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Protective structural and enzymatic roles of catechin and gallic acid against nephrotoxicity and hepatotoxicity ınduced by cisplatin

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This study aims to investigate the structural and enzymatic effects of Catechin (K) and gallic acid (G) in the treatment of nephrotoxicity and hepatotoxicity induced by cisplatin. Cisplatin (cis-[$Pt(NH_3)_2Cl_2$]), while commonly used as a cancer treatment, can have toxic effects on the kidneys and liver. In the study, cisplatin (15 mg/kg) was administered intraperitoneally to rats to induce toxicity, and then Catechin ($C_{15}H_{14}O_6$) (20 mg/kg) and gallic acid (3,4,5-Trihydroxybenzoic acid) (100 mg/kg) were administered by oral gavage for 4 weeks. The experimental results showed that cisplatin treatment caused a loss of body weight, as well as reductions in kidney and liver weights. Significant damage to kidney and liver tissues was observed, along with increased serum levels of urea, creatinine, BUN, AST, ALT, and tissue levels of MDA. Additionally, increased levels of GPx and GSR were recorded. The results suggest that catechin and gallic acid significantly ameliorate cisplatin-induced kidney and liver damage.

Cancer is a major global health challenge and ranks among the leading causes of mortality worldwide, primarily due to the uncontrolled proliferation and abnormal growth of cells caused by DNA damage. Despite advances in early diagnosis and therapeutic strategies, cancer treatment remains complex and often relies heavily on chemotherapeutic agents[1–4]. Cisplatin, a platinum-based chemotherapeutic drug, is one of the most commonly used agents in the treatment of various malignancies, including cancers of the head and neck, lung, ovary, bladder, testis, and others[1–4]. Its mechanism of action involves the formation of DNA crosslinks, leading to apoptosis and inhibition of tumor growth[3,4].

However, the clinical utility of cisplatin is substantially limited by its dose-dependent toxic side effects, particularly nephrotoxicity and hepatotoxicity, which can cause irreversible organ damage and necessitate treatment discontinuation[1,5]. Nephrotoxicity is considered the most significant adverse effect, characterized by renal tubular cell injury mediated largely through oxidative stress pathways. Excessive production of reactive oxygen species (ROS) during cisplatin therapy disrupts cellular homeostasis by damaging lipids, proteins, and DNA, leading to mitochondrial dysfunction and apoptosis in renal cells[4,6–11]. Similarly, cisplatin-induced hepatotoxicity results from oxidative stress-induced mitochondrial damage in hepatocytes, leading to impaired liver function and structural damage[12].

Given the pivotal role of oxidative stress in the pathogenesis of cisplatin-induced toxicity, considerable attention has been directed toward natural antioxidant compounds that can mitigate these harmful effects[1,13,14]. Flavonoids and phenolic acids, which are abundant in plant-based foods, have demonstrated promising antioxidant and anti-inflammatory properties capable of protecting cells against oxidative injury[15–18]. Catechin, a flavonoid, has been reported to enhance endogenous antioxidant defenses by increasing the activities of enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), effectively scavenging harmful ROS including superoxide, hydroxyl, and peroxyl radicals. Gallic acid, a naturally occurring phenolic acid found in many fruits and vegetables, exhibits antioxidant, anticarcinogenic, and antimutagenic effects, contributing to cellular protection against oxidative stress[14,19].

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Despite these beneficial properties, the precise mechanisms and efficacy of catechin and gallic acid in protecting against cisplatin-induced renal and hepatic toxicity remain to be fully elucidated. Therefore, the present study aims to investigate the toxic effects of cisplatin and to evaluate the nephroprotective and hepatoprotective potential of catechin and gallic acid, focusing on their antioxidant and cytoprotective mechanisms in an experimental rat model.

Materials and methods

Ethical approval and animal husbandry

A total of 96 male albino Wistar rats (8–10 weeks old, 150–250 g) were procured from the Animal Laboratory of Aydın Adnan Menderes University. Experimental procedures received prior approval from the Animal Ethics Committee of Adnan Menderes University (approval number: 64583101/2022/92). Animals were housed in groups of four per cage under controlled conditions: 12-h light/dark cycle, temperature maintained at 21 ± 1 °C, and relative humidity between 45 and 65%. Standard rodent chow and tap water were provided ad libitum. A one-week acclimatization period was implemented prior to experimentation to minimize stress-related confounding factors. All animal care and experimental manipulations conformed to institutional, national, and international ethical standards, and the study adhered to the ARRIVE guidelines [REF ARRIVE].

Chemicals and preparation

Cisplatin (TCI Chemicals), gallic acid (Biobasic), and catechin (Cayman Chemical) were utilized. Stock solutions of gallic acid and catechin were freshly prepared daily by dissolving in distilled water to ensure stability and potency prior to administration.

Experimental design and group allocation

Ninety-six rats were randomized into eight groups (n = 12 each) as follows:

Group 1 (Control): Oral administration of 3 mL physiological saline.
Group 2 (Catechin): Catechin 20 mg/kg via oral gavage[20,21].
Group 3 (Gallic Acid): Gallic acid 100 mg/kg via oral gavage[22–24].
Group 4 (CP): Cisplatin 15 mg/kg intraperitoneally. (i.p.)[7,25,26]
Group 5 (CP + Catechin): Cisplatin 15 mg/kg (i.p.) + catechin 20 mg/kg (oral gavage).
Group 6 (CP + Gallic Acid): Cisplatin 15 mg/kg (i.p.) + gallic acid 100 mg/kg (oral gavage).
Group 7 (Catechin + Gallic Acid): Catechin 20 mg/kg + gallic acid 100 mg/kg orally.
Group 8 (CP + Catechin + Gallic Acid): Cisplatin 15 mg/kg (i.p.) + catechin 20 mg/kg + gallic acid 100 mg/kg (oral gavage).

Catechin and gallic acid administrations were separated by a 1-h interval to minimize interaction. All treatments were administered daily for four weeks. Animals were monitored daily for clinical signs, food and water intake, and body weight, which were recorded at baseline and weekly.

Drug administration routes and timing

Cisplatin was delivered intraperitoneally in a single dose of 15 mg/kg consistent with nephrotoxicity induction protocols[27–29]. Gallic acid and catechin were administered orally by gavage once daily. In groups receiving both antioxidants, a 1-h interval between administrations was maintained to prevent potential pharmacokinetic interference.

Anesthesia and euthanasia procedures

At designated time points (weekly, three animals per group), rats were anesthetized with ketamine (100 mg/kg) and xylazine (5 mg/kg) via intraperitoneal injection. Upon reaching deep anesthesia, euthanasia was performed by cervical dislocation following AVMA guidelines to ensure rapid and humane death.

Sample collection and biochemical analysis

Following 12-h fasting, blood was collected via intracardiac puncture into serum-separating tubes. Samples were centrifuged at 3500–4000 rpm for 10 min at 4 °C. Serum aliquots were analyzed for renal (urea, BUN, creatinine) and hepatic (AST, ALT) function markers using validated automated analyzers at the Biochemistry Laboratory of Aydın Adnan Menderes University Research and Training Hospital.

Post-mortem, liver and kidneys were excised, rinsed with cold physiological saline, blotted, and weighed. Tissues were immediately processed or stored at − 80 °C for subsequent biochemical assays. Body weight changes were tracked to assess systemic toxicity.

Preparation of tissue homogenates

Liver and kidney samples were homogenized in cold phosphate-buffered saline (PBS, pH 7.4) at a 1:10 (w/v) ratio using a mechanical homogenizer. Homogenates were centrifuged at 14,000 rpm for 20 min at 4 °C. Supernatants were aliquoted and stored at − 80 °C. Oxidative stress biomarkers—malondialdehyde (MDA) (A.B.T. Laboratory Industry, Atlas Biotechnology, Turkey), Cat. No. ABT10165Ra), glutathione peroxidase (GPx) (A.B.T. Laboratory Industry, Atlas Biotechnology, Turkey), Cat. No. ABT2557Ra), and glutathione reductase (GR) (A.B.T. Laboratory Industry, Atlas Biotechnology, Turkey), Cat. No. ABT3544Ra)—were quantified using standardized enzymatic assay kits at Atlas Biotechnology Laboratory, following manufacturer instructions and previously described protocols[30–32].

Histopathological and morphometric evaluation

Tissue samples were fixed in 10% neutral buffered formalin for 24–48 h, dehydrated, and embedded in paraffin. Sections (4–5 µm) were stained with hematoxylin–eosin (H&E) for general morphology and picro-sirius red for collagen detection using the GBL CONVASTAIN Picro Sirius Red Kit (GBL Microscopy, Turkey Ref No: 5002). Staining procedures conformed to established protocols[33–35].

Histological assessments were performed blinded by two independent histologists and one experienced pathologist. Renal tubular injury was semi-quantitatively scored on a 0–5 scale reflecting the extent of tissue damage[36]. Collagen deposition indicating fibrosis was morphometrically quantified in ten random cortical and medullary fields per kidney section at 40× magnification using Figure J software, with fibrosis severity scored 0–3[37,38].

Liver sections were evaluated for inflammation, congestion, hepatocyte degeneration, sinusoidal dilatation, Kupffer cell proliferation, and necrosis. Semi-quantitative scoring ranged from 0 (normal) to 3 (severe)[39]. Fibrosis grading followed METAVIR-based scales from 0 to 4, assessing collagen fiber distribution and septal formation[40,41]. These scoring systems provide reproducible, standardized evaluation of hepatic and renal pathology[42–44].

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism 8.02 (GraphPad Software, La Jolla, CA). Parametric data were analyzed by one-way ANOVA with Tukey's or Dunnett's post hoc tests; non-parametric data were analyzed using the Kruskal–Wallis test where appropriate. A p-value of < 0.05 was considered statistically significant.

Results

Examination of body weight

A significant decrease in body weight was observed in the CP group compared to the control, catechin, gallic acid, and combined catechin plus gallic acid treatment groups. However, treatment with catechin and gallic acid alleviated the CP-induced body weight loss. The weekly weight changes of the groups are presented in Fig. 1.

An equal number of rats (n = 24) were assigned to each experimental group throughout the study. At the start of the experiment, there were no statistically significant differences in body weight among the groups ($p > 0.05$). Over the 4-week period, all cisplatin-treated groups exhibited significantly lower body weights compared to the control, catechin, gallic acid, and catechin + gallic acid groups ($p < 0.05$). However, the body weights of the groups receiving cisplatin combined with catechin, gallic acid, or both were significantly higher than those of the cisplatin-only group ($p < 0.05$). Among these, the cisplatin + gallic acid group showed significantly lower body weights than the cisplatin + catechin group ($p < 0.05$). The greatest body weight increase was observed in the group treated with cisplatin combined with both catechin and gallic acid ($p < 0.05$). Notably, all cisplatin-treated groups demonstrated a significant increase in body weight starting from the third week ($p < 0.05$).

Examination of kidney weight

Regarding kidney weights, the cisplatin (CP)-treated group exhibited a significant increase compared to the control and other treatment groups (catechin and gallic acid). However, this increase was attenuated in the

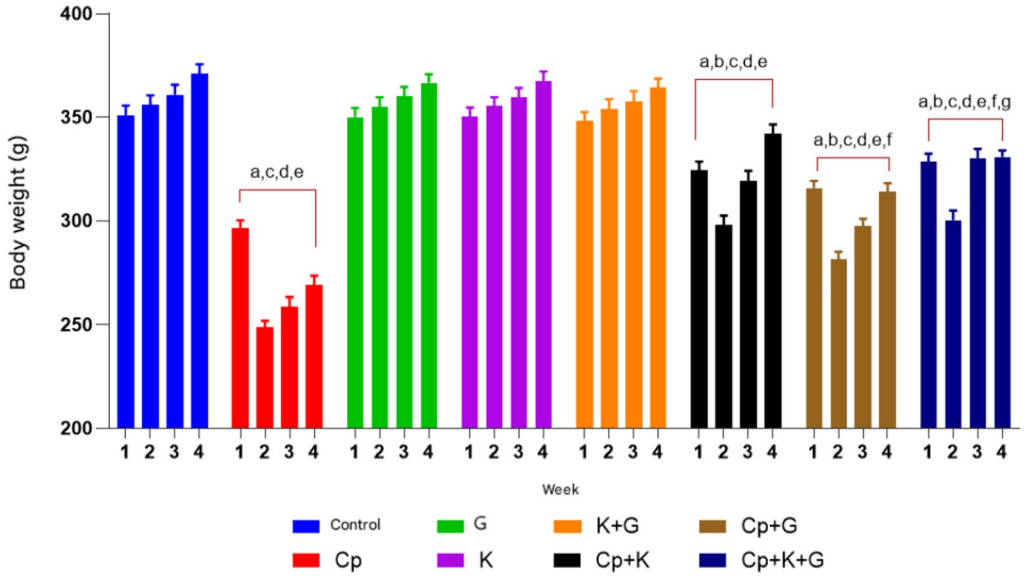

Fig. 1. Weekly weight change graph of groups. (a $p < 0.05$; compared to the control group, b $p < 0.05$; compared to the K group, c $p < 0.05$; compared to the G group, d $p < 0.05$; compared to the K + G group, e $p < 0.05$; compared to the CP group, f $p < 0.05$; compared to the CP + K group, g $p < 0.05$; compared to the CP + G group).

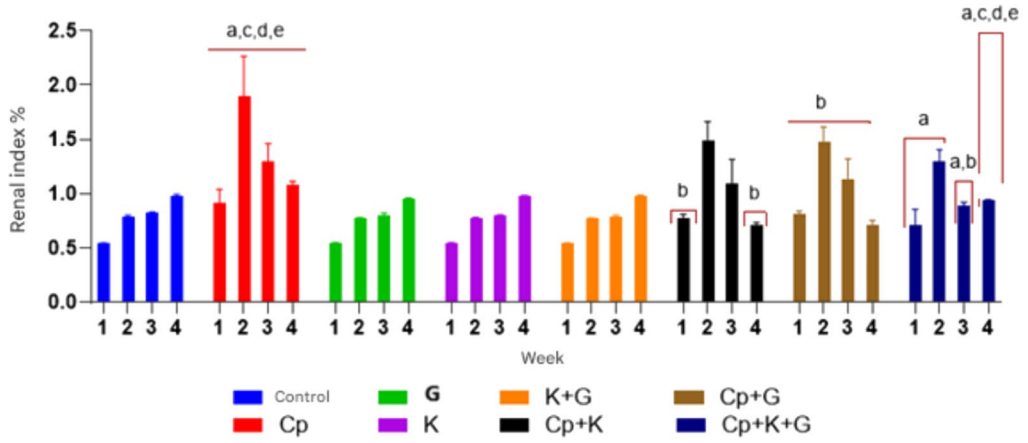

Fig. 2. Graphical representation of kidney indices of experimental groups (kidney indices = kidney weight/body weight). A total of 24 rats were included per week. Kidney indices were calculated as the ratio of the combined weight of the right and left kidneys to the total body weight. (a $p < 0.05$; compared to control group, b $p < 0.05$; compared to CP group, c $p < 0.05$; compared to K group, d $p < 0.05$; compared to G group, e $p < 0.05$; compared to K + G group).

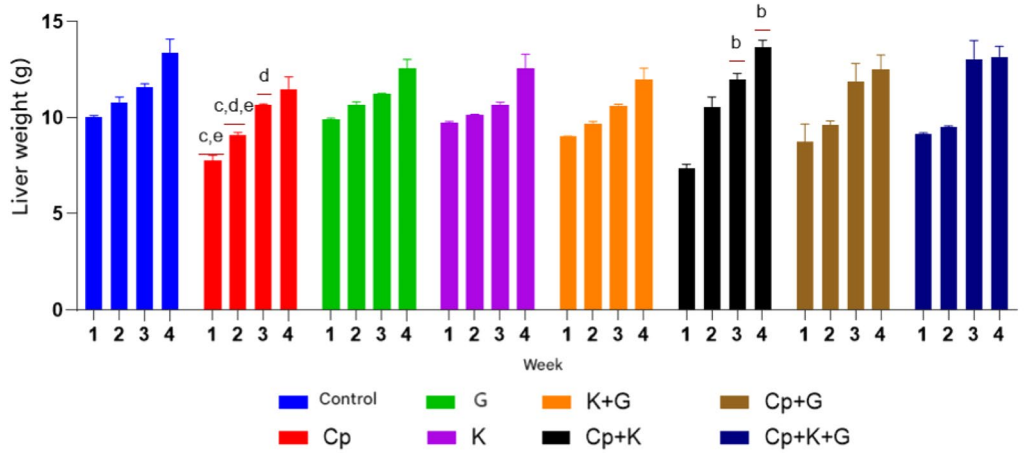

Fig. 3. Liver weight graph of experimental groups. (b $p < 0.05$; compared to CP group, c $p < 0.05$; compared to K group, d $p < 0.05$; compared to G group, e $p < 0.05$; compared to K + G group).

groups treated with catechin and gallic acid. The graphical representation of the kidney indexes (kidney weight/body weight ratio) for the experimental groups is presented in Fig. 2.

Although slight increases in kidney indices were observed in the control, catechin, gallic acid, and catechin + gallic acid groups, these changes were not statistically significant ($p > 0.05$). In the cisplatin-only group, kidney weights and indices at weeks 2, 3, and 4 were significantly higher compared to the control and other treatment groups ($p < 0.05$). At week 4, both the cisplatin + catechin and cisplatin + gallic acid groups exhibited significantly lower kidney indices compared to the cisplatin-only group ($p < 0.05$). Additionally, the cisplatin + catechin + gallic acid group showed significantly reduced kidney indices at weeks 3 and 4 relative to the cisplatin group at weeks 2 and 3 ($p < 0.05$). Overall, while cisplatin administration led to an increase in kidney index values, co-administration with catechin and/or gallic acid effectively mitigated this effect in a time-dependent manner.

