZM alone showed no significant difference compared to control (P>0.05) relative to controls, indicating their safety at the tested doses. Co-administration of CP or ZM with Cd significantly (P<0.05) mitigated the Cd-induced elevations in apoptotic markers, while the combined treatment (Cd+CP+ZM) produced the most pronounced restoration toward control levels. Interestingly, rats receiving both extracts without Cd showed no significant deviation from baseline values, confirming the synergistic cytoprotective efficacy of the plant extracts against Cd-mediated apoptosis.

Table 2: Modulatory effects of *C. papaya* (CP) and *Z. mauritiana* (ZM) extracts on cadmium-induced alterations in renal function test of male Wistar rats

Parameter	Control	Only Cd	Only CP	Only ZM	Cd+CP	Cd+ZM	Cd+CP+ZM	CP+ZM
S. Creatinine (mg/dL)	0.70±0.06	1.50±0.06 ***	0.80±0.06 ns	0.90±0.06 ns	1.10±0.06 ***	0.90±0.06 ns	1.30±0.06 ***	1.00±0.06 *
S. Urea (mg/dL)	32.10±0.23	68.90±0.35 ***	36.40±0.23 ***	38.70±0.23 ***	48.20±0.23 ***	42.10±0.23 ***	55.50±0.29 ***	40.30±0.23 ***
BUN (mg/dL)	15.00±0.12	32.00±0.29 ***	16.80±0.12 ***	18.00±0.12 ***	22.50±0.12 ***	19.50±0.12 ***	24.00±0.12 ***	18.80±0.12 ***
Uric Acid (mg/dL)	2.10±0.06	5.20±0.06 ***	2.20±0.06 ns	2.30±0.06 ns	3.50±0.06 ***	2.60±0.06 ***	3.20±0.06 ***	2.80±0.06 ***
NGAL (ng/mL)	2.00±0.06	8.10±0.06 ***	2.53±0.04 ***	2.70±0.03 ***	4.57±0.03 ***	5.32±0.03 ***	3.54±0.03 ***	3.00±0.03 ***
KIM-1 (ng/mL)	40.00±0.58	86.51±0.58 ***	44.16±0.55 ***	47.21±0.58 ***	55.10±0.58 ***	63.20±0.58 ***	52.31±0.58 ***	46.21±0.58 ***
Semaphorin-3A (ng/mL)	4.00±0.06	13.11±0.06 ***	5.20±0.06 ***	5.50±0.06 ***	7.50±0.06 ***	8.11±0.06 ***	6.70±0.06 ***	5.15±0.06 ***

The values are displayed as the mean±SE (n=7). The significance at the level of P<0.05.S.E: Standard error, S. Creatinine: Serum Creatinine, S. Urea: Serum Urea, BUN: Blood Urea Nitrogen, NGAL: Neutrophil Gelatinase–Associated Lipocalin, KIM-1: Kidney Injury Molecule-1

Table 3: Protective effects of *C. papaya* and *Z. mauritiana* extracts against Cd-induced antioxidant markers in male Wistar rats

Parameter	Control	Only Cd	Only CP	Only ZM	Cd+CP	Cd+ZM	Cd+CP+ZM	CP+ZM
SOD	8.53±0.20	3.23±0.15 ***	7.90±0.12 ns	8.13±0.20 ns	7.23±0.15 ***	7.43±0.15 ***	7.70±0.12 **	7.87±0.15 *
CAT	42.33±1.45	15.00±0.58 ***	39.67±0.88 ns	42.67±0.88 ns	37.67±0.88 *	33.33±0.88 ***	36.67±0.88 **	35.00±0.58 ***
GSH	4.20±0.12	1.10±0.06 ***	3.77±0.09 ns	4.07±0.09 ns	3.47±0.09 ***	3.67±0.09 **	3.30±0.06 ***	3.87±0.09 ns
GPx	12.83±0.20	4.47±0.09 ***	11.60±0.12 ***	13.13±0.15 ns	10.90±0.12 ***	11.30±0.12 ***	9.50±0.12 ***	10.80±0.12 ***
GR	6.50±0.12	2.20±0.06 ***	5.90±0.12 **	6.30±0.12 ns	5.50±0.12 ***	6.00±0.12 *	4.50±0.12 ***	5.50±0.12 **
GST	18.00±0.58	6.00±0.58 ***	16.00±0.58 ns	19.00±0.58 ns	14.00±0.58 ***	13.00±0.58 ***	12.00±0.58 ***	17.00±0.58 ns
TAC	1.20±0.06	0.40±0.06 ***	1.10±0.06 ns	1.30±0.06 ns	0.80±0.06 ***	0.90±0.06 *	0.80±0.06 ***	1.00±0.06 ns

Values are presented as mean±standard error (SE; *n*=7). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test, and differences were considered significant at P<0.05. SOD: superoxide dismutase; CAT: catalase; GSH: reduced glutathione; GPx: glutathione peroxidase; GR: glutathione reductase; GST: Glutathione-S-transferase; TAC: total antioxidant capacity

Table 4: Protective effects of *C. papaya* and *Z. mauritiana* extracts against cadmium-induced Oxidative Stress markers in male Wistar rats

Parameter	Control	Only Cd	Only CP	Only ZM	Cd+CP	Cd+ZM	Cd+CP+ZM	CP+ZM
ROS (%)	98.00±0.58	145.00±1.15 ***	100.00±0.58 ns	99.00±0.58 ns	120.00±1.15 ***	114.00±1.15 ***	110.00±0.58 ***	96.00±0.58 ns
TOS (µmol H_2O_2 equiv./L)	8.00±0.06	25.00±0.29 ***	7.00±0.06 ***	9.00±0.06 ***	16.50±0.06 ***	13.00±0.06 ***	14.00±0.06 ***	10.00±0.06 ***
MDA (nmol/mg protein)	1.80±0.06	4.90±0.06 ***	2.10±0.06 *	2.00±0.06 ns	2.70±0.06 ***	3.10±0.06 ***	2.40±0.06 ***	2.00±0.06 ns
H_2O_2 (µmol/L)	22.00±0.29	48.00±0.29 ***	25.00±0.29 ***	24.00±0.29 ***	32.00±0.29 ***	29.00±0.29 ***	25.00±0.29 ***	24.00±0.29 ***
NO (µmol/L)	30.00±0.29	65.00±0.29 ***	32.00±0.29 ***	31.00±0.29 ns	42.00±0.29 ***	39.00±0.29 ***	36.00±0.29 ***	32.00±0.29 ***
Protein Carbonyls (nmol/mg)	1.30±0.06	3.80±0.06 ***	1.50±0.06 ns	1.40±0.06 ns	1.90±0.06 ***	1.70±0.06 ***	1.60±0.06 *	2.00±0.06 **
TBARS (nmol/mg)	2.50±0.06	5.40±0.06 ***	2.70±0.06 ns	2.60±0.06 ns	3.10±0.06 ***	3.40±0.06 ***	2.90±0.06 ***	2.20±0.06 *

Values are expressed as mean±SE (n=7). Significant differences were considered at P<0.05. ROS: reactive oxygen species; TOS: total oxidant status; MDA: malondialdehyde; H_2O_2: hydrogen peroxide; NO: nitric oxide; TBARS: thiobarbituric acid reactive substances; and protein carbonyls: indicators of protein oxidation

Table 5: Ameliorative effects of *C. papaya* and *Z. mauritiana* extracts on cadmium-induced alterations in apoptotic biomarkers in male Wistar rats

Parameter	Control	Only Cd	Only CP	Only ZM	Cd + CP	Cd + ZM	Cd + CP + ZM	CP + ZM
BAX (fold-change)	1.00±0.06	3.23±0.15 ***	1.20±0.06 ns	1.10±0.06 ns	1.80±0.06 ***	1.60±0.06 ***	1.40±0.06 **	1.10±0.06 ns
BAK (fold-change)	1.10±0.06	2.90±0.12 ***	1.10±0.06 ns	1.00±0.06 ns	1.60±0.06 ***	1.90±0.06 ***	1.40±0.06 *	1.30±0.06 ns
Caspase-3 (U/mg protein)	45.00±1.15	115.00±1.73 ***	50.00±1.15 ns	52.00±1.15 **	72.33±1.45 ***	87.00±1.15 ***	65.00±1.15 ***	50.00±1.15 ns
Caspase-8 (U/mg protein)	35.00±1.15	98.00±1.73 ***	39.67±0.88 ns	39.00±1.15 ns	61.00±1.15 ***	57.00±1.15 ***	55.00±1.15 ***	43.00±1.15 **
Caspase-9 (U/mg protein)	40.00±1.15	105.00±1.73 ***	42.00±1.15 ns	43.00±1.15 ns	68.00±1.15 ***	77.00±1.15 ***	60.00±1.15 ***	49.00±1.15 ***
p53 (pg/mL)	40.00±1.15	130.67±1.76 ***	42.00±1.15 ns	41.00±1.15 ns	78.00±1.15 ***	69.00±1.15 ***	65.00±1.15 ***	47.00±1.15 **
FAS (pg/mL)	50.00±1.15	122.33±1.45 ***	48.00±1.15 ns	46.00±1.15 ns	75.00±1.15 ***	83.00±1.15 ***	70.00±1.15 ***	55.00±1.15 *

Values are presented as mean±SE (n=7). Significant differences were considered at P<0.05. BAX: Bcl-2-associated X protein; BAK: Bcl-2 homologous antagonist/killer; Caspase-3: cysteine-aspartic protease-3; Caspase-8: cysteine-aspartic protease-8; Caspase-9: cysteine-aspartic protease-9; p53: tumor protein p53; FAS: Fas cell surface death receptor

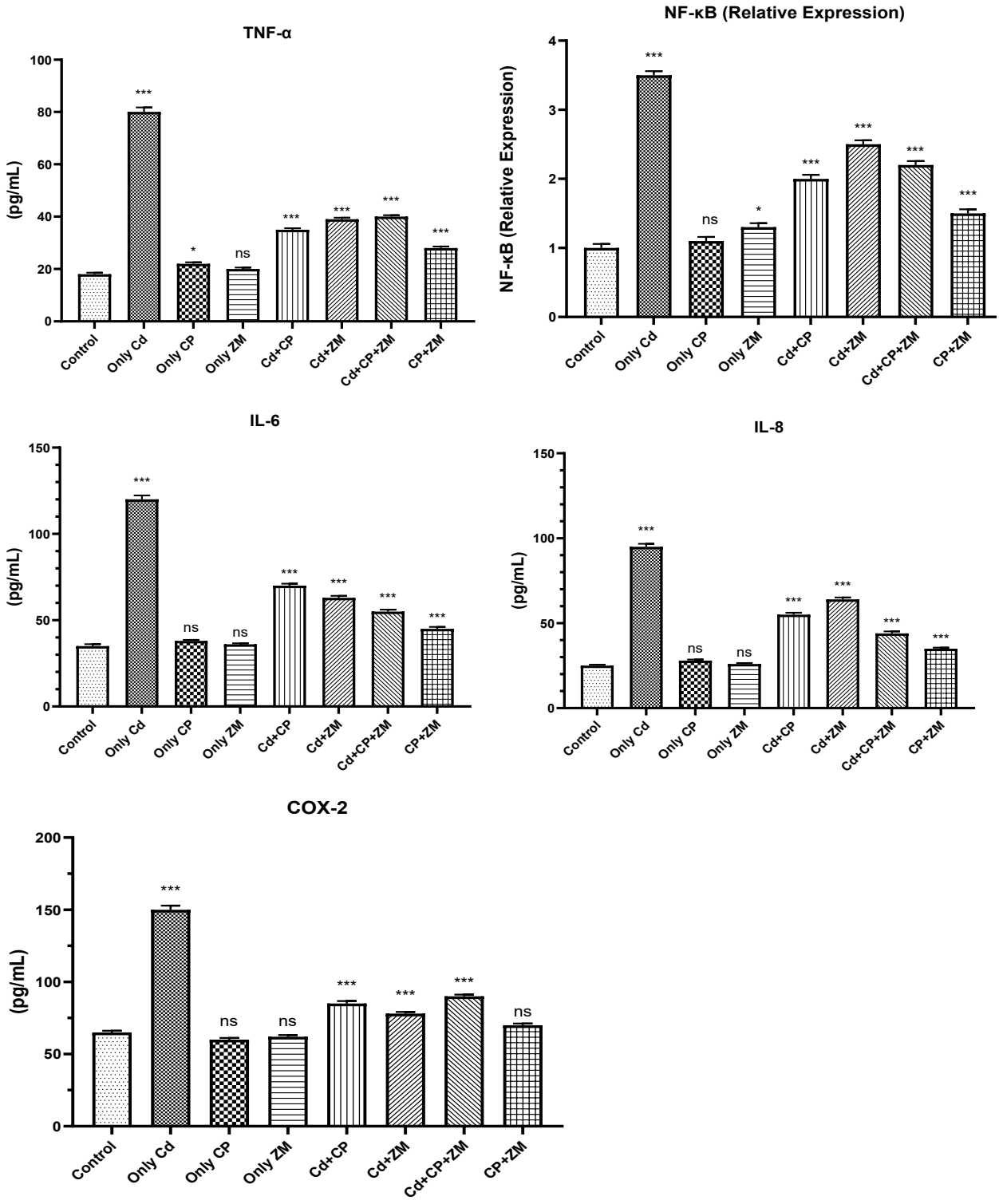

Fig. 3: Effects of *C. papaya* and *Z. mauritiana* extracts on inflammatory biomarkers (COX-2, TNF-α, NF-κB, IL-6, and IL-8) in cadmium-intoxicated rats. Values represent mean±SE (n=7). Significant differences were considered at P<0.05. COX-2: cyclooxygenase-2; TNF-α: tumor necrosis factor-alpha; NF-κB: nuclear factor kappa B; IL-6: interleukin-6; IL-8: interleukin-8.

Effects of *C. papaya* and *Z. mauritiana* on anti-apoptotic markers in Cd-intoxicated rats: *C. papaya* (CP) and *Z. mauritiana* (ZM) extracts on Cd-induced alterations in anti-apoptotic proteins in male Wistar rats were evaluated (Fig. 4). Cd intoxication significantly decreased Survivin, XIAP, Bcl-2, Bcl-xL, and Mcl-1 expression, as observed in increased apoptotic sensitivity. CP or ZM administration alone partially reversed these effects, bringing protein levels closer to the control group. Markedly, co-administration of CP and ZM (Cd+CP+ZM) was a better protective effect, which normalized anti-apoptotic protein expression. These results emphasize the strong synergistic hepatoprotective and anti-apoptotic effects of CP and ZM against Cd-induced apoptosis of cells.