Examination of liver weight

When comparing liver weights, the CP-treated group showed an increase compared to the control and other treatment groups. However, this increase was attenuated in the groups treated with catechin and gallic acid. (The weight graph of the experimental groups is shown in Fig. 3, and the weekly change in liver indices of the groups is presented in Fig. 4).

In the cisplatin-only group, liver weights recorded at weeks 1, 2, and 3 were significantly higher than those observed in several treatment groups at week 3, including the catechin-only, gallic acid-only, and catechin + gallic

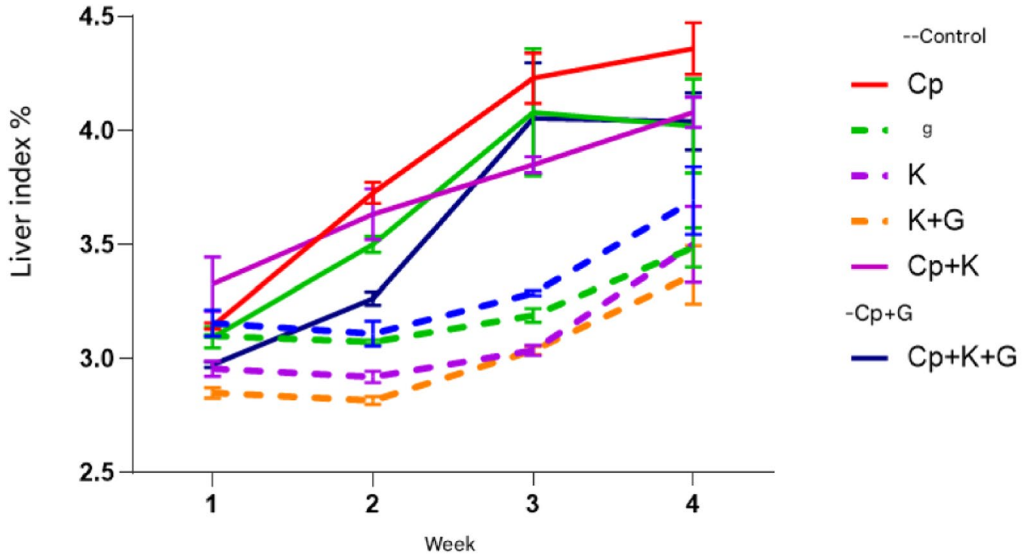

Fig. 4. Weekly change graph of liver indices of the groups ((indexes of the experimental liver = liver weight/body weight ratio).

acid groups ($p < 0.05$). Notably, at weeks 3 and 4, liver weights in the cisplatin + catechin group were significantly higher compared to both the cisplatin-only group and its own values at week 1 ($p < 0.05$).

Histopathologic studies

Examination of kidney tissue stained with hematoxylin–eosin

In microscopic examinations under light microscopy, after hematoxylin–eosin staining, the kidney tissues of the control, gallic acid, catechin, and catechin with gallic acid groups showed normal histological appearances in the glomeruli, proximal and distal tubules, and collecting duct structures (Figs. 5, 6, 7, 8, 9, 10, 11, 12). In a study investigating the effects of cisplatin on the kidneys, varying degrees of damage were observed in the kidney tissue during the treatment period. In the group treated with cisplatin alone, changes such as degeneration, fluid accumulation, necrosis, cell death, and dilation, especially in the proximal and distal tubules, were observed. The damage persisted from the first to the fourth week, with signs of mesangial cell proliferation and hypertrophy in the glomeruli (Figs. 13, 14).

In the group treated with a combination of cisplatin, catechin, and gallic acid, some improvements in kidney tissue were observed throughout the treatment period. However, degeneration, cell shape alteration, and fluid accumulation in the proximal tubules persisted. This treatment combination reduced kidney damage to some extent compared to the cisplatin-alone group, but complete recovery was not achieved. In conclusion, while cisplatin caused significant structural damage to the kidneys, adjunctive treatments such as catechin and gallic acid partially improved this damage (Figs. 15, 16, 17, 18, 19, 20).

The severity of renal damage in the cisplatin-only group was significantly higher than that observed in the control, gallic acid, and gallic acid plus catechin groups ($p < 0.05$). Furthermore, during the first three weeks, damage severity in the cisplatin group was significantly greater than in the catechin-only group ($p < 0.05$). At week 2, the cisplatin group showed more severe renal damage than both the control and the gallic acid plus catechin groups at week 1 ($p < 0.05$). Additionally, renal damage observed in the first week of both the cisplatin and cisplatin plus gallic acid plus catechin groups was significantly greater than that in the control and gallic acid plus catechin groups during the same period ($p < 0.05$) (Table 1).

Examination of liver tissue stained with hematoxylin–eosin

Microscopic evaluation of liver tissues stained with hematoxylin–eosin revealed normal histological architecture in the control, gallic acid, catechin, and catechin plus gallic acid groups (Figs. 21, 22, 23, 24). In the cisplatin-treated group, hepatic alterations such as hepatocellular degeneration, sinusoidal dilatation, and an increased number of Kupffer cells were observed from the first week onward. As the treatment period progressed, granular and vacuolar degeneration, binucleated hepatocytes, and increased karyomegaly became evident (Figs. 25, 26). In the catechin- and gallic acid-treated groups, these pathological changes developed more slowly and were less pronounced. Notably, both treatments partially mitigated cisplatin-induced hepatic damage, with a gradual reduction in granular and vacuolar degeneration and Kupffer cell proliferation over time (Figs. 27, 28, 29, 30, 31, 32).

Liver tissue damage severity was evaluated based on sinusoidal dilation, inflammatory cell infiltration, congestion, degeneration or cytoplasmic vacuolization, increased Kupffer cell count, and hepatic necrosis. Histopathological changes were scored as follows: 0 = normal, 1 = mild, 2 = moderate, and 3 = severe[35].

No statistically significant differences in liver damage severity were observed among the control, catechin, gallic acid, and catechin plus gallic acid treatment groups. However, the cisplatin-treated group exhibited significantly

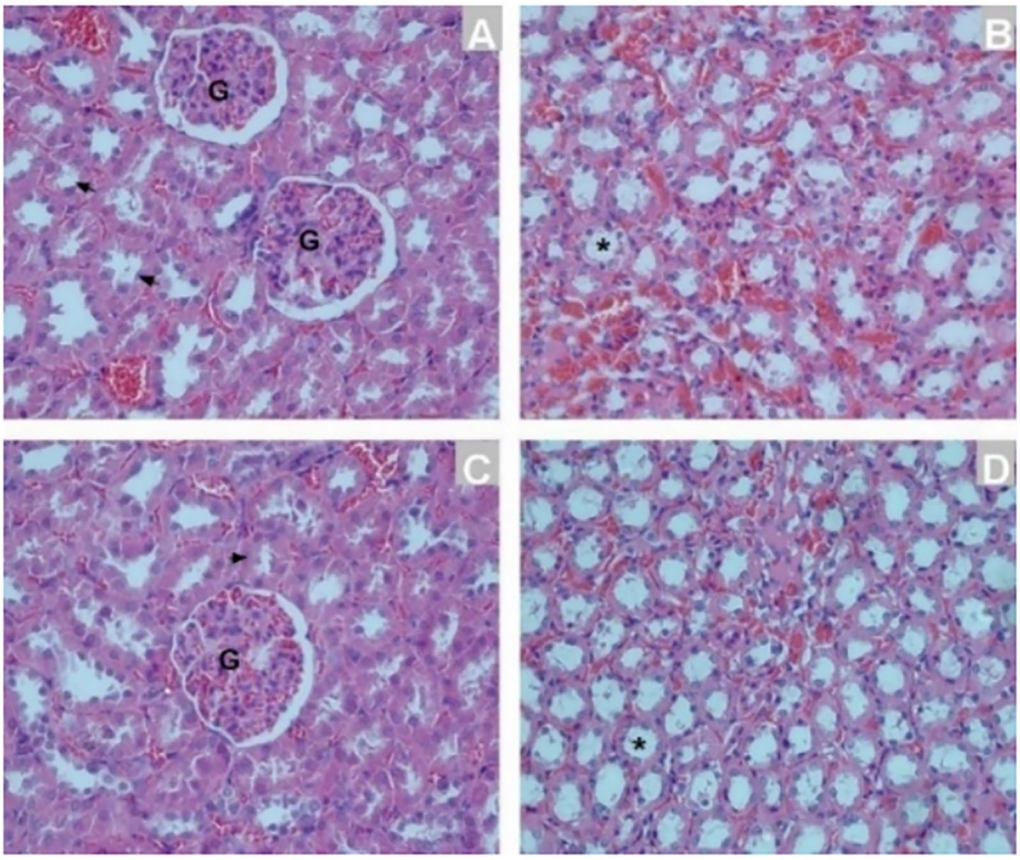

Fig. 5. Cortex (**A**,**C**)-medulla (**B**,**D**) Figure of the kidney in the 1st and 2nd week of the control group (H-E; ×40) (Glomerulus: G, Proximal tubule: Arrowhead, Collecting tubule: * sign).

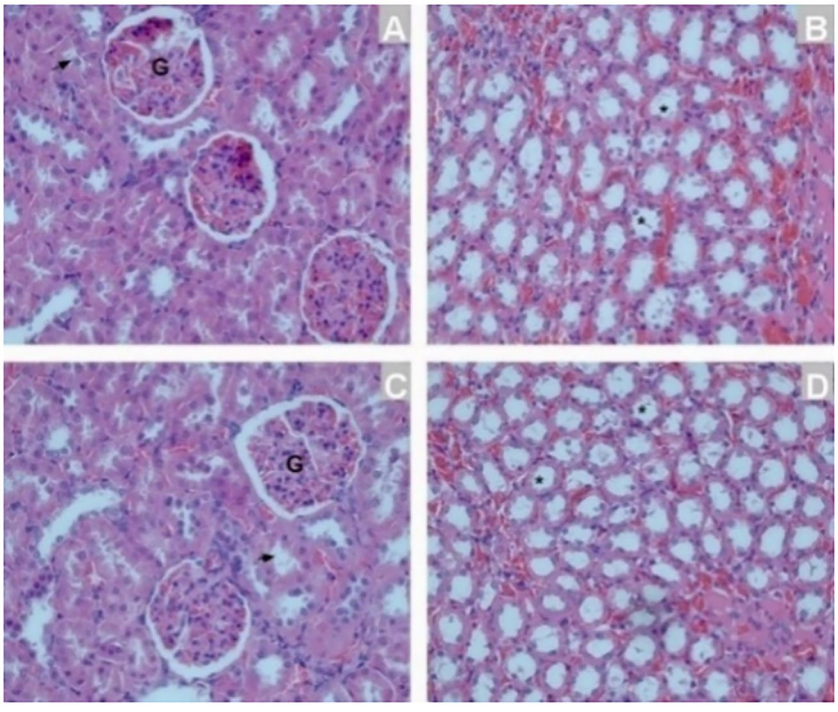

Fig. 6. Cortex (**A**,**C**)-medulla (**B**,**D**) Figure of the kidney in the 3rd and 4th week of the control group (H-E; ×40) (Glomerulus: G, Proximal tubule: Arrowhead, Collecting tubule: * sign).

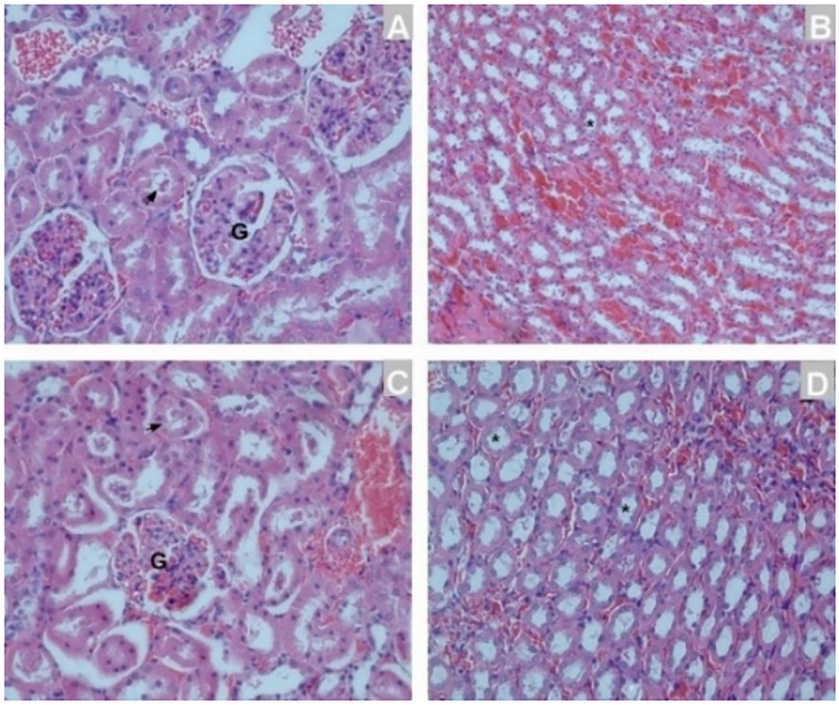

Fig. 7. Cortex (**A**,**C**)-medulla (**B**,**D**) Figure of the kidney at 1st and 2nd week of catechin group (H-E; ×40). (Glomerulus: G, Proximal tubule: Arrowhead, Collecting tubule: * sign).

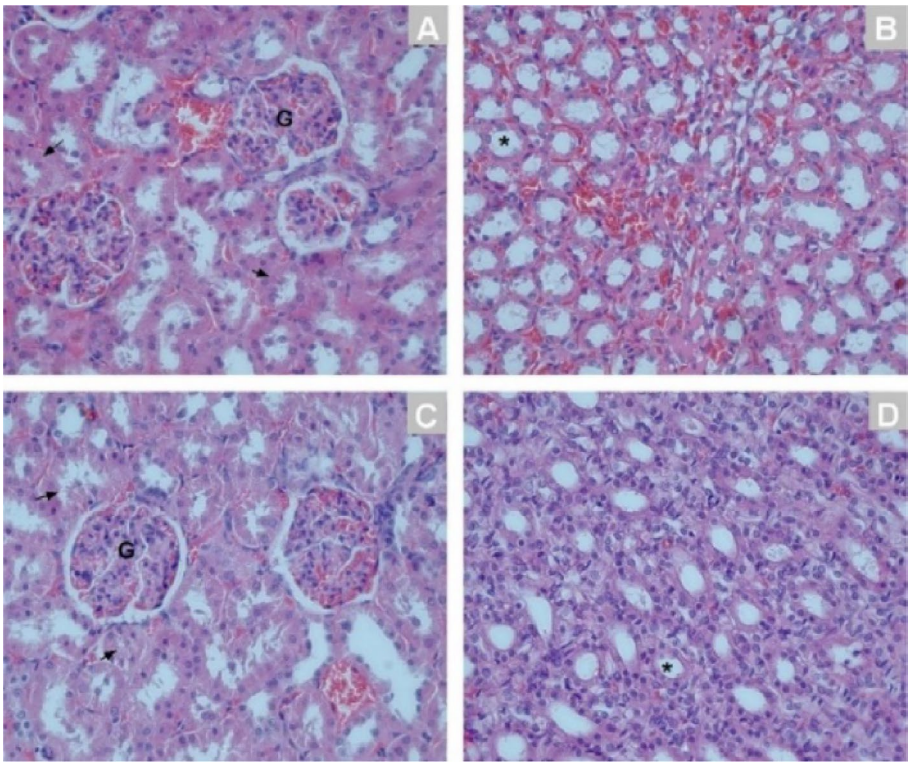

Fig. 8. Cortex (**A**,**C**)-medulla (**B**,**D**) Figure of the kidney at 3rd and 4th week of catechin group (H-E; ×40) (Glomerulus: G, Proximal tubule: Arrowhead, Collecting tubule: * sign).

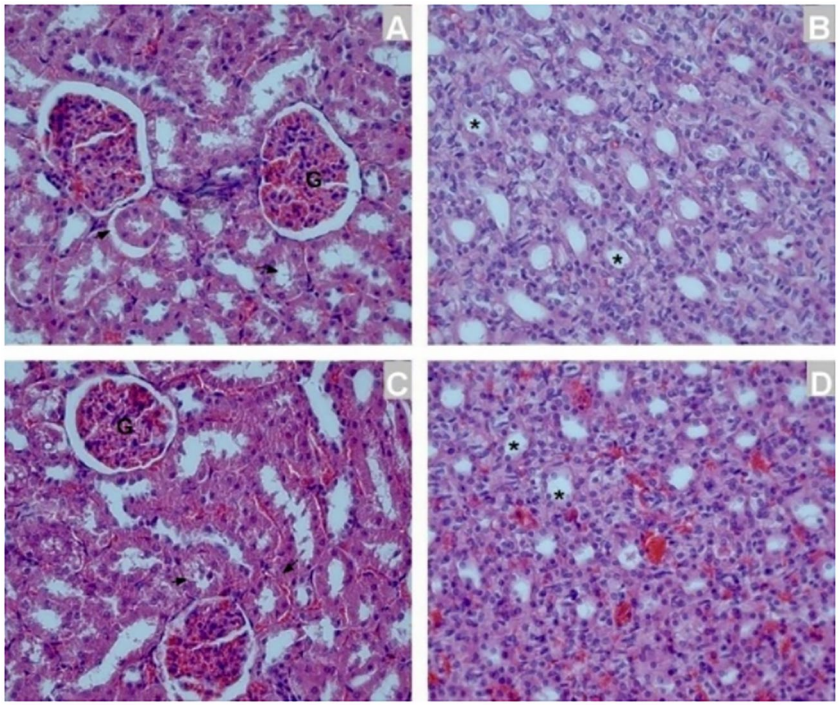

Fig. 9. Cortex (**A**,**C**)-medulla (**B**,**D**) Figure of the kidney at 1 and 2 weeks of gallic acid group (H-E; ×40) (Glomerulus: G, Proximal tubule: Arrowhead, Collecting tubule: * sign).