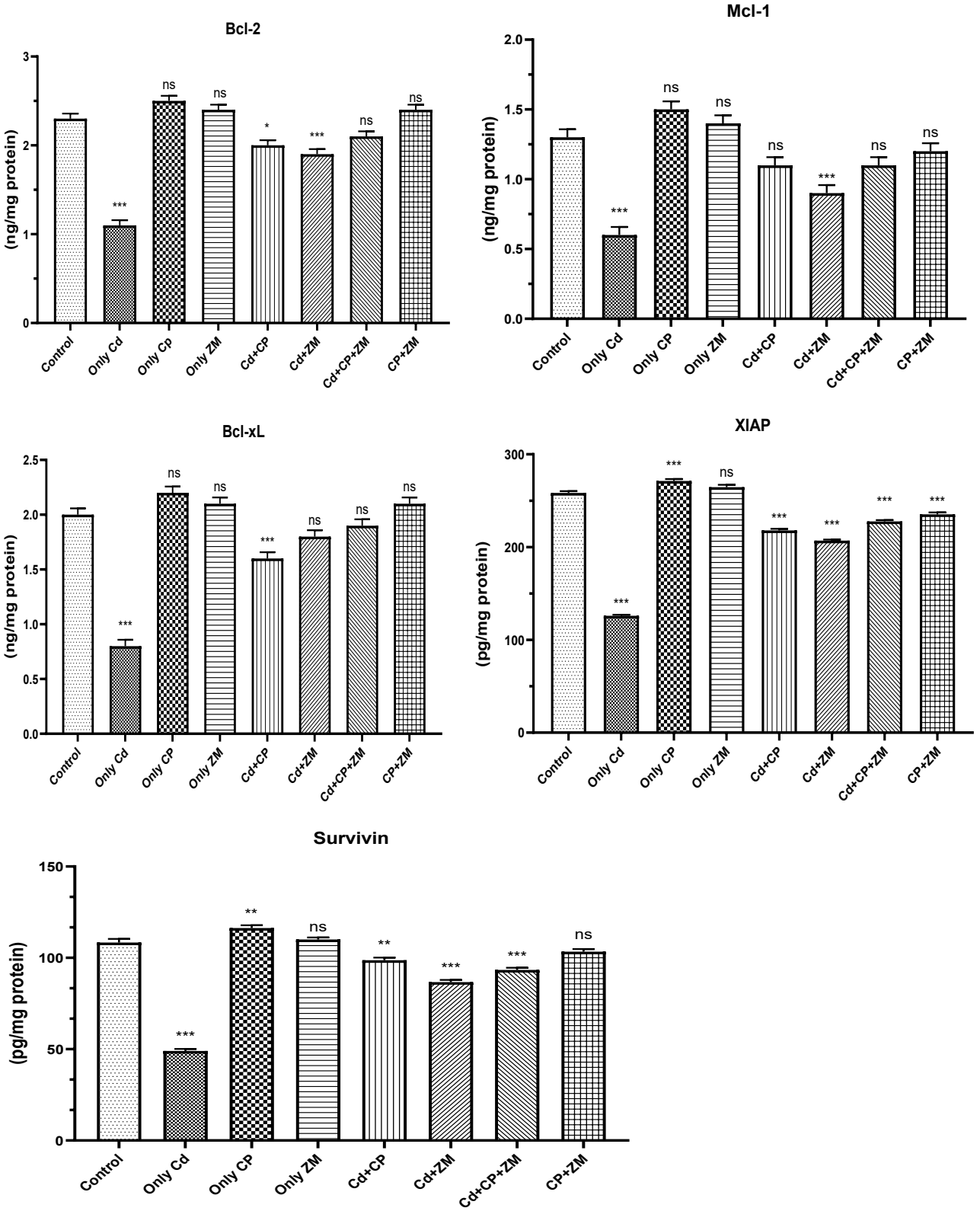

Fig. 4: Effects of *C. papaya* (CP) and *Z. mauritiana* (ZM) extracts on anti-apoptotic protein markers in cadmium-intoxicated male Wistar rats. Values represent mean±SE (n=7). Survivin: inhibitor of apoptosis protein; XIAP: X-linked inhibitor of apoptosis protein; Bcl-2: B-cell lymphoma 2; Bcl-xL: B-cell lymphoma-extra-large; Mcl-1: myeloid cell leukemia-1.

Effects of *C. papaya* and *Z. mauritiana* on hematological parameters in cadmium-intoxicated male Wistar rats: Cadmium exposure significantly changed hematological values in male Wistar rats by decreasing WBCs, RBCs, hemoglobin, hematocrit, platelet count, and lymphocyte percentage but enhancing RDW, MPV and neutrophil percentage when compared to controls ($P<0.05$). Treatment with *C. papaya* (CP) or *Z. mauritiana* (ZM) partially restored these parameters toward control values, whereas co-administration of CP and ZM most showed significantly greater improvement compared to individual treatment groups ($P<0.05$). Hematological markers, indicating a synergistic protective effect against Cd-induced hematotoxicity (Table 6).

Molecular docking analysis of phytoconstituents from *C. papaya* and *Z. mauritiana*: All major phytoconstituents from *C. papaya* and *Z. mauritiana* were docked into the active sites of Cd-binding proteins, NF-κB (1NFK) and metallothionein (2AZ5). Among them, chlorogenic acid (−11.842 kcal/mol) exhibited the highest affinity for 1NFK, while quercetin (−10.976 kcal/mol) showed the most stable interaction with 2AZ5. Both ligands established multiple hydrogen bonds and hydrophobic contacts with residues essential for Cd coordination. The docking revealed strong and stable accommodation within the active sites, indicating that these phytochemicals may disrupt Cd–protein complex formation and underlie their observed protective efficacy (Fig. 5).

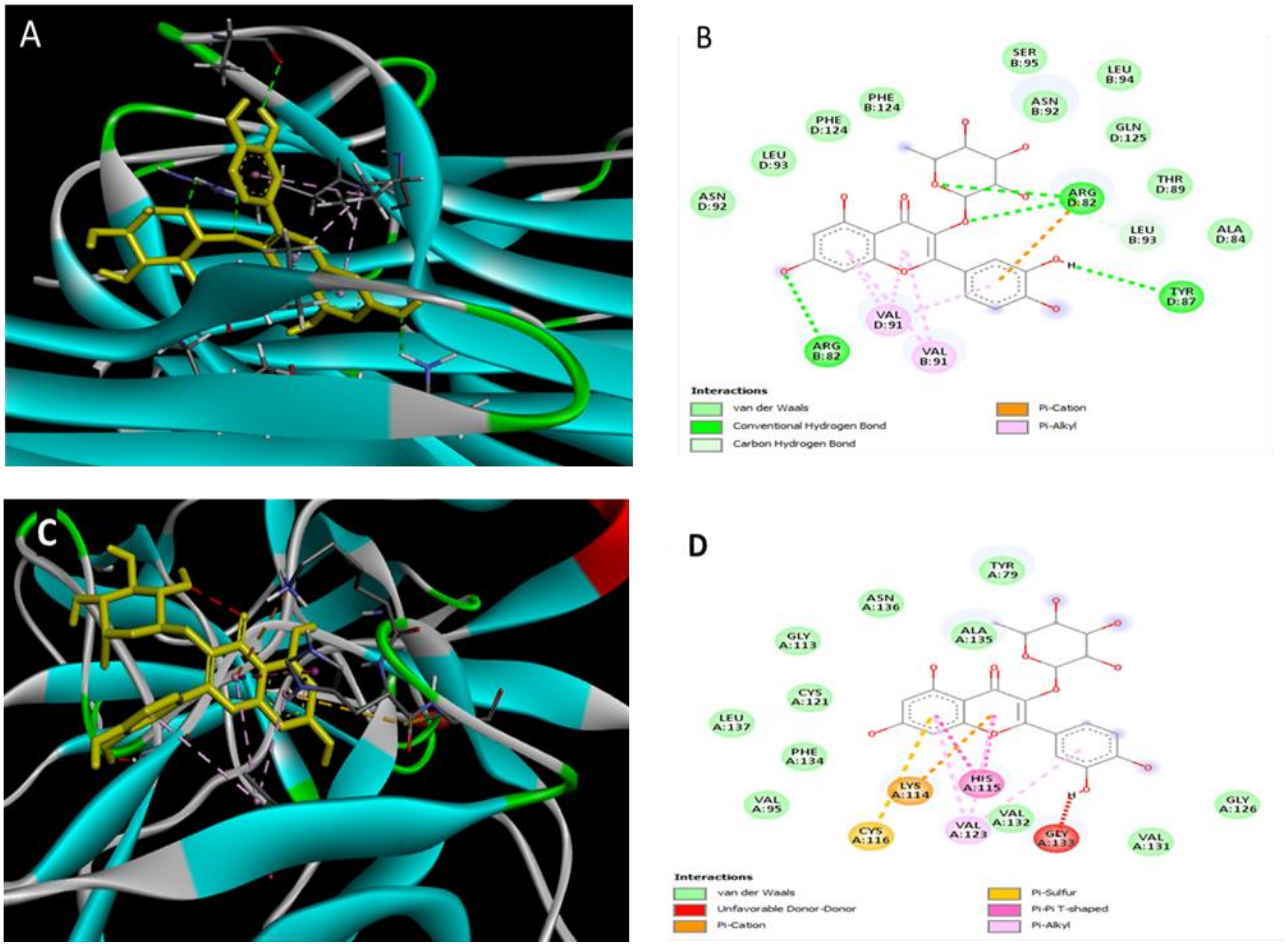

Fig. 5: Molecular docking and interaction analysis of quercetin and chlorogenic acid with target proteins. [A] Best docked pose of quercetin within the active site of 2AZ5; [B] 2D interaction map depicting hydrogen bonding and hydrophobic interactions of quercetin with amino acid residues of 2AZ5; [C] Docking pose of chlorogenic acid bound to the DNA-binding domain of NF-κB (1NFK); [D] 2D interaction plot illustrating key interactions of the chlorogenic acid–1NFK complex.