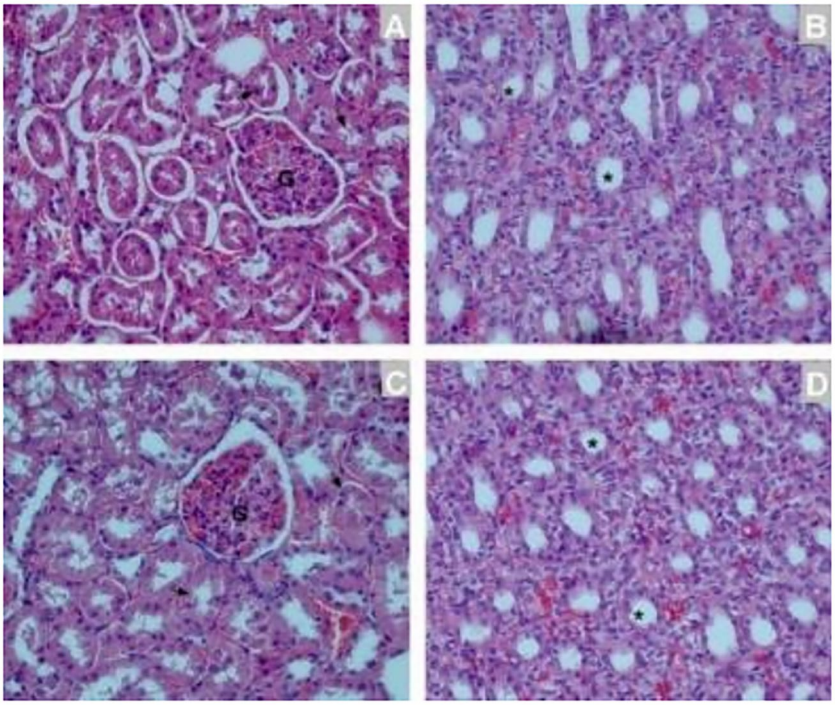

Fig. 10. Cortex (**A**,**C**)-medulla (**B**,**D**) Figure of the kidney at 3 and 4 weeks of gallic acid group (H-E; ×40) (Glomerulus: G, Proximal tubule: Arrowhead, Collecting tubule: * sign).

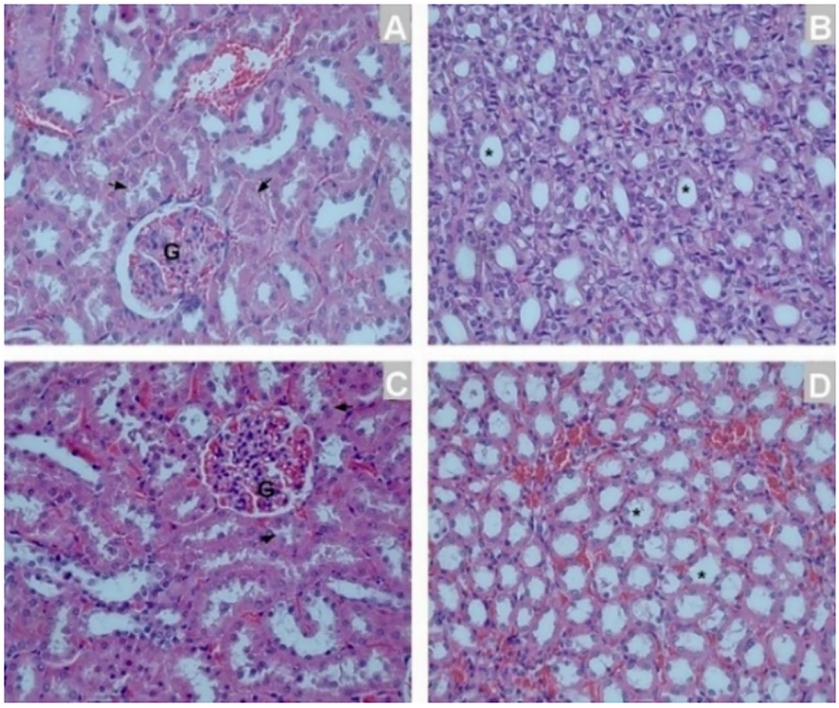

Fig. 11. Cortex (**A**,**C**)-medulla (**B**,**D**) Figure of the kidney in the 1st and 2nd week of the gallic acid group administered together with catechin (H-E; ×40) (Glomerulus: G, Proximal tubule: Arrowhead, Collecting tubule: * sign).

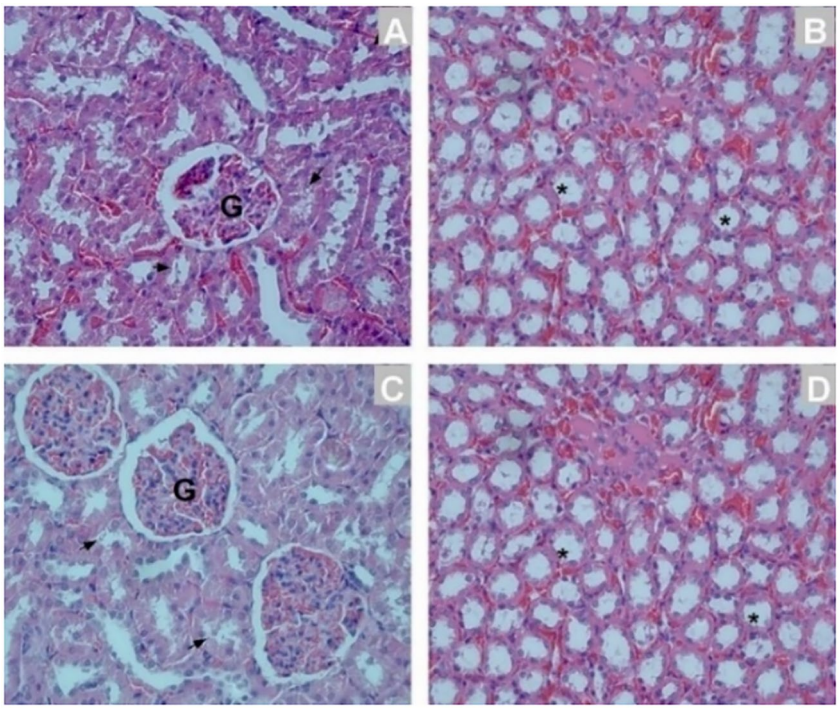

Fig. 12. Cortex (**A**,**C**)-medulla (**B**,**D**) Figure of the kidney in the 3rd and 4th week of the gallic acid group administered with catechin (H-E; ×40) (Glomerulus: G, Proximal tubule: Arrowhead, Collecting tubule: * sign).

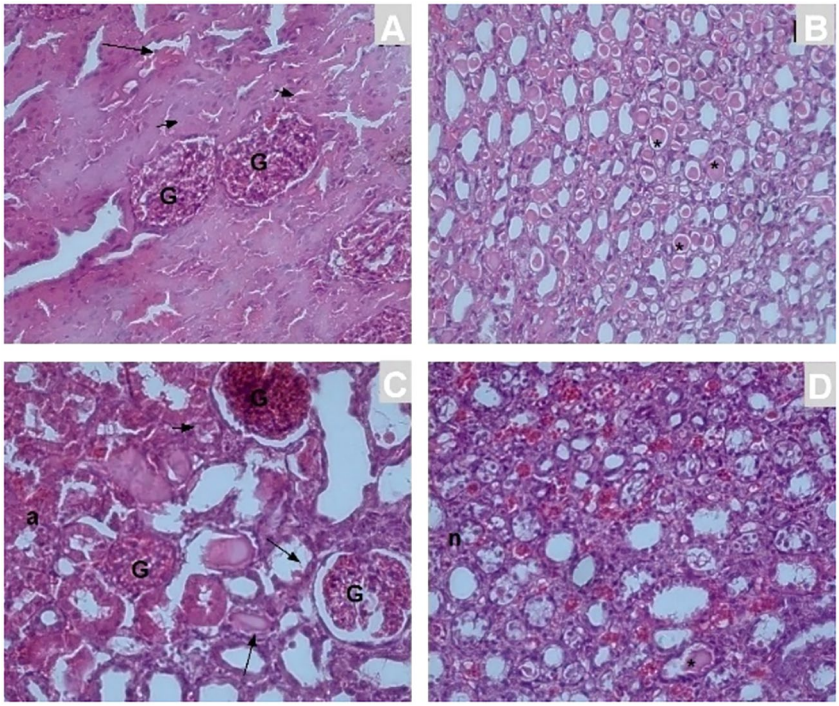

Fig. 13. Cortex (**A**,**C**)-medulla (**B**,**D**) Figures of the kidney at 1 and 2 weeks of the cisplatin alone group (H-E; ×40) (Glomerulus: G, Proximal tubule: arrowhead and arrow, Collecting tubule: * sign, Acidophilic body: a, Necrotic area).

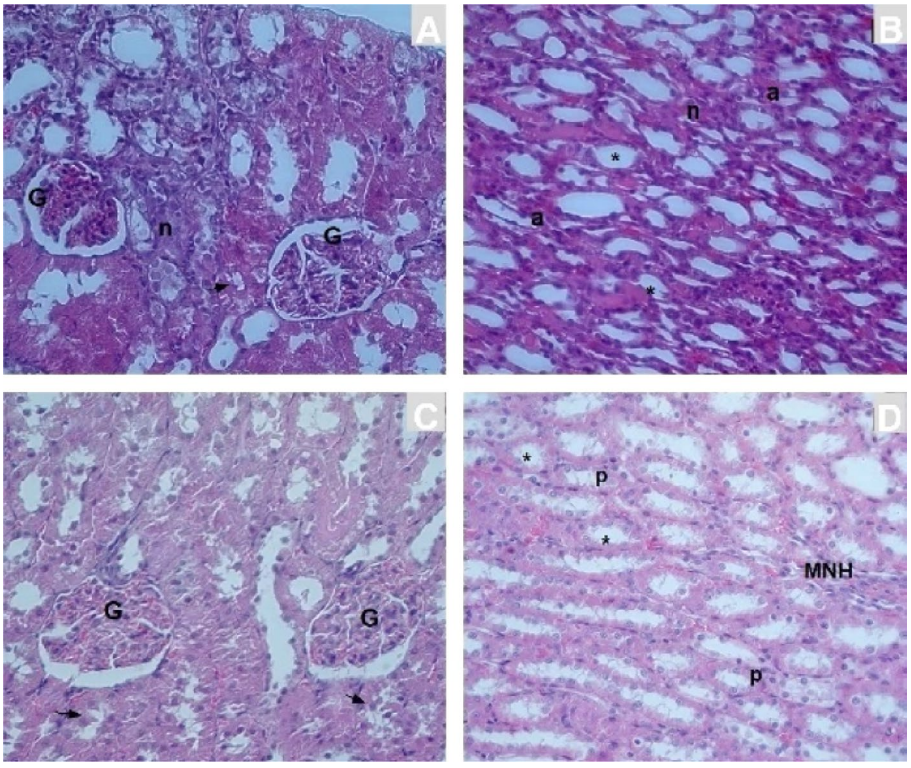

Fig. 14. Cortex (**A**,**C**)-medulla (**B**,**D**) Figures of the kidney at the 3rd and 4th week of the cisplatin alone group (H-E; ×40) (Glomerulus: G, Proximal tubule: Arrowhead, Collecting tubule: * sign, Acidophilic body: a, Necrotic area: n, Pyknotic nucleus: p, MNH increase: MNH).

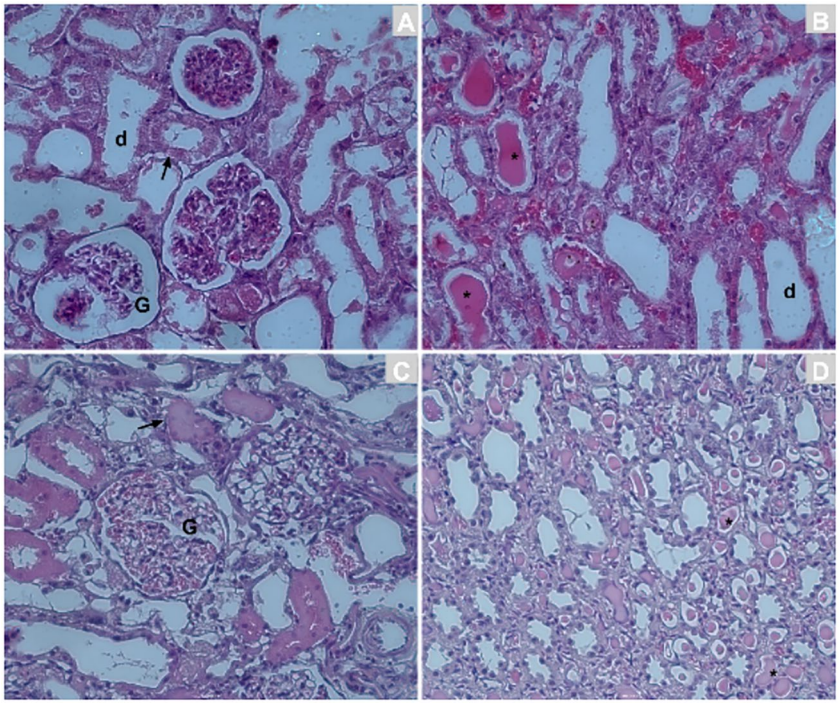

Fig. 15. Cortex (**A**,**C**)-medulla (**B**,**D**) Figure of the kidney in the 1st and 2nd week of the group in which cisplatin was administered together with catechin (H-E; ×40) (Glomerulus: G, Proximal tubule: Arrowhead, Collecting tubule: * sign, Dilatation: d).

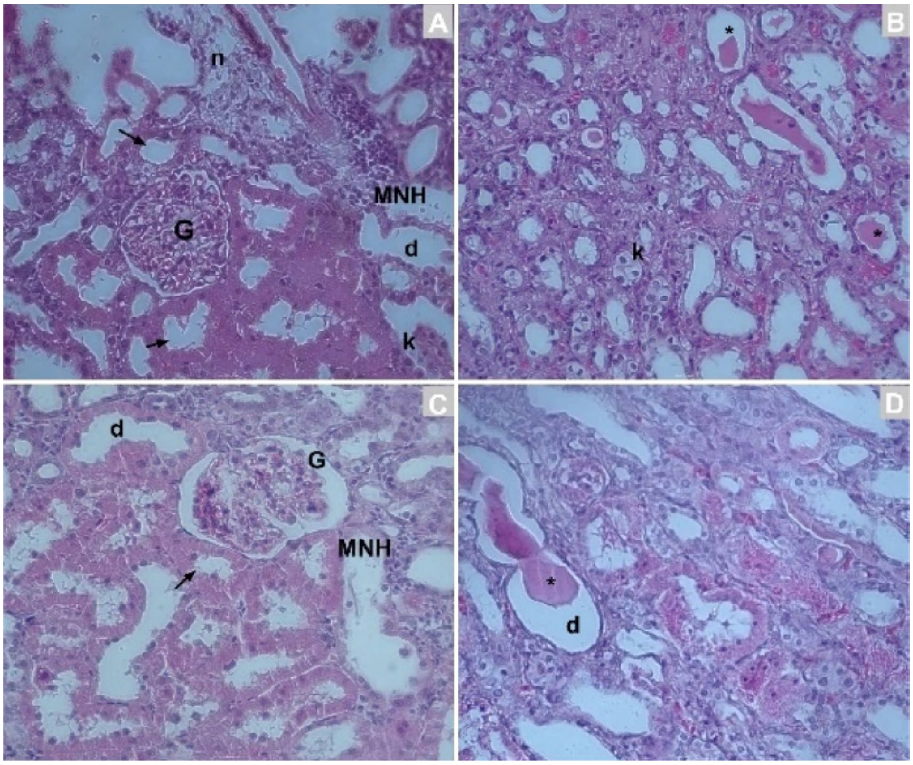

Fig. 16. Cortex (**A**,**C**)-medulla (**B**,**D**) Figure of the kidney in the 3rd and 4th week of the group in which cisplatin was administered together with catechin (H-E; ×40) (Glomerulus: G, Proximal tubule: Arrowhead, Collecting tubule: * sign, Hyperchromatic nucleus: h, Dilatation: d, Necrosis: n, MNH increase: MNH, Cariomegaly: k).

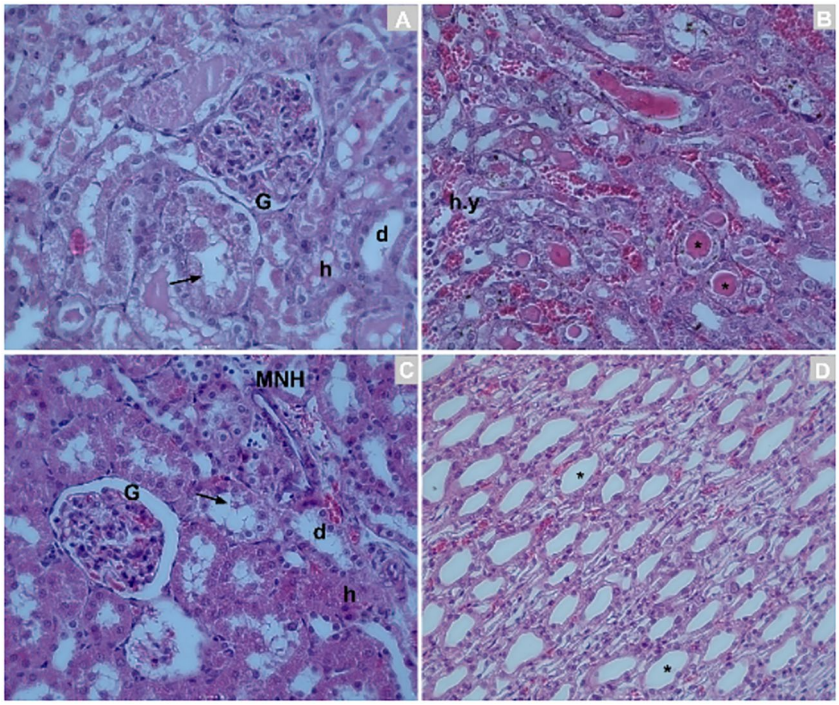

Fig. 17. Cortex (**A**,**C**)-medulla (**B**,**D**) Figure of the kidney at 1 and 2 weeks of the group in which cisplatin was administered together with gallic acid (H-E; ×40) (Glomerulus: G, Proximal tubule: Arrowhead, Collecting tubule: * sign, Hyperchromatic nucleus: h, Necrosis: n, Dilatation: d).

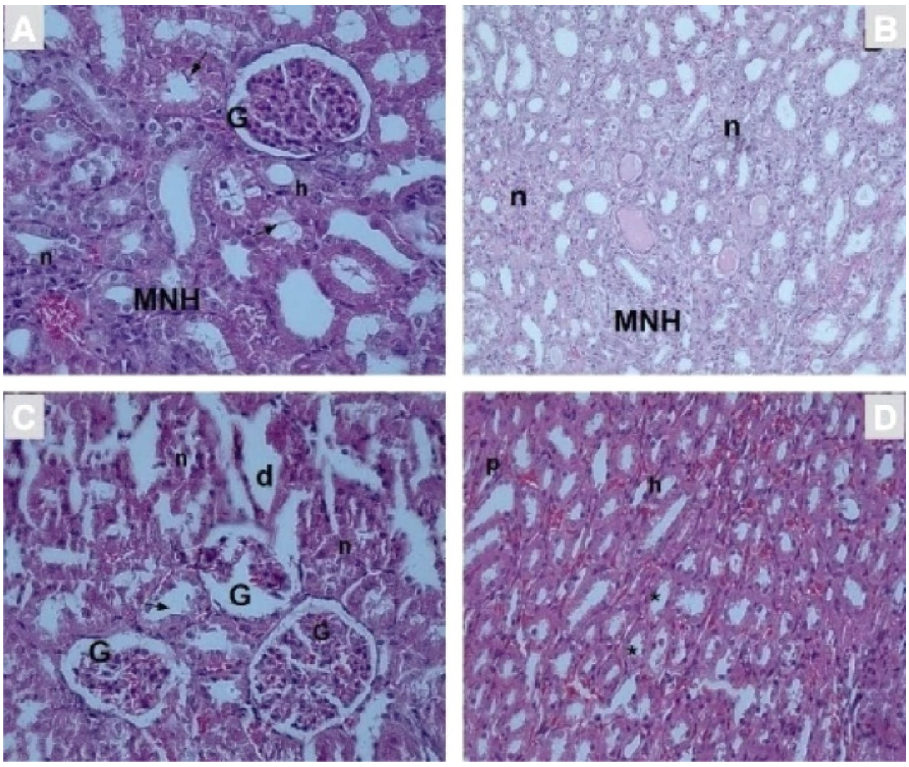

Fig. 18. Cortex (**A**,**C**)-medulla (**B**,**D**) Figure of the kidney at the 3rd and 4th week of the group in which cisplatin was administered together with gallic acid (H-E; ×40) (Glomerulus: G, Proximal tubule: Arrowhead, Collecting tubule: * sign, Hyperchromatic nucleus: h, Pyknotic nucleus: p, Necrosis: n, Dilatation: d).

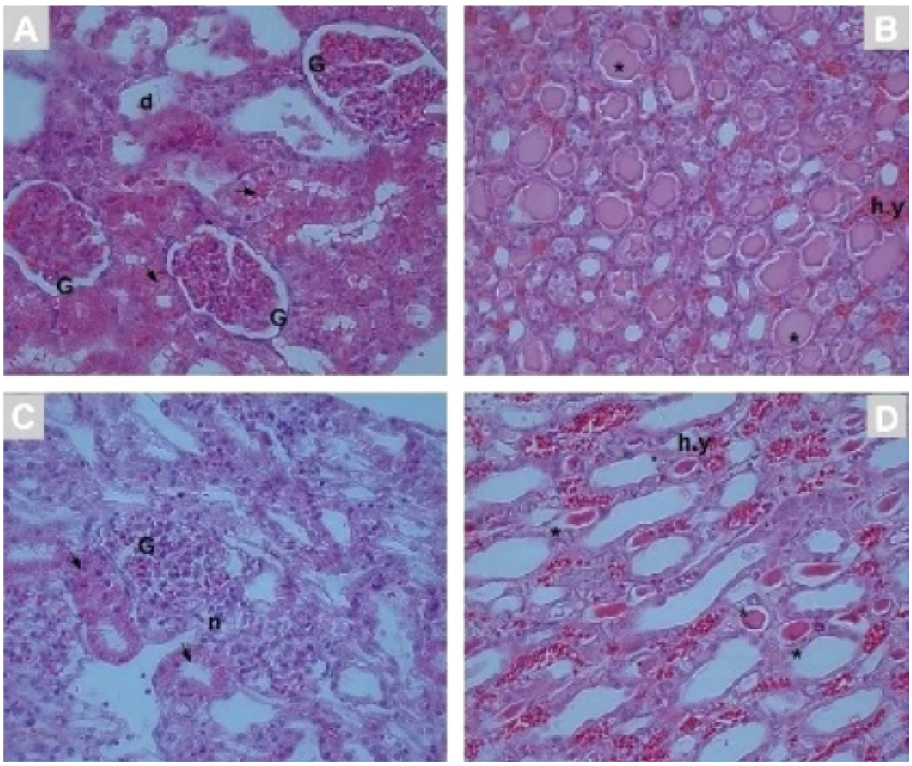

Fig. 19. Cortex (**A**,**C**)-medulla (**B**,**D**) Figure of the kidney in the 1st and 2nd week of the group in which cisplatin was administered together with catechin and gallic acid (H-E; ×40) (Glomerulus: G, Proximal tubule: Arrow, Collecting tubule: * sign, Necrosis: n, Dilatation: d, Hyperemic structure: h.y).

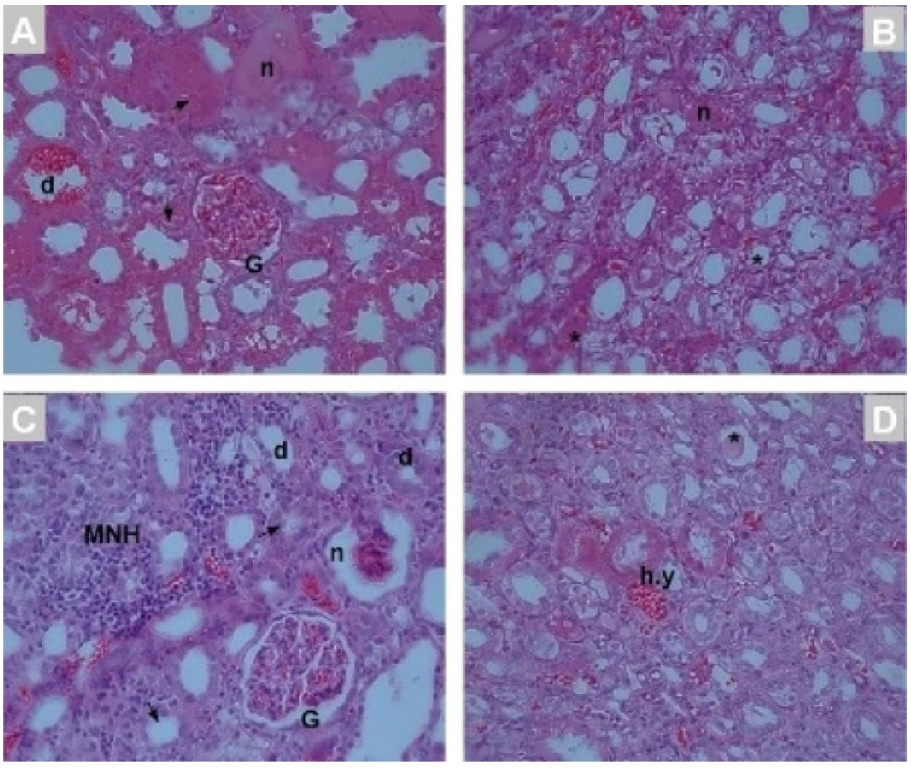

Fig. 20. Kidney tissue of the group in which cisplatin was administered with catechin and gallic acid at 3 and 4 weeks (H-E; ×40) (Glomerulus: G, Proximal tubule: Arrow, Collecting tubule: * sign, Necrosis: n, Dilatation: d, Hyperemic structure: h.y, mononuclear cell infiltration: MNH).

Groups	Damage score (Week 1)	Damage score (Week 2)	Damage score (Week 3)	Damage score (Week 4)
Control	0.60 ± 0.54	0.80 ± 0.44	0.80 ± 0.44	0.80 ± 0.44
CP	5.00 ± 0.21[a,b,c,d]	4.40 ± 0.54[a,d]	3.40 ± 0.54	3.20 ± 0.33
Catechin	0.80 ± 0.44	0.80 ± 0.44	0.80 ± 0.44	1.00 ± 0.46
Gallic acid	0.80 ± 0.44	0.80 ± 0.44	0.80 ± 0.44	0.80 ± 0.45
Catechin + gallic acid	0.60 ± 0.60	0.80 ± 0.43	0.81 ± 0.45	0.80 ± 0.44
Catechin + CP	4.20 ± 0.44[a,d]	3.20 ± 0.43	2.60 ± 0.55	2.40 ± 0.55
Gallic acid + CP	4.40 ± 0.54[a,d]	3.40 ± 0.54	2.80 ± 0.45	2.60 ± 0.55
Catechin + gallic acid + CP	2.40 ± 0.54	2.60 ± 0.53	3.40 ± 0.54	3.60 ± 0.54

Table 1. Graph of changes in renal tissue damage scores in experimental groups. [a]$p < 0.05$; compared to the control group, [b]$p < 0.05$; compared to the K group, [c]$p < 0.05$; compared to the G group, [d]$p < 0.05$; compared to the K + G group. 0: no damage, 1: < 10%, 2: 11–25%, 3: 26–45%, 4: 46–75%, 5: > 76%)[32].

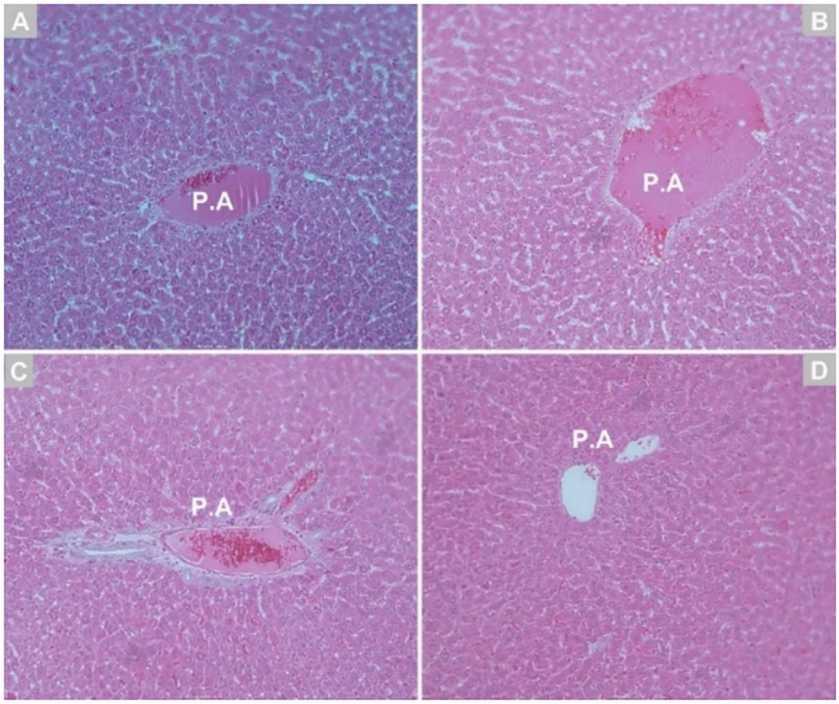

Fig. 21. Liver tissue of the control group at week 1 (**A**), week 2 (**B**), week 3 (**C**), week 4 (**D**) (H-E; 20X) (portal area: P.A).

higher liver damage scores in the first week compared to the other groups. Despite this, no statistically significant differences were detected among the various cisplatin-treated subgroups. These findings are quantitatively summarized in Table 2, which presents the weekly liver tissue damage scores for all experimental groups.

Examination of kidney tissue stained with Picro-Sirius Red

Microscopic examination under light microscopy following Picro-Sirius Red staining revealed that kidney tissues from the control, gallic acid, catechin, and catechin plus gallic acid groups exhibited collagen deposition as thin, linear staining around the proximal and distal tubules, glomerular corpuscles, and collecting ducts. Intense collagen staining was particularly evident around the blood vessels (Figs. 33, 34, 35, 36, 37, 38, 39, 40).

In the cisplatin-treated group, fine collagen staining was observed in the renal cortex and color changes were noted around medullary vessels during the first week. By the second week, thickening of the glomerular structures, increased perivascular collagen deposition, and focal color alterations in the medulla were evident (Fig. 41). In the third week, cortical staining became faint, while collagen deposition in the medulla became more pronounced. By the fourth week, mild collagen staining was observed in the glomeruli (Fig. 42).

In the cisplatin plus catechin group, similar collagen staining patterns were observed in the renal corpuscles and tubules during the first week, as well as around the medullary vessels. By the second week, intense perivascular staining was evident, while no notable alterations were detected in the medulla. In the third week, both intense staining and fine linear collagen deposition around the tubules were observed (Figs. 43, 44).

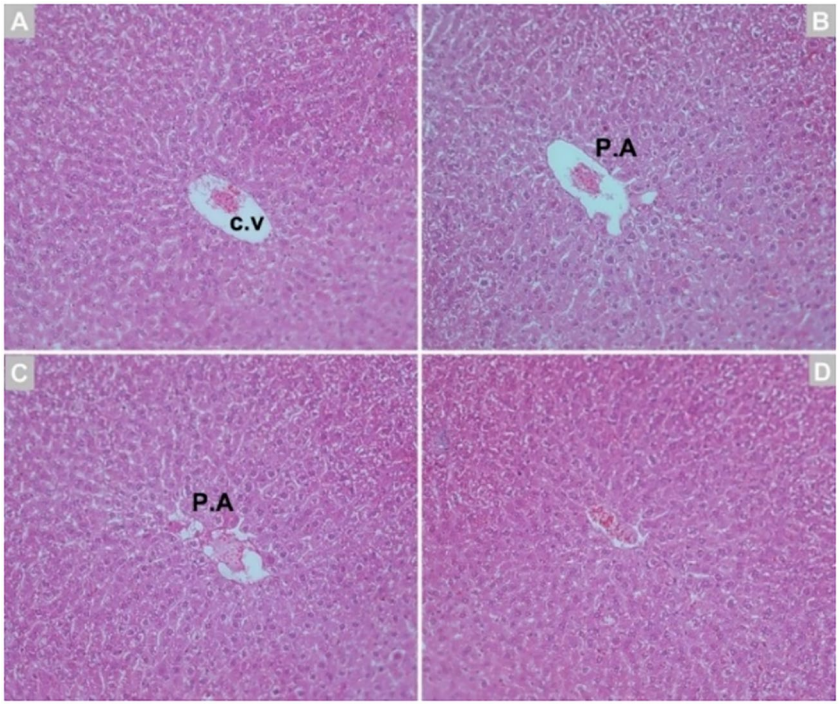

Fig. 22. Liver tissue of catechin group at week 1 (**A**), week 2 (**B**), week 3 (**C**), week 4 (**D**) (H-E; 20X) (portal area: P.A, central vein: c.v).

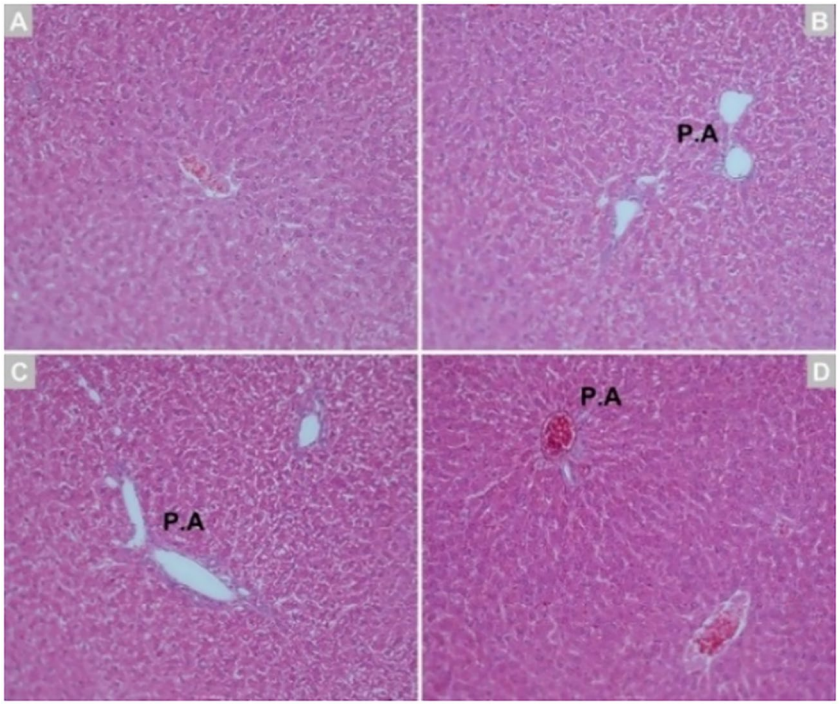

Fig. 23. Liver tissue of gallic acid group at week 1 (**A**), week 2 (**B**), week 3 (**C**), week 4 (**D**) (H-E; 20X) (Portal area: P.A).