Table 6: Ameliorative effects of *C. papaya* and *Z. mauritiana* extracts on cadmium-induced alterations in hematology parameters in male Wistar rats

Parameter (Unit)	Control	Only Cd	Only CP	Only ZM	Cd+CP	Cd+ZM	Cd+CP+ZM	CP+ZM
WBC (x10³/µL)	8.50±0.12	5.20±0.12 ***	9.10±0.12 *	8.90±0.12 ns	7.40±0.12 ***	7.20±0.12 ***	6.80±0.12 ***	8.10±0.12 ns
RBC (x10⁶/µL)	8.10±0.12	6.30±0.12 ***	8.60±0.12 *	8.40±0.12 ns	7.50±0.12 *	7.70±0.12 ns	7.10±0.12 ***	8.00±0.12 ns
Hb (g/dL)	14.80±0.12	11.20±0.12 ***	15.20±0.12 ns	14.90±0.12 ns	13.70±0.12 ***	12.30±0.12 ***	12.90±0.12 ***	14.30±0.12 *
HCT (%)	44.00±0.58	35.00±0.58 ***	46.00±0.58 ns	45.00±0.58 ns	41.00±0.58 *	39.00±0.58 ***	39.00±0.58 ***	43.00±0.58 ns
RDW (%)	14.20±0.12	16.80±0.12 ***	13.30±0.12 ***	13.50±0.12 **	15.00±0.12 ***	13.10±0.12 ***	13.50±0.12 **	13.80±0.12 ns
PLT (x10³/µL)	850.00±11.55	520.00±11.55 ***	910.00±11.55 *	890.00±11.55 ns	765.00±11.55 ***	715.00±11.55 ***	695.00±11.55 ***	825.00±11.55 ns
MPV (fL)	6.70±0.12	7.50±0.12 ***	6.50±0.12 ns	6.40±0.12 ns	7.00±0.12 ns	6.90±0.12 ns	6.10±0.12 *	6.30±0.12 ns
Neutrophils (%)	24.00±0.58	32.00±0.58 ***	22.00±0.58 ns	21.00±0.58 *	27.00±0.58 *	26.00±0.58 ns	29.00±0.58 ***	25.00±0.58 ns
Lymphocytes (%)	68.00±0.58	55.00±0.58 ***	72.00±0.58 ***	70.00±0.58 ns	62.00±0.58 ***	60.00±0.58 ***	63.00±0.58 ***	66.00±0.58 ns

WBC=White Blood Cells; RBC=Red Blood Cells; Hb=Hemoglobin; HCT=Hematocrit; RDW=Red Cell Distribution Width; PLT=Platelets; MPV=Mean Platelet Volume. Cd=Cadmium; CP=*Carica papaya*; ZM=*Ziziphus mauritiana*. Values are expressed as mean±SE (n=7)

Comet assay depicting the ameliorative effects of *C. papaya* and *Z. mauritiana* on cadmium-induced genotoxicity in hepatorenal cells of male Wistar rats:

Liver Comet-assay: The degree of hepatic DNA damage was evaluated using the alkaline comet assay, and representative fluorescence micrographs (Fig. 6). Control rats (G1) displayed intact nuclei with no significant difference compared to control. DNA migration, whereas Cd exposure (G2) induced extensive comet tail formation, indicating severe hepatocellular DNA fragmentation. Administration of *C. papaya* (G3) or *Z. mauritiana* (G4) alone preserved normal nuclear morphology with no significant difference compared to control (P>0.05). DNA migration, demonstrating their genomic safety and potent antioxidant potential. Co-treatment with Cd and *C. papaya* (G5) or *Z. mauritiana* (G6) significantly reduced DNA strand breaks than the Cd group. Co-administration of both extracts with Cd (G7) was most effective in diminishing comet parameters, with values approaching control levels, while the extract combination without Cd (G8) retained normal nuclear integrity. The results prove that Cd causes extensive genotoxic damage, while *C. papaya* and *Z. mauritiana*, especially in combination, have strong protective effects against DNA damage caused by Cd.

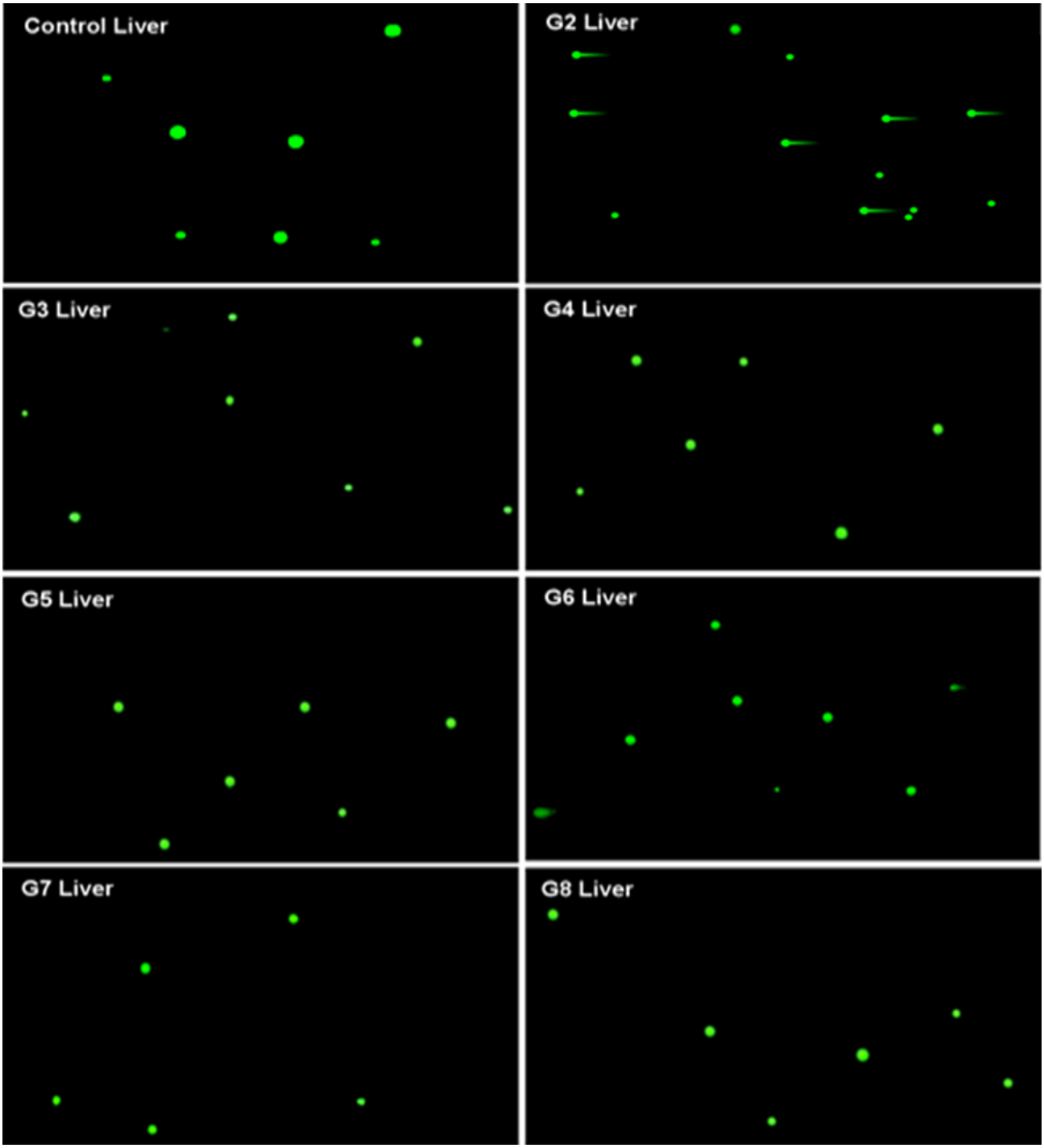

Fig.6: Comet-assay (DNA damages) in hepatic cells of rats from different experimental groups after 28 days of treatment. Control (G1); Cd-exposed (G2, 5 mg/kg b.w.); *C. papaya*-treated (G3, 300 mg/kg b.w.); *Z. mauritiana*-treated (G4, 300 mg/kg b.w.); Cd + *C. papaya* (G5); Cd+ *Z. mauritiana* (G6); Cd + *C. papaya* + *Z. mauritiana* (G7); and *C. papaya* + *Z. mauritiana* (G8).