In the cisplatin plus gallic acid group, the first week was characterized by intense collagen accumulation around collagen levels gradually decreased vascular structures, accompanied by fine linear staining within the medulla. During the second week, thickening of the renal corpuscles, increased vascular wall thickness, and a reticulated collagen staining pattern in the medulla were noted. By the third week, enhanced collagen deposition

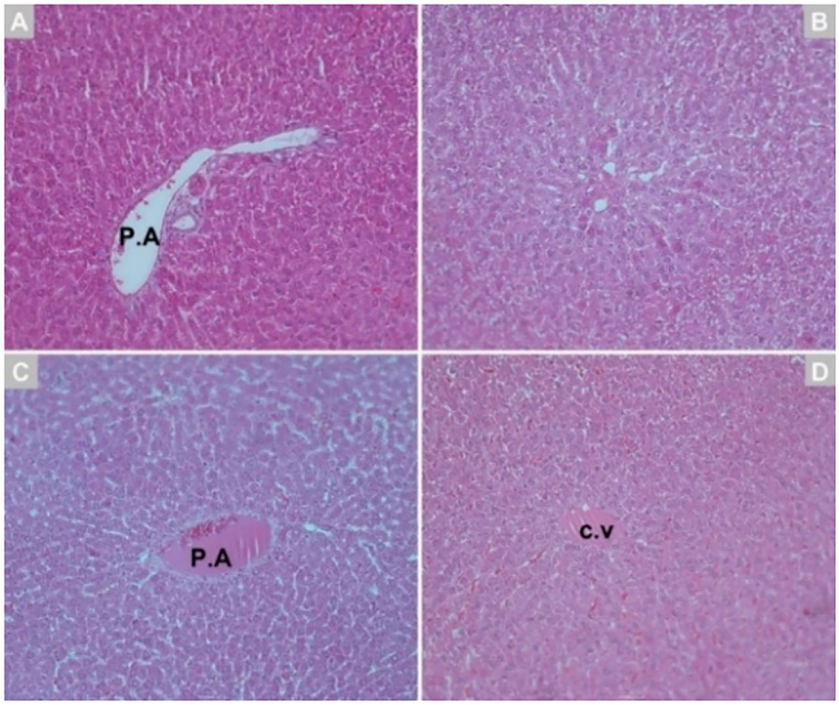

Fig. 24. Liver tissue of catechin and gallic acid group at week 1 (**A**), week 2 (**B**), week 3 (**C**), week 4 (**D**) (H-E; 20X) (Portal area: P.A, Central vein: c.v).

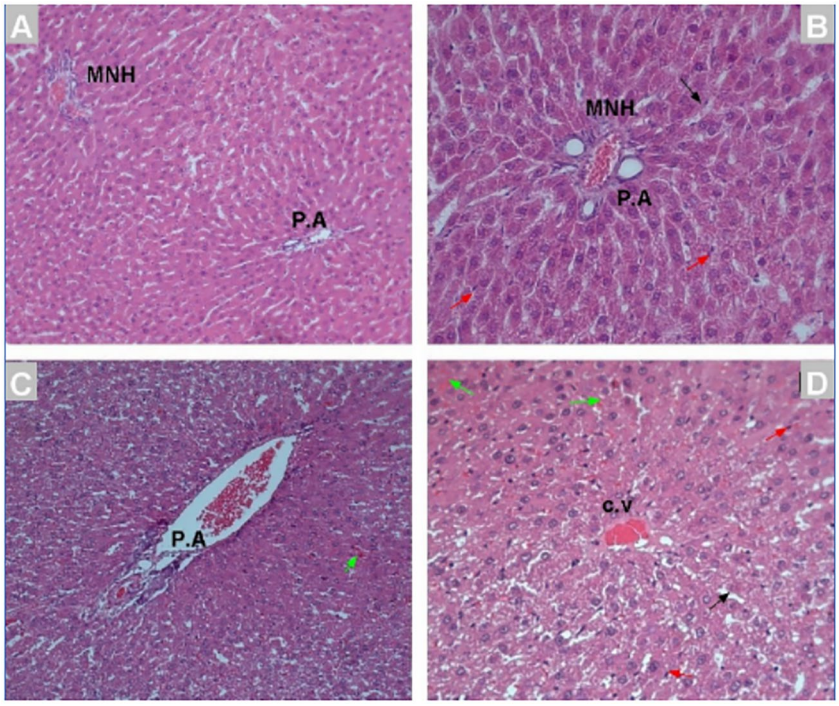

Fig. 25. Liver tissue (H-E; ×20–×40) of the cisplatin alone group at 1 (**A**,**B**) and 2 (**C**,**D**) weeks (Portal area: P.A, MNH infiltration: MNH, Kupffer cell: Red arrow, Dilatation: Black arrow, Acidophilic body: Green arrow).

and further alterations around medullary vessels were observed. In the fourth week, the overall staining intensity declined (Figs. 45, 46).

In the group treated with a combination of cisplatin, catechin, and gallic acid, intense collagen staining was observed during the first week, followed by fine linear collagen deposition around the tubules in the second week.

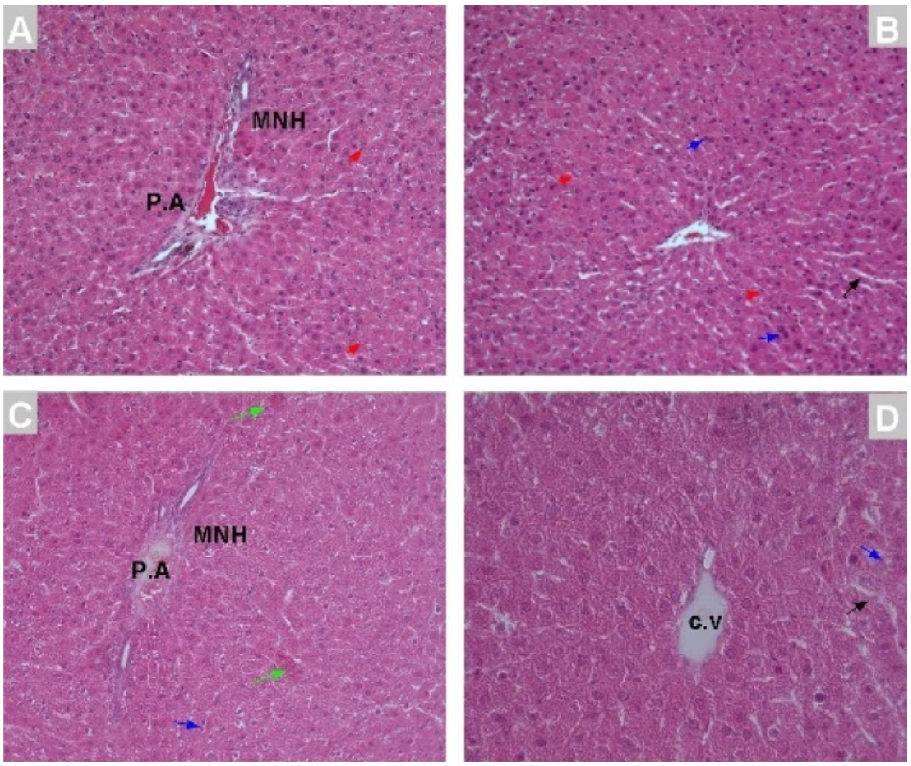

Fig. 26. Liver tissue (H-E; ×20–×40) of the group in which cisplatin was administered alone at weeks 3 and 4 (Portal area: P.A, MNH infiltration: MNH, Kupffer cell: Red arrow, Dilatation: Black arrow, Acidophilic body: Green arrow, Binucleated hepatocyte: Blue arrow, Central vein: c.v).

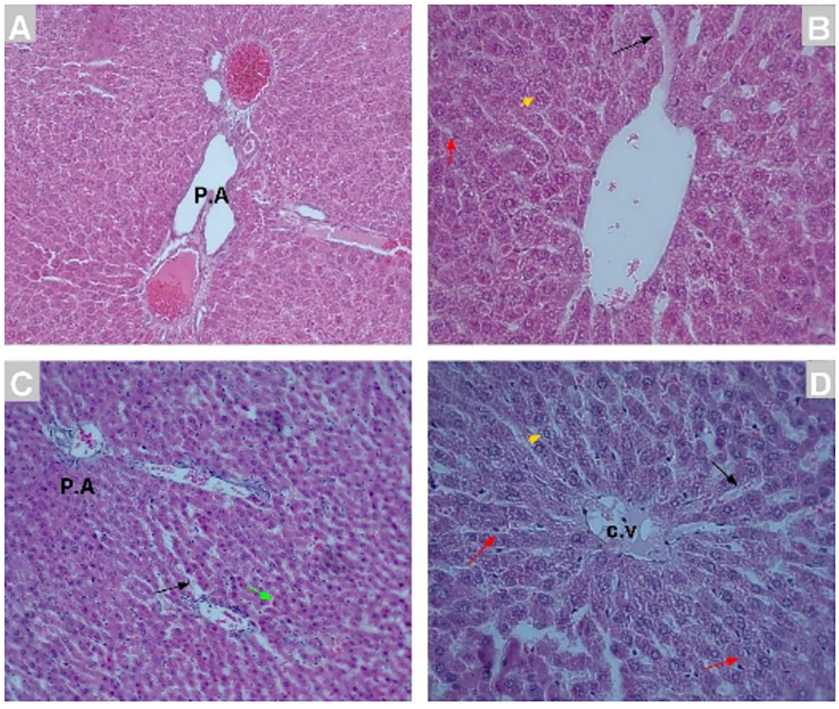

Fig. 27. Liver tissue of the group in which cisplatin was administered with catechin at the 1st and 2nd week (H-E; ×20–×40) (Portal field: P.A, Kupffer cell: Red arrow, Dilatation: Black arrow, Acidophilic body: Green arrow, Central vein: c.v, Granular degeneration: Orange arrow).

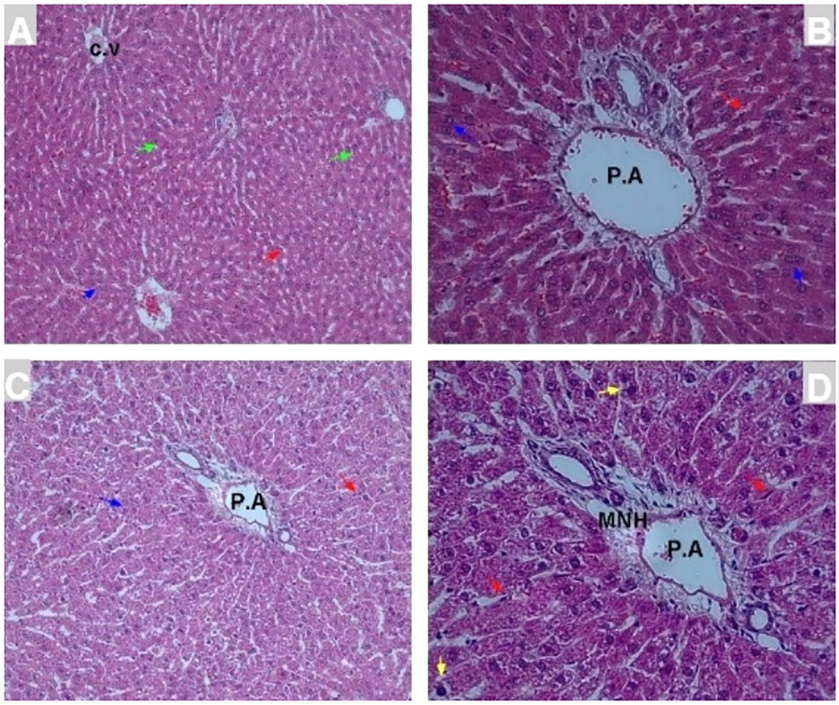

Fig. 28. Liver tissue of the group in which cisplatin was administered with catechin at the 3rd and 4th week (H-E; ×20–×40) (Portal field: P.A, Kupffer cell: Red arrow, Dilatation: Black arrow, Acidophilic body: Green arrow, Central vein: c.v, Hyperchromatic nucleus: Yellow arrow, Binucleated hepatocyte: Blue arrow).

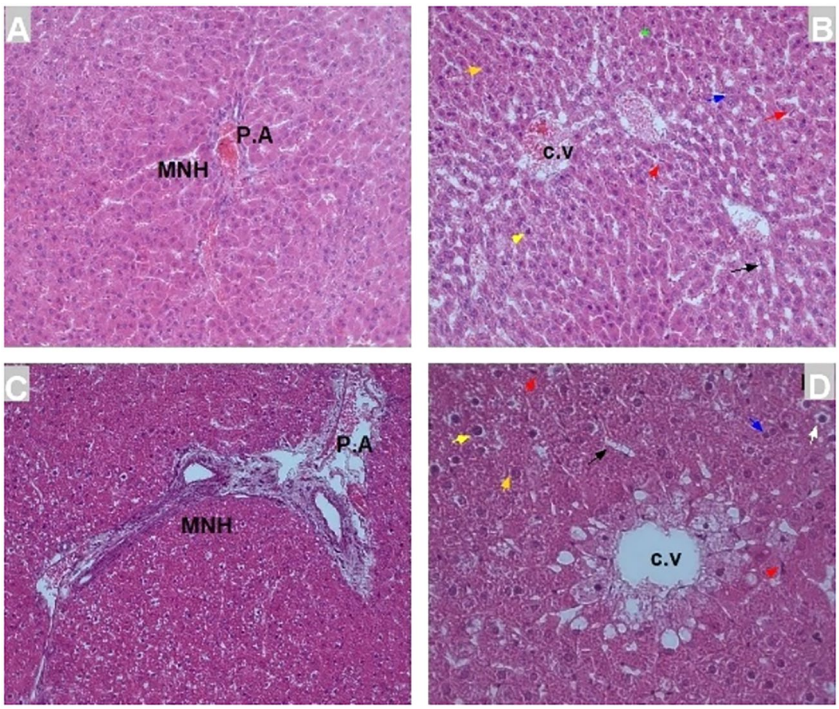

Fig. 29. Liver tissue of the group in which cisplatin was administered with gallic acid at 1st (**A**,**B**) and 2nd (**C**,**D**) weeks (H-E; ×20–×40) (Portal field: P.A, Kupffer cell: Red arrow, Dilatation: Black arrow, Acidophilic body: Green arrow, Central vein: c.v, Hyperchromatic nucleus: Yellow arrow, Binucleated hepatocyte: Blue arrow).

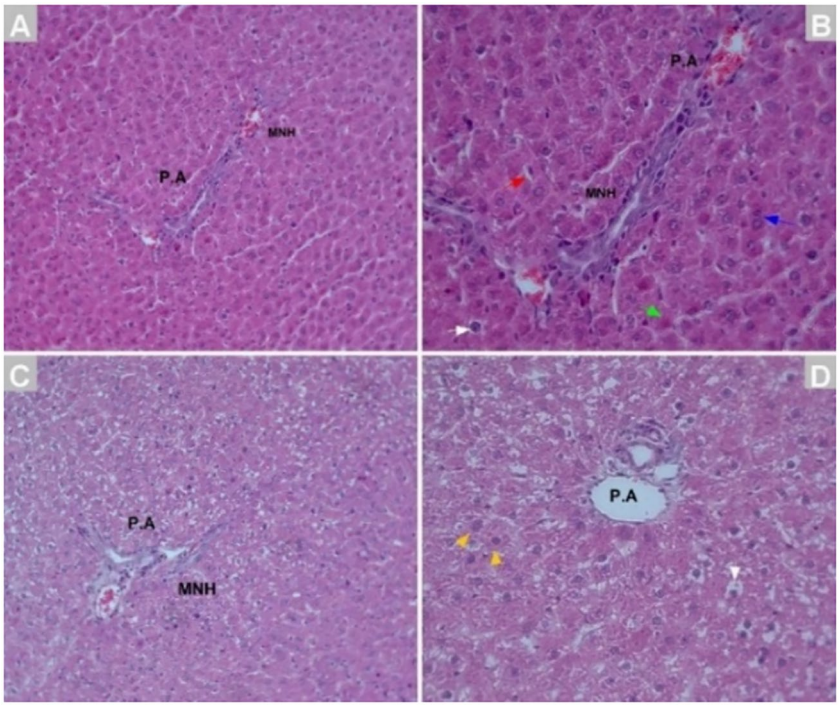

Fig. 30. Liver tissue of the group in which cisplatin was administered with gallic acid at 3 (**A**,**B**) and 4 (**C**,**D**) weeks (H-E; ×20–×40) (Portal field: P.A, Kupffer cell: Red arrow, Binucleated hepatocyte: Blue arrow, Granular degeneration: Orange arrow, Cariomegaly: White arrow, Acidophilic body: Green arrow, MNH increase: MNH).

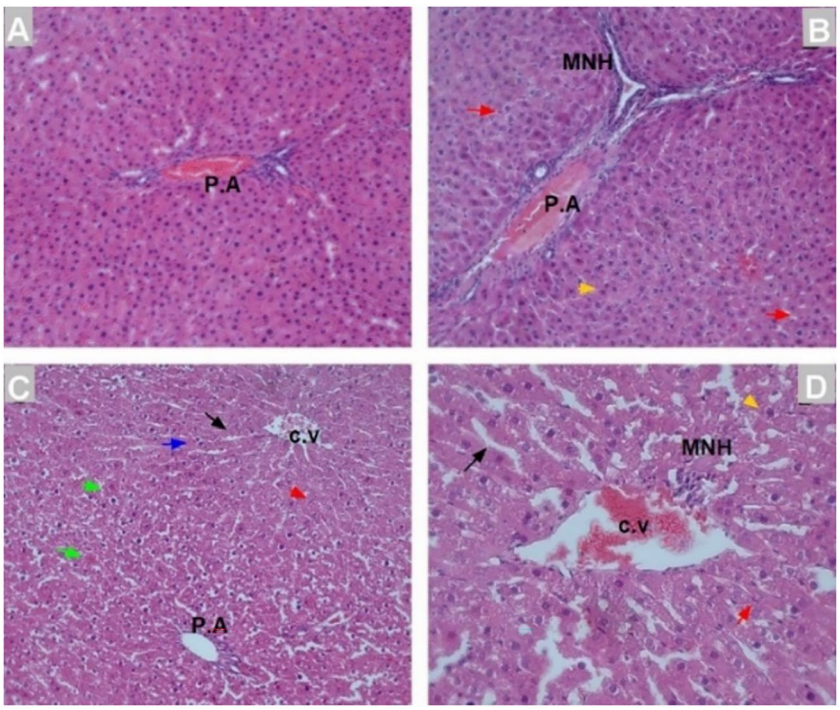

Fig. 31. Liver tissue (H-E; ×20–×40) of the 1st (**A**,**B**) and 2nd (**C**,**D**) week of the group in which cisplatin was administered with catechin and gallic acid (Portal field: P.A, Kupffer cell: Red arrow, Binucleated hepatocyte: Blue arrow, Granular degeneration: Orange arrow, Acidophilic body: Green arrow, MNH increase: MNH, Dilatation: Black arrow, Central vein: c.v).

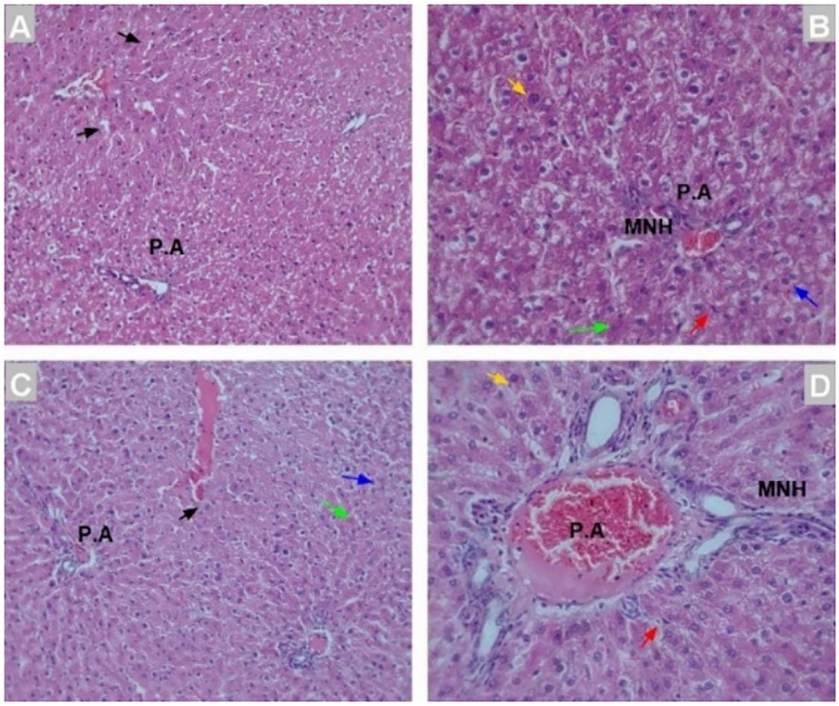

Fig. 32. Liver tissue (H-E; ×20–×40) of the 3rd (**A**,**B**) and 4th (**C**,**D**) weeks of the group in which cisplatin was administered with catechin and gallic acid (Portal field: P.A, Kupffer cell: Red arrow, Binucleated hepatocyte: Blue arrow, Granular degeneration: Orange arrow, Acidophilic body: Green arrow, MNH increase: MNH, Dilatation: Black arrow, Central vein: c.v).

Groups	Damage score (Week 1)	Damage score (Week 2)	Damage score (Week 3)	Damage score (Week 4)
Control	0.20 ± 0.44	0.20 ± 0.44	0.40 ± 0.54	0.60 ± 0.54
CP	3.00 ± 0.05[a,b,c,d]	2.60 ± 0.54	2.40 ± 0.54	2.40 ± 0.55
Catechin	0.20 ± 0.44	0.20 ± 0.44	0.40 ± 0.54	0.60 ± 0.54
Gallic acid	0.20 ± 0.44	0.20 ± 0.45	0.40 ± 0.55	0.60 ± 0.54
Catechin + gallic acid	0.20 ± 0.40	0.20 ± 0.44	0.60 ± 0.54	0.60 ± 0.44
Catechin + CP	2.60 ± 0.54	2.40 ± 0.54	2.20 ± 0.44	1.60 ± 0.55
Gallic acid + CP	2.60 ± 0.54	2.40 ± 0.54	2.20 ± 0.45	1.60 ± 0.54
Catechin + gallic acid + CP	2.40 ± 0.54	2.20 ± 0.44	2.00 ± 0.08	1.40 ± 0.54

Table 2. Graph of changes in liver tissue damage scores in experimental groups. [a]$p < 0.05$; compared to the control group, [b]$p < 0.05$; compared to the K group, [c]$p < 0.05$; compared to the G group, [d]$p < 0.05$; compared to the K + G group.

No staining was detected in either the cortex or medulla during the third week. However, increased collagen accumulation around the blood vessels was noted in the fourth week. The corresponding semi-quantitative collagen scores observed in kidney tissue across the study groups are shown in Table 3 (Figs. 47, 48).

Examination of liver tissue stained with Picro-Sirius Red

Picro-Sirius Red staining under light microscopy revealed normal collagen distribution in the liver tissues of the control, gallic acid, catechin, and catechin + gallic acid groups. Collagen staining appeared in physiologic amounts between the central vein, portal areas, and hepatocytes (Figs. 49, 50, 51, 52, 53, 54, 55, 56).

In the cisplatin-treated group, fine collagen striations were observed around the central vein and within the portal area during the first week, accompanied by mild sinusoidal staining. In the second week, increased collagen deposition was detected around hepatocytes and in the portal region. By the third week, collagen accumulation persisted in the portal area but decreased between hepatocytes. In the fourth week, collagen levels declined in the portal area, while pericentral collagen remained unchanged (Figs. 57, 58).

In the cisplatin + catechin group, increased collagen deposition was observed in the portal area and between hepatocytes in the first week. This accumulation gradually decreased during the second and third weeks and continued to decline in the fourth week (Figs. 59, 60).

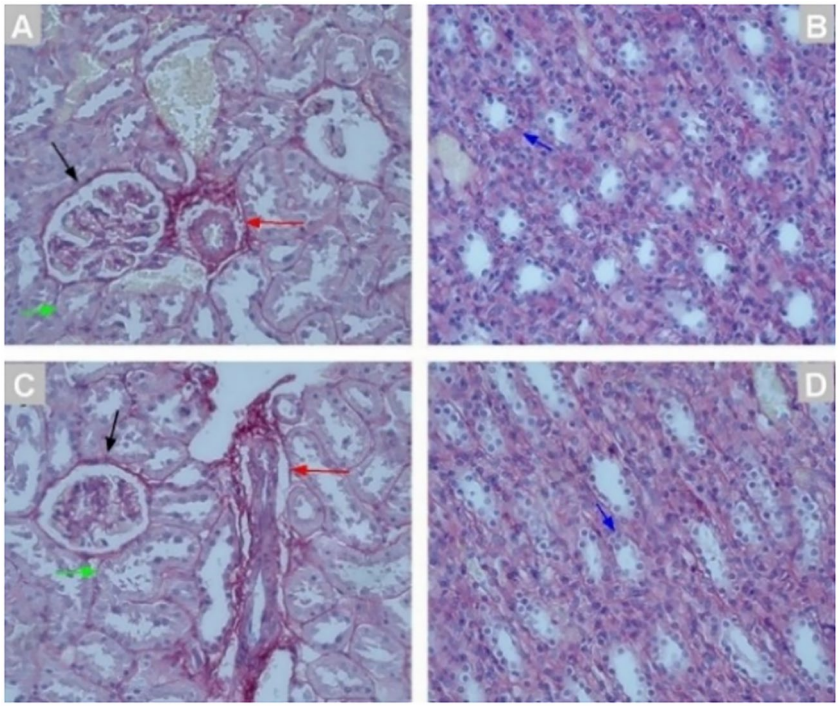

Fig. 33. Cortex-medulla view of kidney tissue of the control group at 1 (**A**,**B**) and 2 (**C**,**D**) weeks (Picro-Sirius red; ×40) (Kidney body: Black arrow, Vascular periphery: Red arrow, Proximal tubule: Green arrow, Collecting tubule: Blue arrow).

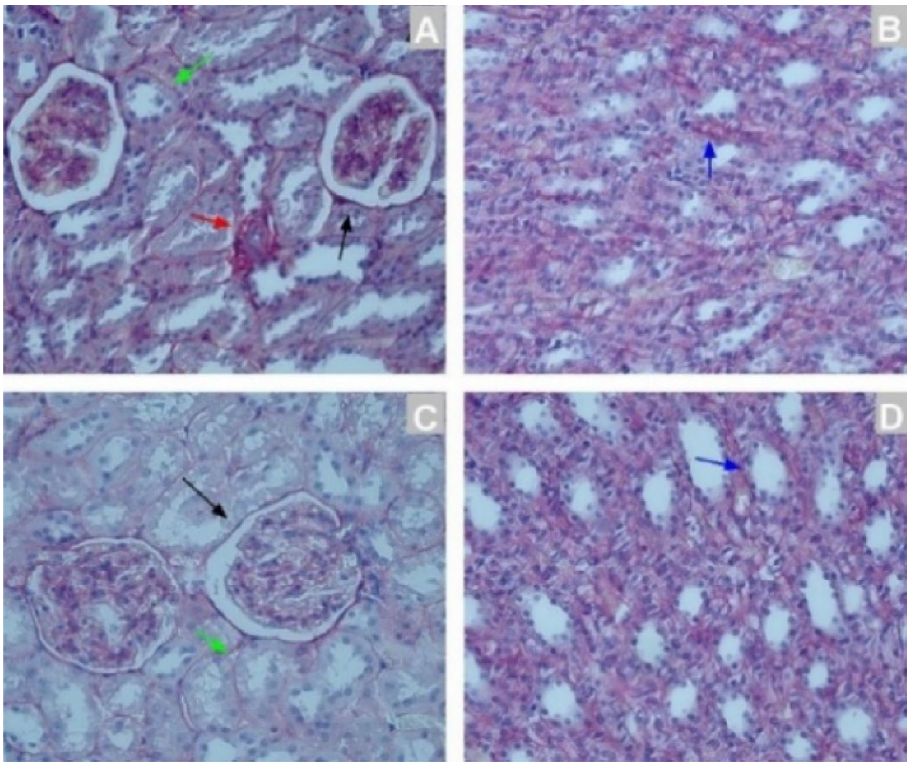

Fig. 34. Cortex-medulla view of kidney tissue of the control group at 3rd (**A**,**B**) and 4th (**C**,**D**) week (Picro-Sirius red; ×40) (Kidney body: Black arrow, Vascular periphery: Red arrow, Proximal tubule: Green arrow, Collecting tubule: Blue arrow).

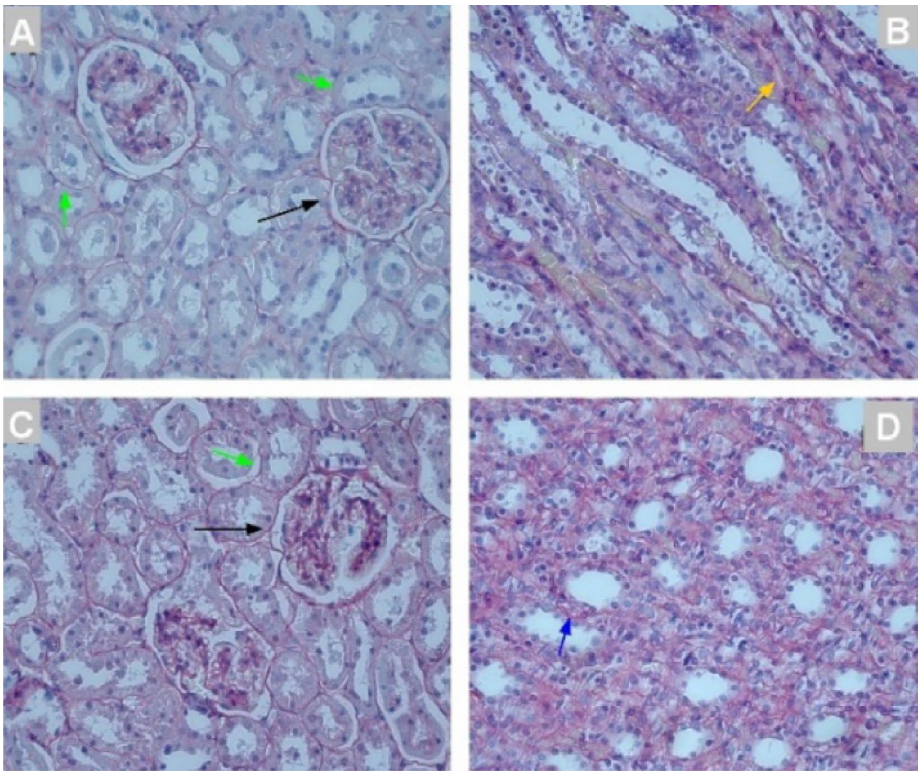

Fig. 35. Cortex-medulla view of kidney tissue of catechin group at 1st (**A**,**B**) and 2nd (**C**,**D**) week (Picro-Sirius red; ×40) (Kidney body: Black arrow, Yellow arrow, Proximal tubule: Green arrow, Collecting tubule: Blue arrow).

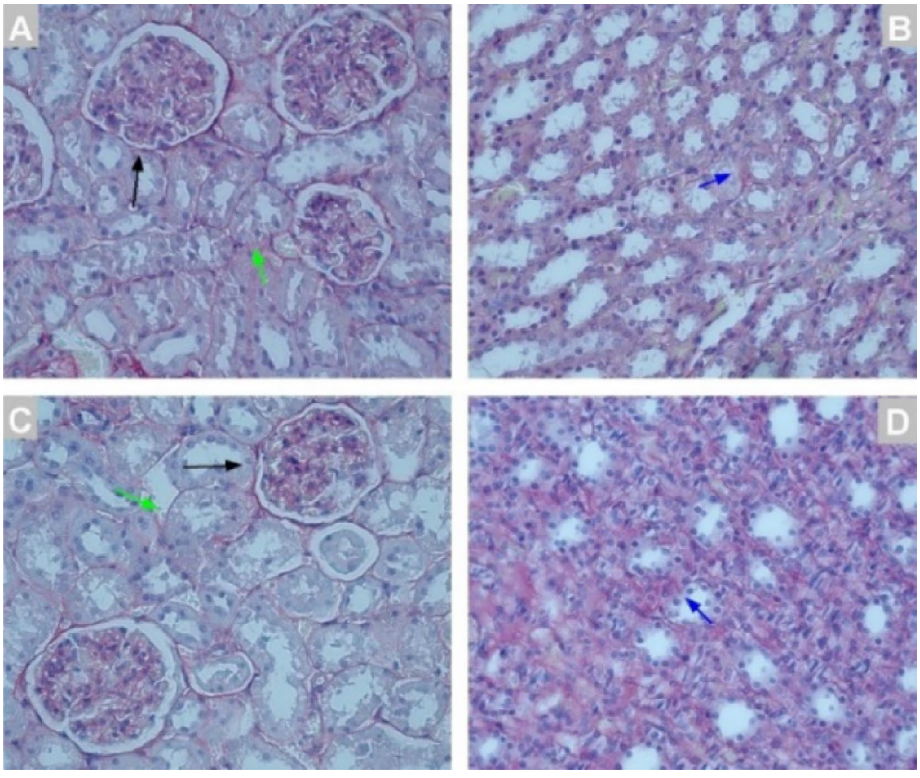

Fig. 36. Cortex-medulla view of kidney tissue of catechin group at 3rd (**A**,**B**) and 4th (**C**,**D**) week (Picro-Sirius red; ×40) (Kidney body: Black arrow, Proximal tubule: Green arrow, Collecting tubule: Blue arrow).

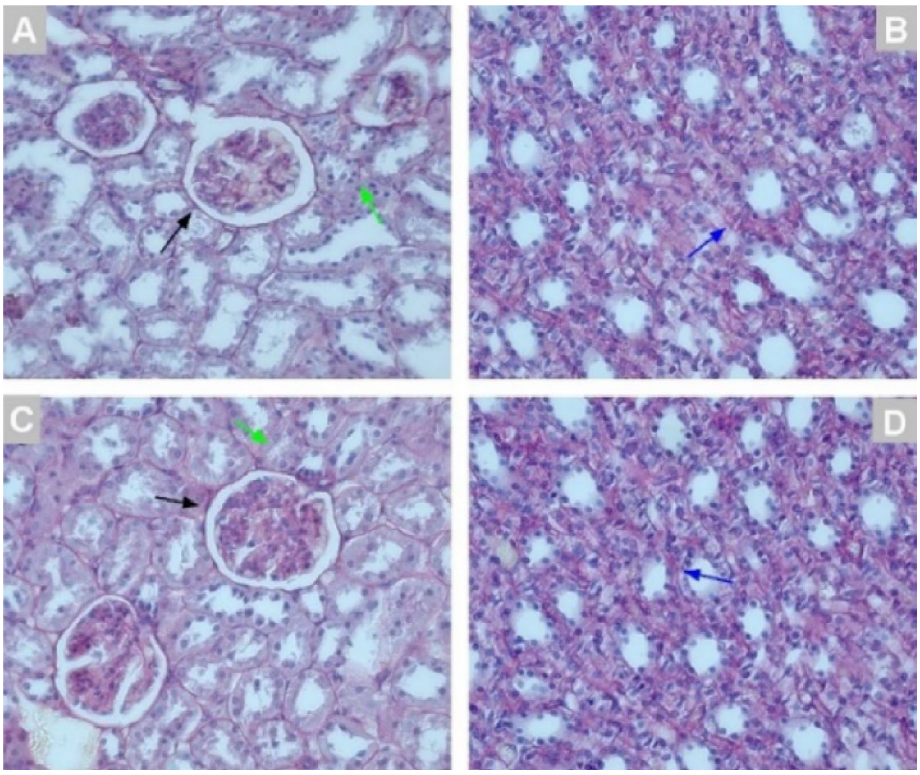

Fig. 37. Cortex-medulla view of kidney tissue of gallic acid group at 1st (**A**,**B**) and 2nd (**C**,**D**) week (Picro-Sirius red; ×40) (Kidney body: Black arrow, Proximal tubule: Green arrow, Collecting tubule: Blue arrow).