Kidney Comet-assay: Renal DNA integrity was determined by the alkaline comet assay, and representative images (Fig. 7). Intact nuclei and no DNA migration were observed in control rats (G1), whereas Cd exposure (G2) caused significant comet tail development indicative of massive DNA strand breaks and intense kidney genotoxicity. Individual treatments with *C. papaya* (G3) or *Z. mauritiana* (G4) showed normal nuclear morphology, which confirms their genomic safety and antioxidant capability. Co-treatment with cd plus *C. papaya* (G5) or *Z. mauritiana* (G6) significantly short-lived DNA migration relative to Cd treatment alone, showing partial reversal of Cd-harm. Treatment with both extracts and Cd together (G7) provided maximum protection, restoring nuclear integrity near control levels, while the extract combination by itself (G8) maintained normal DNA structure. Collectively, these results substantiate the nephroprotective efficacy of *C. papaya* and *Z. mauritiana* against Cd-induced oxidative genotoxic stress. Cd exposure significantly increased DNA damage, as evidenced by increased tail length and DNA percentage. Treatment groups showed a significant reduction in these parameters ($P<0.05$).

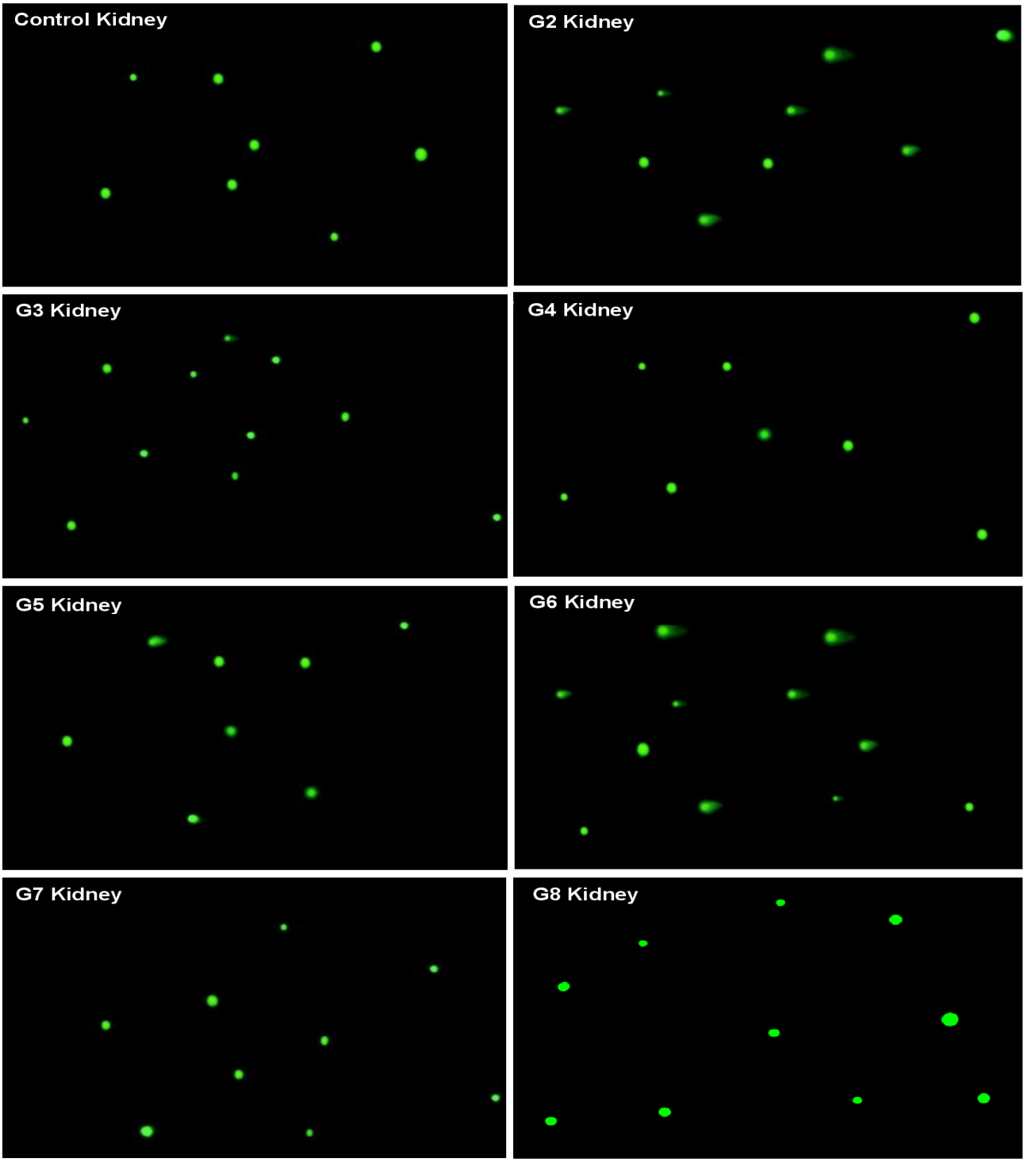

Fig. 7: Representative fluorescence micrographs of renal cells showing DNA damage assessed by the comet assay in the experimental groups described in Fig. 6. Cd exposure induced distinct comet tails, while *C. papaya* and/or *Z. mauritiana* co-treatment effectively preserved nuclear integrity, reflecting marked nephroprotection.

Histopathological examination of liver and kidneys of cadmium intoxicated male Wistar rats:

Liver histopathology: Liver sections from experimental rats (H&E, 200×) are photomicrographed (Fig. 8) where control rats (G1) exhibited normal hepatic architecture with radiating hepatocyte cords and intact central veins. Cd exposure (G2) induced severe hepatocellular degeneration, necrosis, sinusoidal congestion, and inflammatory infiltration. Treatment with *C. papaya* (G3) or *Z. mauritiana* (G4) alone preserved normal hepatic morphology. Co-treatment with *C. papaya* + Cd (G5) or *Z. mauritiana* + Cd (G6) mitigated Cd-induced lesions, showing mild degeneration and reduced inflammation. The combined extract group (*C. papaya* + *Z. mauritiana*, G8) displayed normal hepatic histoarchitecture, while rats administered both extracts with Cd (G7) exhibited near-normal hepatocytes, indicating marked hepatoprotection.