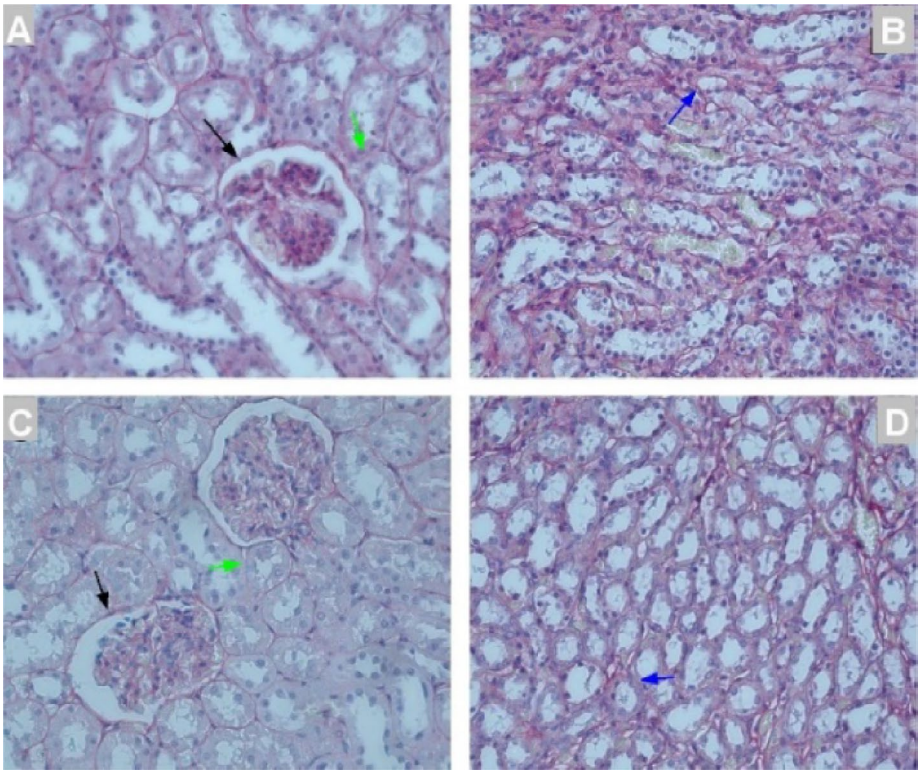

Fig. 38. Cortex-medulla view of kidney tissue of gallic acid group at 3rd (**A**,**B**) and 4th (**C**,**D**) week (Picro-Sirius red; ×40) (Kidney body: Black arrow, Proximal tubule: Green arrow, Collecting tubule: Blue arrow).

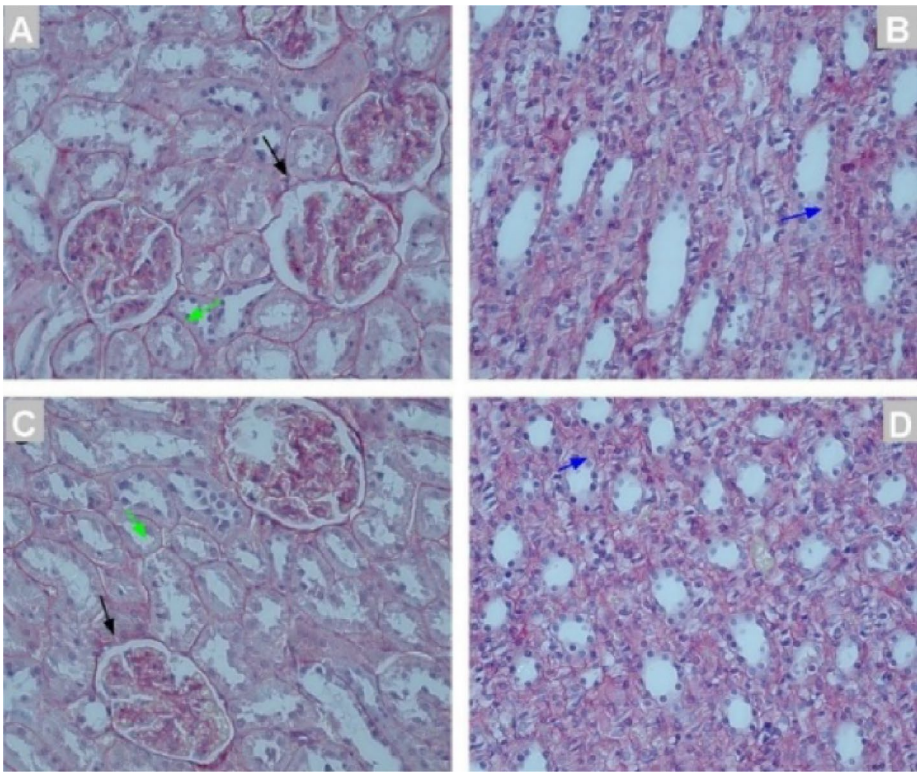

Fig. 39. Cortex-medulla view of kidney tissue of catechin and gallic acid group at 1st (**A**,**B**) and 2nd (**C**,**D**) week (Picro-Sirius red; ×40) (Kidney body: Black arrow, Proximal tubule: Green arrow, Collecting tubule: Blue arrow).

In the cisplatin + gallic acid group, collagen accumulation was reduced in the first week, with staining intensity between hepatocytes resembling the control group. An increase in collagen was noted during the second week, followed by a slight reduction in the third week, and a further decrease in the fourth week (Figs. 61, 62).

In the cisplatin + catechin + gallic acid group, collagen deposition increased between the portal area and hepatocytes in the first week, and this elevation persisted into the second week. However, collagen levels gradually decreased in the third and fourth weeks. Detailed weekly collagen staining scores of the liver tissue for each group are presented in Table 4 (Figs. 63, 64).

Biochemical findings

Serum biochemistry values

Serum urea, BUN, and creatinine levels In the cisplatin-only group, serum levels of urea, creatinine, and BUN were significantly elevated compared to the control and other treatment groups. However, in the groups receiving cisplatin in combination with catechin, gallic acid, or both, urea levels were lower than those observed in the cisplatin-only group. Notably, in the group treated with the combination of cisplatin, catechin, and gallic acid, urea levels demonstrated a progressive increase from the first to the final week of the study (Figs. 65, 66, 67).

Examination of serum AST, ALT values This study evaluated the effects of cisplatin on liver enzyme levels across different experimental groups. Serum AST and ALT levels were significantly elevated in the cisplatin-only group compared to the control and other treatment groups ($p < 0.05$). Co-administration of cisplatin with catechin, gallic acid, or their combination led to a significant reduction in AST and ALT levels ($p < 0.05$). In the cisplatin-only group, AST and ALT levels peaked during the first week and declined progressively, reaching their lowest values by the fourth week ($p < 0.05$). A similar trend was observed in the groups treated with cisplatin plus catechin and/or gallic acid, with the highest enzyme levels recorded in the first week and the lowest in the fourth week ($p < 0.05$). These results suggest that catechin and gallic acid may exert a protective effect against cisplatin-induced hepatotoxicity (Figs. 67, 68, 69).

Examination of tissue biochemistry values

When GPx and GR levels derived from kidney tissue were evaluated, the cisplatin-only group exhibited significantly lower values compared to the control and treatment groups. In the groups receiving cisplatin in combination with either catechin or gallic acid, GPx and GR activities increased, indicating a partial restoration of antioxidant defense mechanisms. However, in the group treated with cisplatin alongside both catechin and gallic acid, GPx and GR values showed a progressive decline from the first to the fourth week.

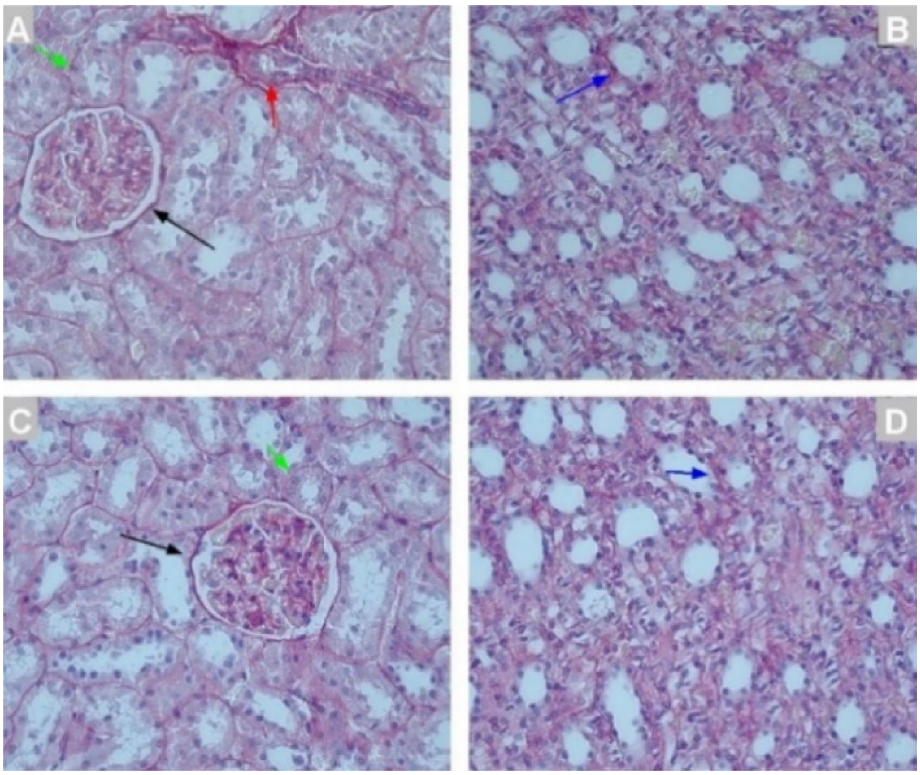

Fig. 40. Cortex-medulla view of kidney tissue of catechin and gallic acid group at 3rd (**A**,**B**) and 4th (**C**,**D**) week (Picro-Sirius red; ×40) (Kidney body: Black arrow, Proximal tubule: Green arrow, Collecting tubule: Blue arrow, Vascular circumference: Red arrow).

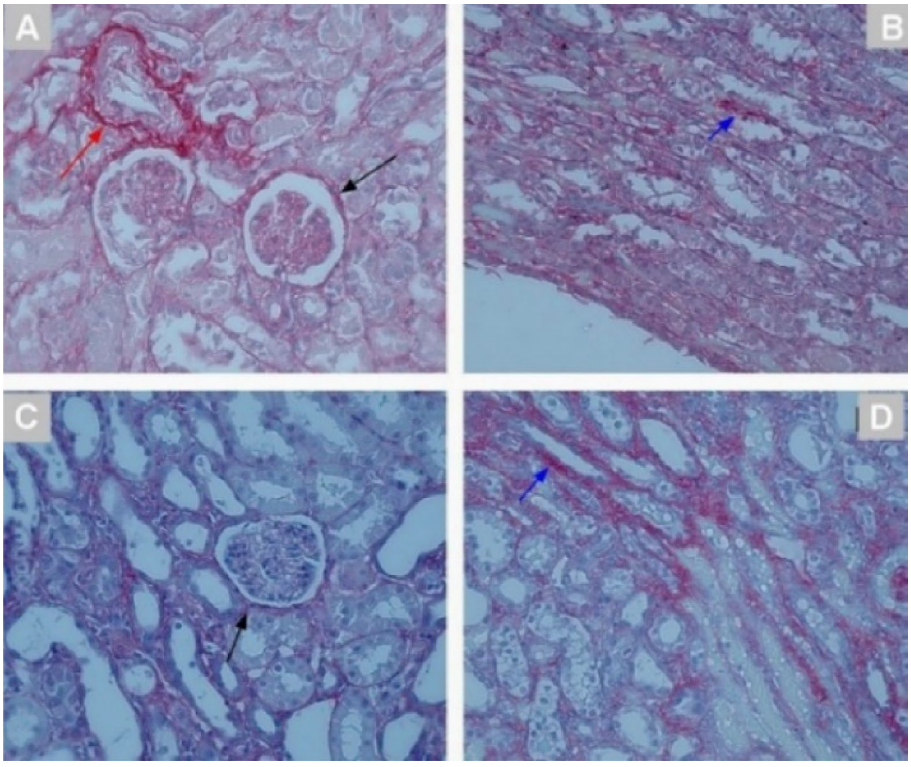

Fig. 41. Cisplatin group renal tissue cortex-medulla view of week 1 (**A**,**B**) and week 2 (**C**,**D**) (Picro-Sirius red; ×40) (Kidney body: Black arrow, Collecting tubule: Blue arrow, proximal tubule: Green arrow).

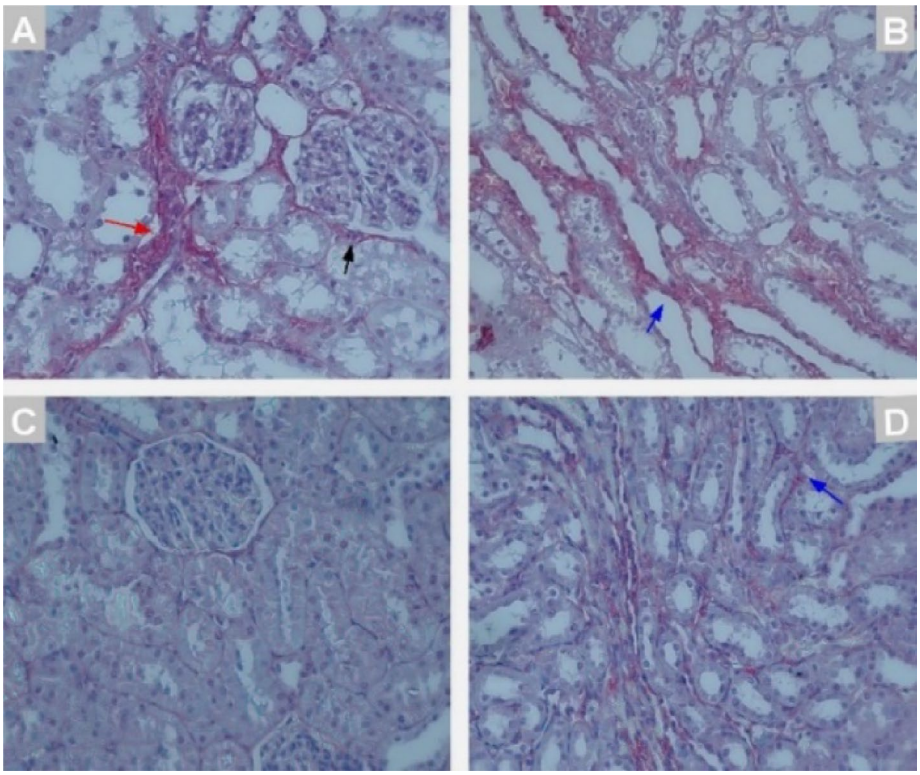

Fig. 42. Cisplatin group renal tissue cortex-medulla appearance at week 3 (**A**,**B**) and 4 (**C**,**D**) (Picro-Sirius red; ×40) (Kidney body: Black arrow, Collecting tubule: Blue arrow, Vascular circumference: Red arrow).

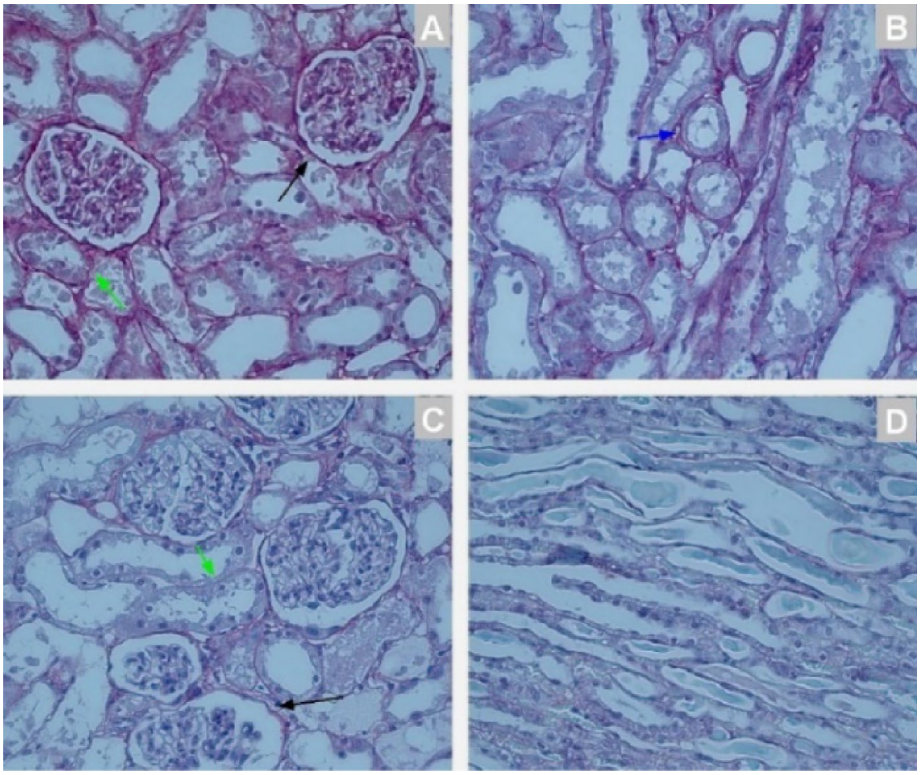

Fig. 43. Cortex-medulla view of kidney tissue of the 1st (**A**,**B**) and 2nd (**C**,**D**) week of the group in which cisplatin was combined with catechin (Picro-Sirius red; ×40) (Kidney body: Black arrow, Collecting tubule: Blue arrow, proximal tubule: Green arrow).