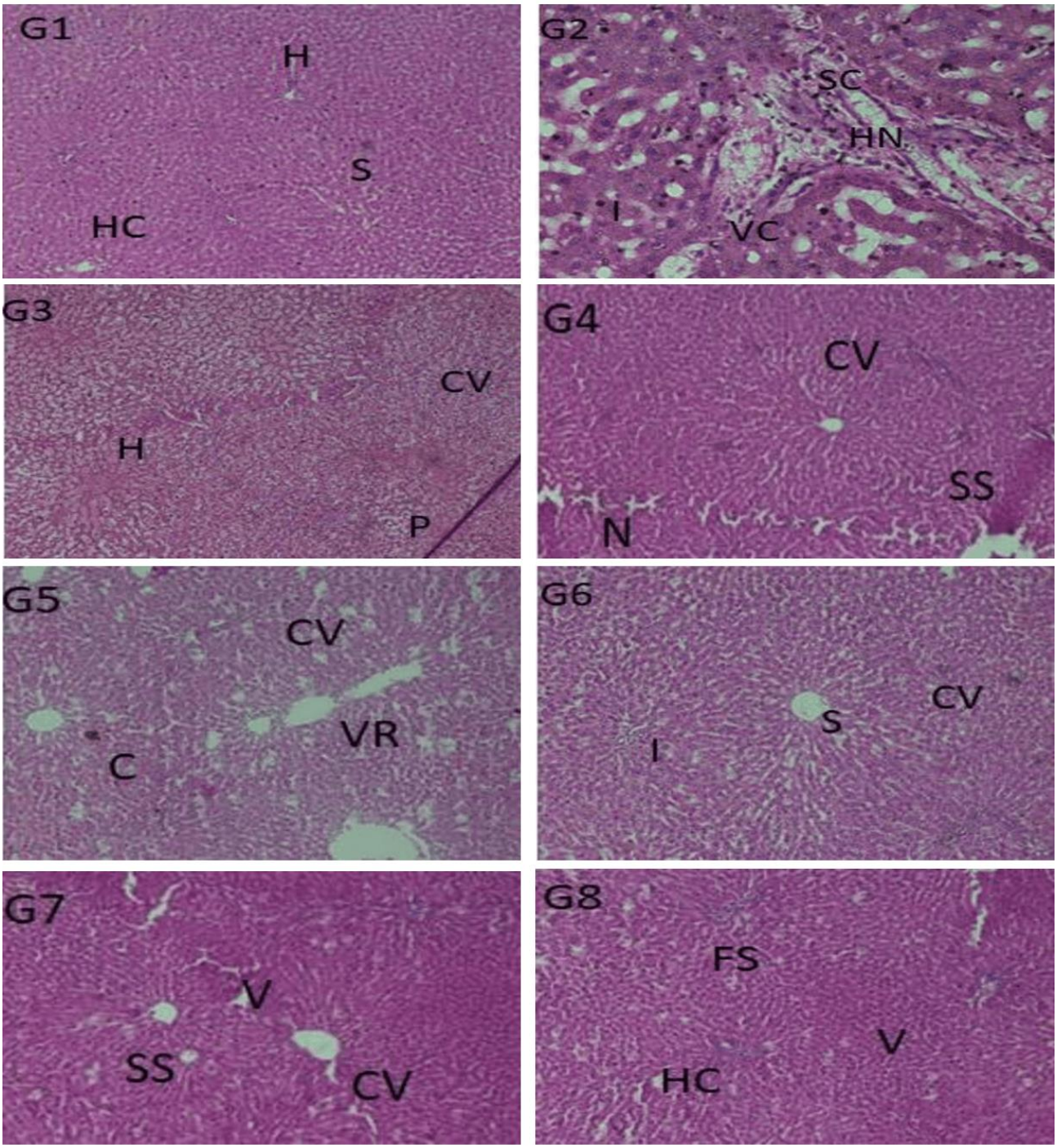

Fig. 8: Liver histopathology of rats exposed to cadmium and treated with plant extracts for 28 days (H&E, 100×). (G1) shows normal hepatic cords (HC), hepatocytes (H), and sinusoids (S). (G2) exhibits sinusoidal congestion (SC), hepatocellular necrosis (HN), vacuolated cytoplasm (VC), and inflammatory infiltration (I). (G3) shows mild hemorrhage (H), cytoplasmic vacuolation (CV), and pyknotic nuclei (P). (G4) reveals normal central vein (CV) and sinusoidal spaces (SS) with focal necrosis (N). (G5) shows mild congestion (C) around the vein region (VR). (G6) exhibits minimal infiltration (I) with distinct sinusoids (S) and central vein (CV). (G7) displays mild vacuolation (V), widened sinusoidal spaces (SS), and preserved hepatic structure, indicating marked protection. (G8) shows organized hepatic cords (HC), fine fibrous septa (FS), and mild vacuolization (V), reflecting normal hepatic morphology.

Kidneys histopathology: Histopathological alterations in kidney tissues of rats exposed to Cd and treated with plant extracts for 28 days (H&E, 200×) (Fig. 9). The control group (G1) exhibited normal renal corpuscles with intact glomeruli and well-defined tubular epithelium. Cd exposure (G2) induced pronounced tubular necrosis, epithelial desquamation, cast formation, and glomerular congestion, indicating severe nephrotoxicity. Administration of *C. papaya* (G3) or *Z. mauritiana* (G4) alone preserved normal renal histoarchitecture with minimal cellular changes. Co-treatment with *C. papaya* + Cd (G5) or *Z. mauritiana* + Cd (G6) markedly ameliorated Cd-induced lesions, exhibiting mild tubular degeneration and reduced vascular congestion. Rats receiving both extracts along with Cd (G7) displayed near-normal renal morphology, suggesting synergistic nephroprotection, whereas the combined extract group (*C. papaya* + *Z. mauritiana*, G8) demonstrated completely preserved renal histoarchitecture comparable to controls.

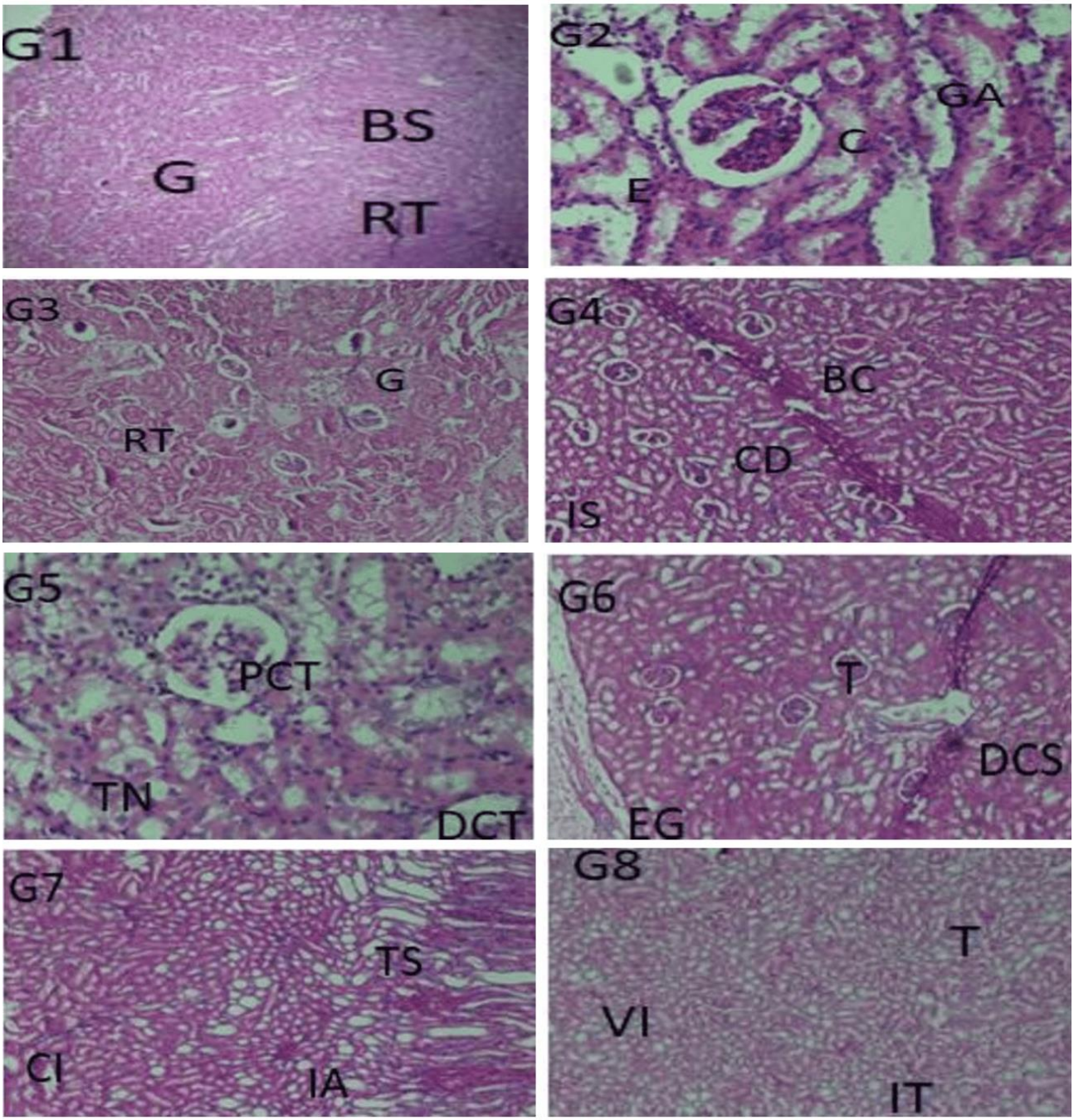

Fig. 9: Kidney histopathology of rats exposed to cadmium and treated with plant extracts for 28 days (H&E, 100×). (G1) shows normal renal architecture with intact glomeruli (G), Bowman's space (BS), and well-organized renal tubules (RT). (G2) exhibits marked glomerular atrophy (GA), tubular degeneration with interstitial edema (E), and vascular congestion (C). (G3) displays preserved renal tubules (RT) and glomeruli (G). (G4) reveals distinct Bowman's capsule (BC), collecting ducts (CD), and expanded interstitial spaces (IS). (G5) shows tubular necrosis (TN) within proximal (PCT) and distal convoluted tubules (DCT). (G6) exhibits tubular degeneration (T), erythrocyte accumulation within glomeruli (EG), and dilation in capsular space (DCS). (G7) presents improved cellular integrity (CI), preserved tubular structure (TS), and reduced interstitial area (IA), reflecting notable restoration. (G8) demonstrates well-maintained renal tubules (T), preserved vascular integrity (VI), and normal interstitial tissue (IT), denoting near-normal renal morphology.

DISCUSSION

Despite considerable advances in understanding cadmium toxicity, cadmium (Cd) exposure remains a major global health concern, contributing to hepatorenal, hematological, and molecular impairments with significant morbidity. Cd is a widespread environmental and industrial toxicant known to induce excessive generation of reactive oxygen species (ROS), leading to oxidative stress, inflammation, and apoptosis in vital organs such as the liver and kidneys (Jomova *et al*., 2023). In the present study, the protective effects of ethanolic extracts of *C. papaya* (CP) and *Z. mauritiana* (ZM) were evaluated using a multi-level approach. Phytochemical analysis confirmed the presence of bioactive compounds including flavonoids and phenolic acids, such as quercetin, gallic acid, chlorogenic acid, and rutin, which are known for their antioxidant and metal-chelating properties (Soria-Lopez *et al*., 2025). These compounds likely contribute to redox homeostasis by scavenging ROS, stabilizing cellular membranes, and reducing cadmium bioavailability through metal chelation (Sai *et al*., 2019).

Cd exposure resulted in marked hepatic dysfunction, as evidenced by elevated serum transaminases and bilirubin along with reduced albumin levels, reflecting hepatocellular injury and impaired synthetic capacity. These alterations are consistent with Cd-induced lipid peroxidation and mitochondrial dysfunction reported previously (Pan *et al*., 2018). The amelioration of these parameters following treatment with CP and ZM, supported by histopathological recovery, indicates restoration of hepatic integrity. Similarly, Cd-induced renal dysfunction, attributed to accumulation in tubular epithelial cells and disruption of transport mechanisms (Satarug *et al*., 2019), was significantly attenuated by the extracts, as reflected by normalization of renal biomarkers and improved tissue architecture.

Dyslipidemia observed in Cd-exposed rats aligns with previous findings demonstrating disruption of lipid metabolism under heavy metal stress (Matović *et al*., 2015). The reversal of lipid abnormalities by CP and ZM may be associated with improved hepatic metabolic function and reduced oxidative burden. Oxidative stress plays a central role in Cd toxicity, as indicated by depletion of endogenous antioxidant systems and increased oxidative biomarkers (Renu *et al*., 2022). The restoration of antioxidant enzyme activities in treated groups suggests activation of endogenous defense systems. A plausible mechanism involves activation of the Nrf2-ARE signaling pathway by phytochemicals such as quercetin, which enhances transcription of antioxidant genes including SOD, CAT, and GPx.

Inflammatory responses induced by Cd, characterized by upregulation of NF-κB and pro-inflammatory cytokines (Jiaxin *et al*., 2020) were significantly reduced following treatment. This anti-inflammatory effect may be attributed to inhibition of NF-κB signaling, possibly mediated by reduced oxidative stress and direct interaction of phytochemicals with inflammatory pathways. Furthermore, Cd-induced apoptotic signaling, evidenced by increased pro-apoptotic markers and suppression of anti-apoptotic proteins (Zhang *et al*., 2017), reflects mitochondrial dysfunction and activation of intrinsic apoptotic pathways. The restoration of apoptotic balance by CP and ZM suggests preservation of mitochondrial integrity and regulation of caspase activation, consistent with the known cytoprotective effects of flavonoids.

Hematological disturbances induced by Cd exposure, including alterations in erythrocyte and leukocyte parameters, are indicative of systemic oxidative stress and impaired hematopoiesis (Xiong *et al*., 2022). The normalization of these parameters following treatment may be associated with improved antioxidant status and stabilization of cellular membranes, thereby protecting blood cells from oxidative damage.

Molecular docking analysis provided supportive, albeit predictive, insights into the interaction of major phytochemicals with key molecular targets such as NF-κB. These interactions may contribute to modulation of inflammatory and apoptotic pathways; however, such findings should be interpreted cautiously due to the inherent limitations of in silico approaches (Konappa *et al*., 2020; Al-Khayri *et al*., 2022). The observed reduction in DNA damage, as evidenced by comet assay, further supports the protective role of the extracts against Cd-induced genotoxicity, likely mediated through antioxidant and DNA repair mechanisms (Samak *et al*., 2025). These findings are consistent with the observed histopathological improvements and biochemical restoration.

Notably, the combined treatment exhibited enhanced protective efficacy compared to individual treatments. This effect may be attributed to complementary mechanisms of action among the phytochemicals present in both extracts. Flavonoids such as quercetin are potent free radical scavengers, whereas phenolic acids such as gallic acid and chlorogenic acid possess strong metal-chelating properties. The combined action of ROS scavenging and cadmium chelation may reduce oxidative damage more effectively than individual treatments. Additionally, simultaneous activation of antioxidant pathways (e.g., Nrf2-ARE) and inhibition of inflammatory signaling (e.g., NF-κB) may further contribute to the observed enhanced efficacy.

This study has several limitations, including the use of only male animals, absence of dose-response analysis, use of crude extracts, and lack of pharmacokinetic evaluation. Future studies should focus on bioassay-guided fractionation, chronic toxicity models, and validation of molecular targets using gene expression or knockout approaches.

Conclusions: In conclusion, *C. papaya* and *Z. mauritiana* extracts exhibited significant protective effects against cadmium-induced toxicity, with combined treatment showing enhanced efficacy. These findings highlight their potential as natural therapeutic agents, warranting further mechanistic and clinical investigations. The extracts also mitigated the inflammation and apoptosis and maintained tissue integrity and genomic stability. Their high affinity to inflammation- and apoptosis-related targets was further suggested by molecular docking. Overall, the synergistic effects of the combined extracts were antioxidant, anti-inflammatory, anti-apoptotic, and genoprotective, and they have potential as natural therapeutic agents against hepatorenal toxicity caused by heavy-metals.

Authors contribution: SH and AR conceived and designed the study, performed the experimental work, collected and analyzed the data, and prepared the initial draft of the manuscript; AR supervised the research, provided critical guidance throughout the study, and reviewed and approved the final version of the manuscript; AA assisted in methodology development and provided technical support; FA guided in molecular analysis and contributed to manuscript revision.

Data availability statement: The data supporting the findings of this study are available within the manuscript. Additional data are securely maintained by the corresponding author and are available upon reasonable request.

Declaration of competing interest: The author declares that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Acosta-Dacal A, Melián Henríquez A, Corbera JA, *et al*., 2025. Comprehensive profiling of essential elements and organic and inorganic contaminants in Dromedary Camels from the Canary Islands: A baseline for nutritional and environmental assessment. Veterinary Sciences 12(9):829.

Akmal TA, Dina CF, Yudhana A, *et al*., 2024. Protective effect of bajakah tampala (*Spatholobus littoralis* Hassk.) on dexamethasone-induced renal toxicity in albino rats. Pakistan Veterinary Journal 44(2): 458-464.

Al-Khayri JM, Sahana GR, Nagella P, *et al*., 2022. Flavonoids as potential anti-inflammatory molecules: A review. Molecules 27(9):2901.

Alara OR, Abdurahman NH and Alara JA, 2022. *Carica papaya*: comprehensive overview of the nutritional values, phytochemicals and pharmacological activities. Advances in traditional medicine 22(1):17-47.

Benjamin B, 2019. Overview of laboratory animal lifestyle, care, and management: a case study of Wistar rats. Journal of Applied Sciences and Environmental Management 23(8):1431-1435.

Cañas Portilla AI, Castaño A and Organization WH, 2024. Human health effects of benzene, arsenic, cadmium, nickel, lead and mercury: Report of an expert consultation.

Chen H, Zhu C and Zhou X, 2023. Effects of lead and cadmium combined heavy metals on liver function and lipid metabolism in mice. Biological Trace Element Research 201(6):2864-2876.

De Ruyck J, Brysbaert G, Blossey R, *et al*., 2016. Molecular docking as a popular tool in drug design, an in silico travel. Advances and Applications in Bioinformatics and Chemistry 1-11.

Dwivedi S, Kushalan S, Paithankar JG, *et al*., 2022. Environmental toxicants, oxidative stress and health adversities: interventions of phytochemicals. Journal of Pharmacy and Pharmacology 74(4):516-536.

Eissa LA, Kenawy HI, El-Karef A, *et al*., 2018. Antioxidant and anti-inflammatory activities of berberine attenuate hepatic fibrosis induced by thioacetamide injection in rats. Chemico-biological Interactions 294:91-100.

El-Boshy ME, Risha EF, Abdelhamid FM, *et al*., 2015. Protective effects of selenium against cadmium induced hematological disturbances, immunosuppressive, oxidative stress and hepatorenal damage in rats. Journal of Trace Elements in Medicine and Biology 29:104-110.

El-Bahr S, 2015. Effect of curcumin on hepatic antioxidant enzymes activities and gene expressions in rats intoxicated with aflatoxin B1. Phytotherapy research 29(1):134-140.

Elbaghdady HAM, Alwaili MA and El-Demerdash RS, 2018. Regenerative potential of bone marrow mesenchymal stem cells on cadmium chloride-induced hepato-renal injury and testicular dysfunction in sprague dawley rats. Ecotoxicology and Environmental Safety 164:41-49.

Ganapathy S, Muraleedharan A, Sathidevi PS, *et al*., 2016. CometQ: An automated tool for the detection and quantification of DNA damage using comet assay image analysis. Computer Methods and Programs in Biomedicine 133:143-154.

Hadzhi T, Monama L, Gololo S and Mathe E, 2024. Pharmacological review of Ziziphus plants and jujuboside A & B compounds. Horticulture International Journal 8(2):55-61.

Idris SY, Kakkaba HA, Enam SJ, et al., 2020. Selenium supplementation ameliorated paraquat-induced haemotoxicity in male rats. Savannah Veterinary Journal 3:17-21.

Ijaz MU, Nadeem A, Hayat MF, *et al*., 2024. Evaluation of possible ameliorative role of robinetin to counteract polystyrene microplastics instigated renal toxicity in rats. Pakistan Veterinary Journal 44(2):400-404.

Jacob Filho W, Lima CC, Paunksnis MRR, *et al*., 2018. Reference database of hematological parameters for growing and aging rats. The Aging Male 21(2):145-148.

Jiaxin S, Shengchen W, Yirong C, *et al*., 2020. Cadmium exposure induces apoptosis, inflammation and immunosuppression through CYPs activation and antioxidant dysfunction in common carp neutrophils. Fish and Shellfish Immunology 99:284-290.

Jomova K, Raptova R, Alomar SY, *et al*., 2023. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Archives of Toxicology 97(10):2499-2574.

Khadam S, Afzal U, Gul H, *et al*., 2019. Phytochemical screening and bioactivity assessment of leaves and fruits extract of Carica papaya. Pakistan Journal of Pharmaceutical Sciences 32(5):1941-1948.

Konappa N, Udayashankar AC, Krishnamurthy S, *et al*., 2020. GC–MS analysis of phytoconstituents from Amomum nilgiricum and molecular docking interactions of bioactive serverogenin acetate with target proteins. Scientific Reports 10(1):16438.

Laib I, Ali BD, Alsalme A, *et al*., 2024. Therapeutic efficacy of *Helianthemum lippii* extract and silver nanoparticles synthesized from the extract against cadmium-induced renal nephrotoxicity in wistar rats. Pharmaceuticals 17(8):982.

M Brzóska M, Borowska S and Tomczyk M, 2016. Antioxidants as a potential preventive and therapeutic strategy for cadmium. Current Drug Targets 17(12):1350-1384.

Matović V, Buha A, Đukić-Ćosić D, *et al*., 2015. Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys. Food and Chemical Toxicology 78:130-140.

Mossa ATH, Swelam ES and Mohafrash SM, 2015. Sub-chronic exposure to fipronil induced oxidative stress, biochemical and histopathological changes in the liver and kidney of male Wistar rats. Toxicology Reports 2:775-784.

Moukadiri H, Noukrati H, Ben Youcef H, *et al*., 2024. Impact and toxicity of heavy metals on human health and latest trends in removal process from aquatic media. International Journal of Environmental Science and Technology 21(3):3407-3444.

Nordberg M and Nordberg GF, 2022. Metallothionein and cadmium toxicology—Historical review and commentary. Biomolecules 12(3):360.

Omidiwura B, 2017. Qualitative and quantitative analysis of pawpaw (*Carica papaya*) leaf extract and its antimicrobial effect in animal production. Nigerian Journal of Animal Production 44(3):76-83.

Pan YX, Luo Z, Zhuo MQ, *et al*., 2018. Oxidative stress and mitochondrial dysfunction mediated Cd-induced hepatic lipid accumulation in zebrafish Danio rerio. Aquatic Toxicology 199:12-20.

Pan Y, Ding L, Xie S, *et al*., 2021. Spatiotemporal simulation, early warning, and policy recommendations of the soil heavy metal environmental capacity of the agricultural land in a typical industrial city in China: Case of Zhongshan City. Journal of Cleaner Production 285:124849.

Park JH, Lee BM and Kim HS, 2021. Potential protective roles of curcumin against cadmium-induced toxicity and oxidative stress. Journal of Toxicology and Environmental Health Part B 24(3):95-118.

Pecoraro L, Wang X, Shah D, *et al*., 2021. Biosynthesis pathways, transport mechanisms and biotechnological applications of fungal siderophores. Journal of Fungi 8(1):21.

Rehman ZU, Khan S, Brusseau ML, *et al*., 2017. Lead and cadmium contamination and exposure risk assessment via consumption of vegetables grown in agricultural soils of five-selected regions of Pakistan. Chemosphere 168:1589-1596.

Renu K, Mukherjee AG, Wanjari UR, *et al*., 2022. Misuse of cardiac lipid upon exposure to toxic trace elements—A focused review. Molecules 27(17):5657.

Sai K, Thapa R, Devkota HP, *et al*., 2019. Phytochemical screening, free radical scavenging and α-amylase inhibitory activities of selected medicinal plants from Western Nepal. Medicines 6(2):70.

Samak DH, Abd-Ellatieff HA, Khalil RH, *et al*., 2025. Mitigation of cadmium toxicity in African catfish using biological Nano chitosan: insights into biochemical, genotoxic, and histopathological effects. BMC Veterinary Research 21(1):278.

Satarug S, Boonprasert K, Gobe GC, *et al*., 2019. Chronic exposure to cadmium is associated with a marked reduction in glomerular filtration rate. Clinical Kidney Journal 12(4): 468-475.

Satarug S, Vesey DA and Gobe GC, 2017. Health risk assessment of dietary cadmium intake: do current guidelines indicate how much is safe? Environmental Health Perspectives 125(3):284-288.

Sinzato YK, Rodrigues T, Cruz LL, *et al*., 2023. Assessment of oxidative stress biomarkers in rat blood. Bio-Protocol 13(5):e4626.

Soria-Lopez A, Pecio Ł, Saber FR, *et al*., 2025. Metabolic profiling via UPLC/MS/MS and in vitro cholinesterase, amylase, glucosidase, and tyrosinase inhibitory effects of Carica papaya L. extracts reveal promising nutraceutical potential. Food Analytical Methods 18(1):57-74.

Souza-Arroyo V, Fabián JJ, Bucio-Ortiz L, *et al*., 2022. The mechanism of the cadmium-induced toxicity and cellular response in the liver. Toxicology 480:153339.

Unsal V, Dalkıran T, Çiçek M and Kölükçü E, 2020. The role of natural antioxidants against reactive oxygen species produced by cadmium toxicity: a review. Advanced Pharmaceutical Bulletin 10(2):184.

Urani C, Melchioretto P, Bruschi M, *et al*., 2015. Impact of cadmium on intracellular zinc levels in HepG2 cells: quantitative evaluations and molecular effects. BioMed Research International 2015(1): 949514.

Xiong L, Fan C, Song J, *et al*., 2022. Associations of long-term cadmium exposure with peripheral white blood cell subtype counts and indices in residents of cadmium-polluted areas. Chemosphere 308:135946.

Yang Y, Wu YN, Zhang YH, *et al*., 2025. Distribution characteristics and pollution assessment of lead and Cadmium content in selected dairy farms in Jiangsu, China. Veterinary Sciences 12(11):1042.

Zhang R, Yi R, Bi Y, *et al*., 2017. The effect of selenium on the Cd-induced apoptosis via NO-mediated mitochondrial apoptosis pathway in chicken liver. Biological Trace Element Research 178(2):310-319.