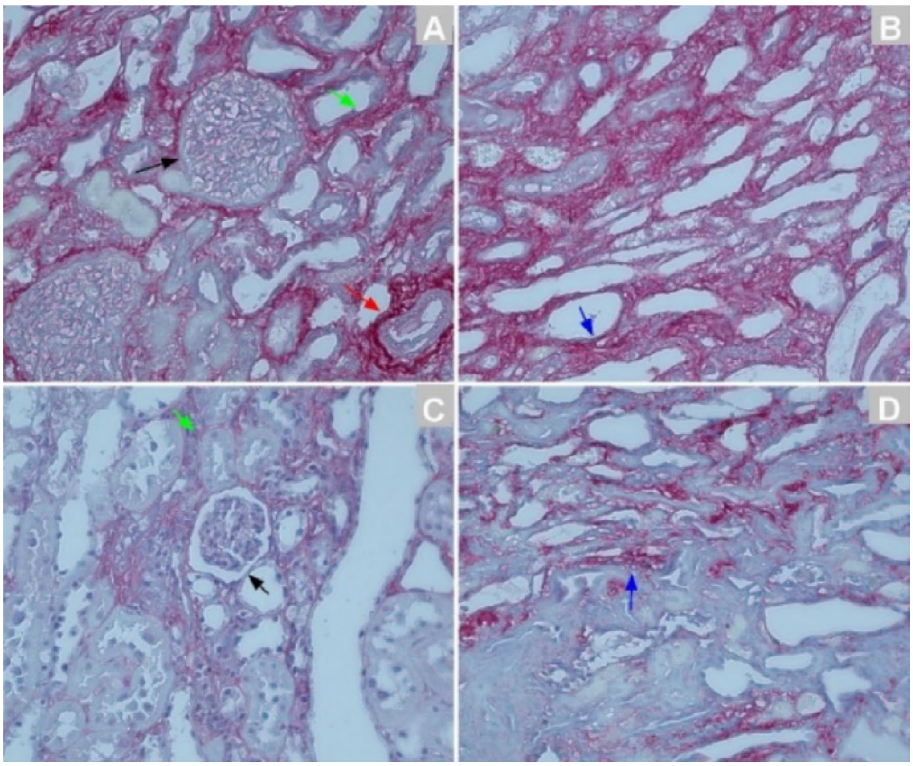

Fig. 44. Cortex-medulla view of kidney tissue of the 3rd (**A**,**B**) and 4th (**C**,**D**) week of the group in which cisplatin was combined with catechin (Picro-Sirius red; 20X) (Kidney body: Black arrow, Proximal tubule: Green arrow, Collecting tubule: Blue arrow, Vascular circumference: Red arrow).

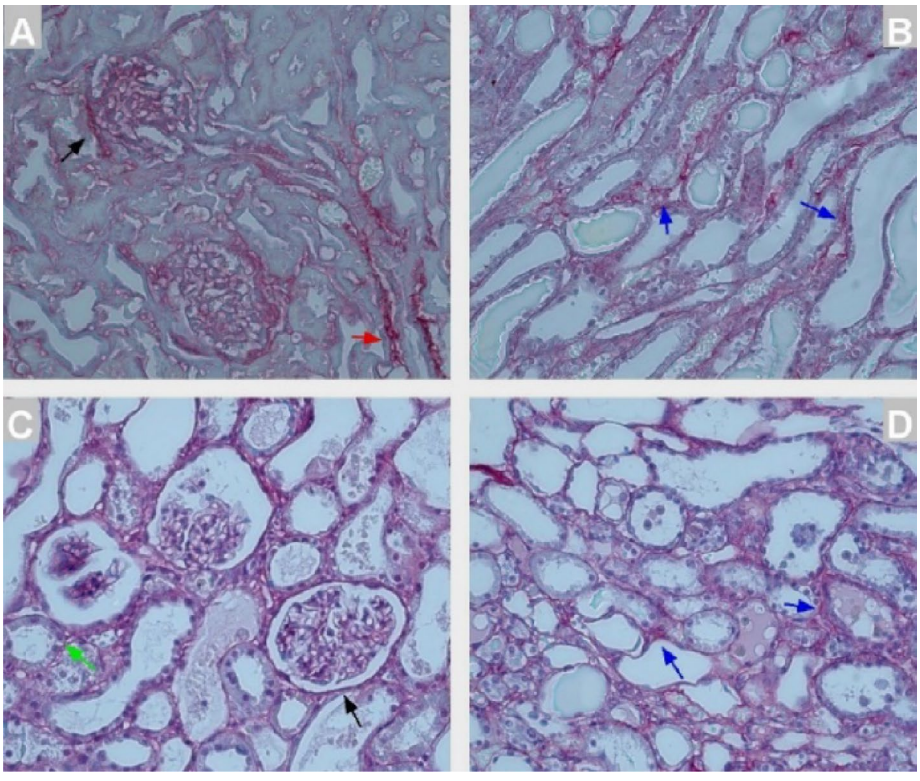

Fig. 45. Cortex-medulla view of kidney tissue of the 1st (**A**,**B**) and 2nd (**C**,**D**) week of the group in which cisplatin was administered with gallic acid (Picro-Sirius red; ×40) (Kidney body: Black arrow, Proximal tubule: Green arrow, Collecting tubule: Blue arrow, Vascular circumference: Red arrow).

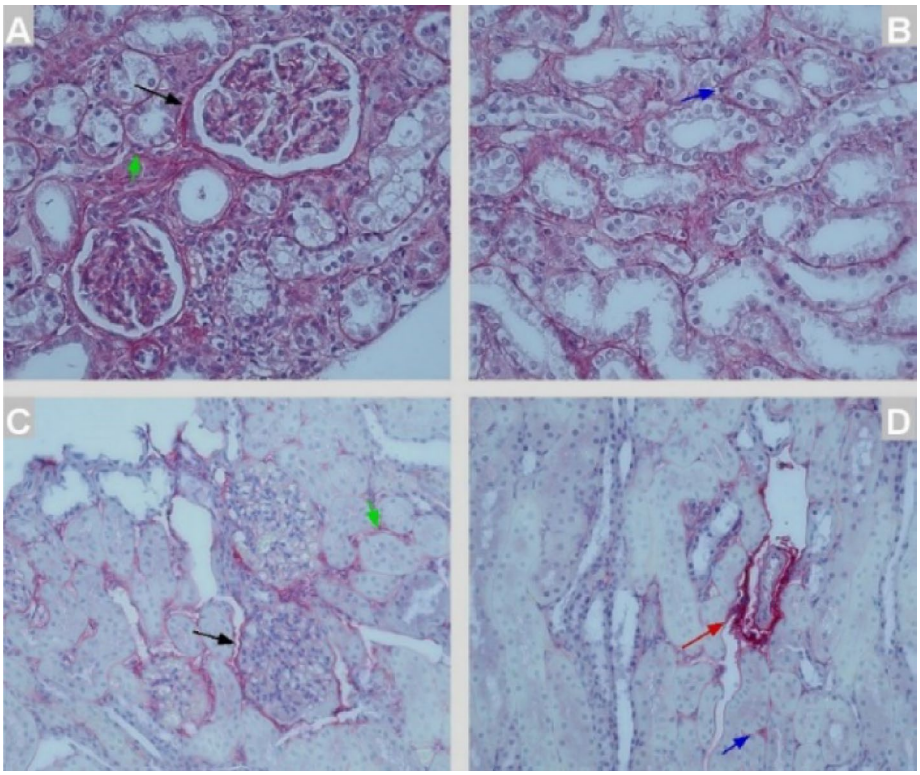

Fig. 46. Cortex-medulla view of kidney tissue of the 3rd (**A**,**B**) and 4th (**C**,**D**) week of the group in which cisplatin was administered with gallic acid (Picro-Sirius red; ×40) (Kidney body: Black arrow, Proximal tubule: Green arrow, Collecting tubule: Blue arrow, Vascular circumference: Red arrow).

Groups	Collagen score (Week 1)	Collagen score (Week 2)	Collagen score (Week 3)	Collagen score (Week 4)
Control	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
CP	3.00 ± 0.00	3.00 ± 0.00	1.00 ± 0.00	2.00 ± 0.00
Catechin	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
Gallic acid	2.00 ± 0.00	2.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
Catechin + gallic acid	2.00 ± 0.00	2.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
Catechin + CP	3.00 ± 0.00	2.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
Gallic acid + CP	3.00 ± 0.00	3.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
Catechin + gallic acid + CP	2.00 ± 0.00	2.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00

Table 3. Collagen staining score change graph of kidney tissue of experimental groups.

Regarding MDA levels in kidney tissue, cisplatin-treated groups demonstrated significantly higher values compared to the control and other treatment groups, reflecting enhanced lipid peroxidation. Conversely, catechin- or gallic acid-treated groups showed a marked reduction in MDA levels. In the group co-treated with cisplatin, catechin, and gallic acid, MDA levels gradually increased from the first to the final week (Figs. 70, 71, 72).

When the GPx and GR values in liver tissue were examined, the GPx and GR values in the cisplatin-treated groups were found to be lower compared to the control and treatment groups. However, in the groups treated with cisplatin combined with catechin and gallic acid, an increase in GPx and GR values was recorded from the first week to the last week (Figs. 73, 74, 75).

Discussion

In our study, the body weight of the rats significantly decreased in the cisplatin-treated group, whereas weight gain was observed in the groups receiving catechin and gallic acid along with the weight loss. The weight loss observed in the cisplatin group could be related to acidosis, anorexia, and reduced food intake due to increased catabolism or damage to the renal tubules[8,43]. Similarly, a study reported that garlic extract had a protective effect against cisplatin toxicity and prevented weight loss[45]. Other studies have also reported that cisplatin treatment with various antioxidants and plant extracts prevented weight loss and maintained body weight[46,47]. In our study,

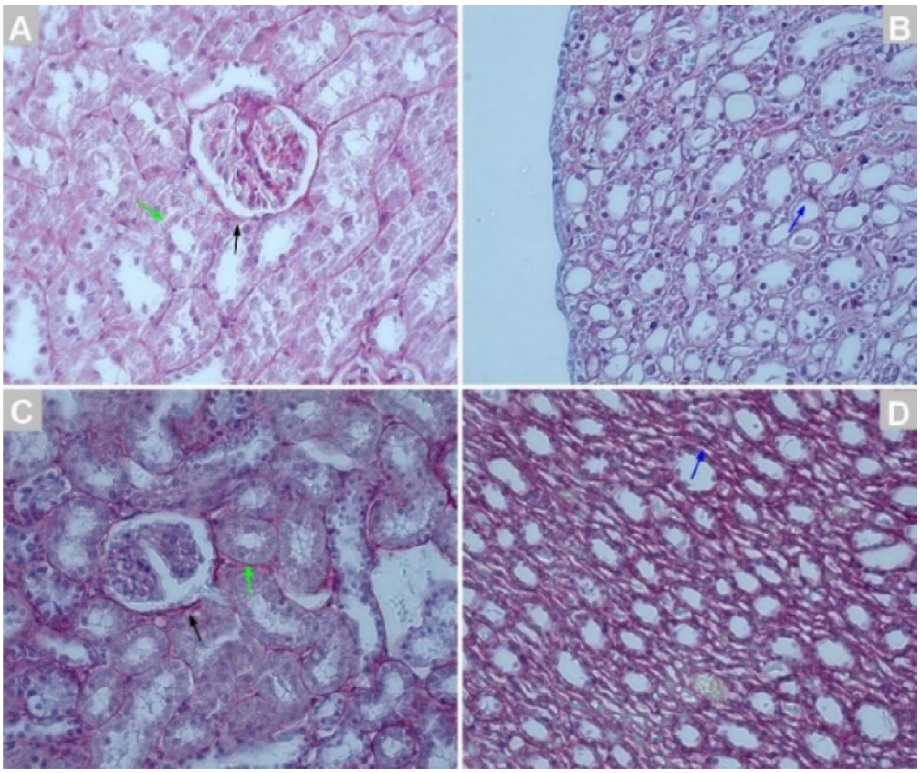

Fig. 47. Cortex-medulla view of kidney tissue of the 1st (**A**,**B**) and 2nd (**C**,**D**) week of cisplatin treatment with catechin and gallic acid (Picro Sirius red; ×40) (Kidney body: Black arrow, Proximal tubule: Green arrow, Collecting tubule: Blue arrow).

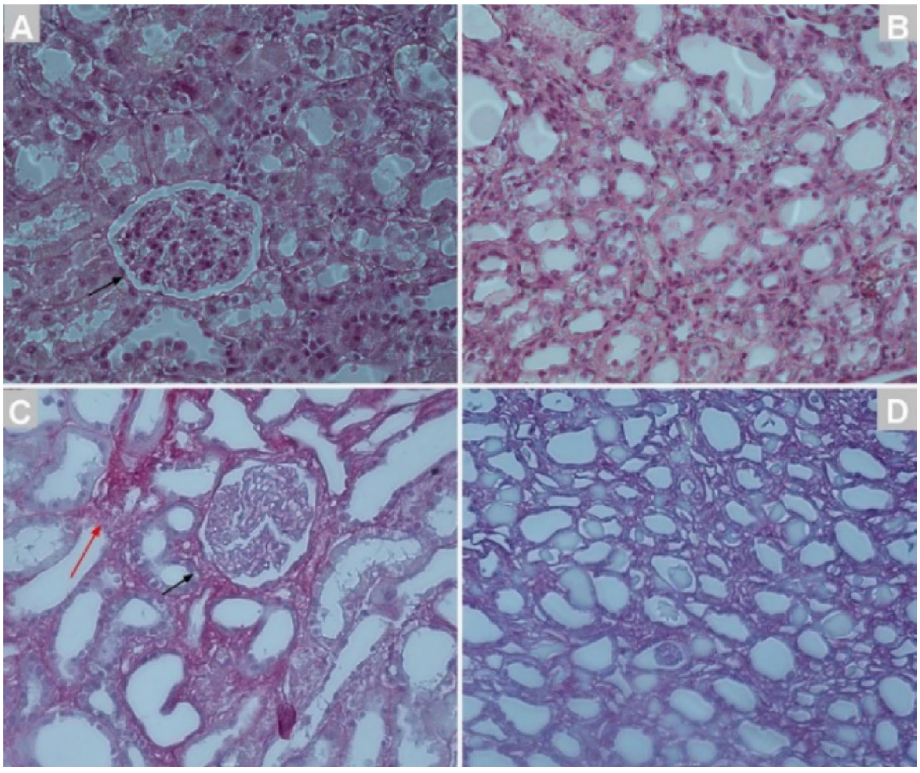

Fig. 48. Cortex-medulla view of kidney tissue of the 3rd (**A**,**B**) and 4th (**C**,**D**) week of cisplatin treatment with catechin and gallic acid (Picro Sirius red; ×40) (Kidney body: Black arrow, Vascular periphery: Red arrow).

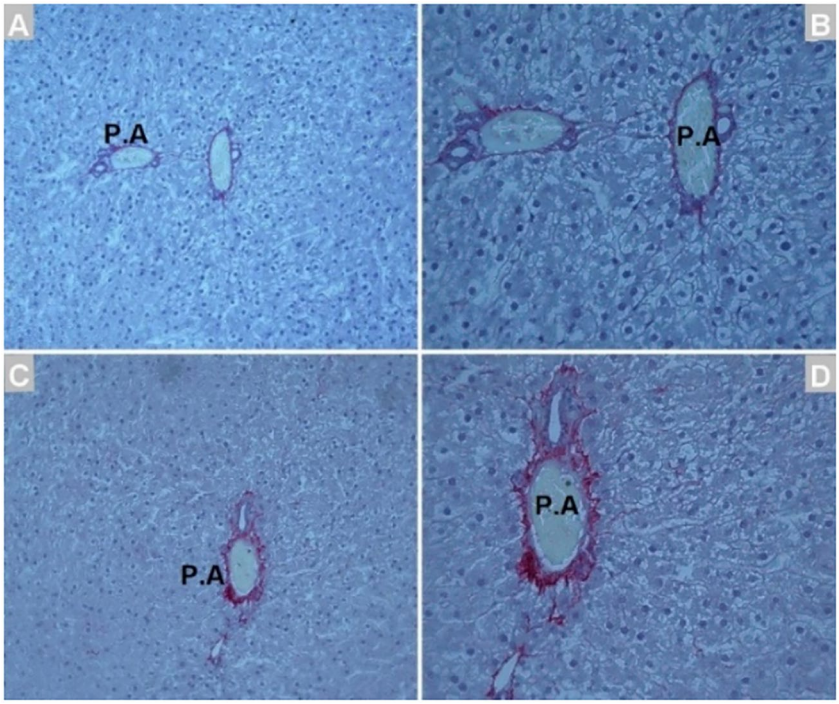

Fig. 49. The appearance of collagen amount in the liver tissue of the control group at 1 (**A**,**B**) and 2 (**C**,**D**) weeks (Picro sirius red; ×20–×40).

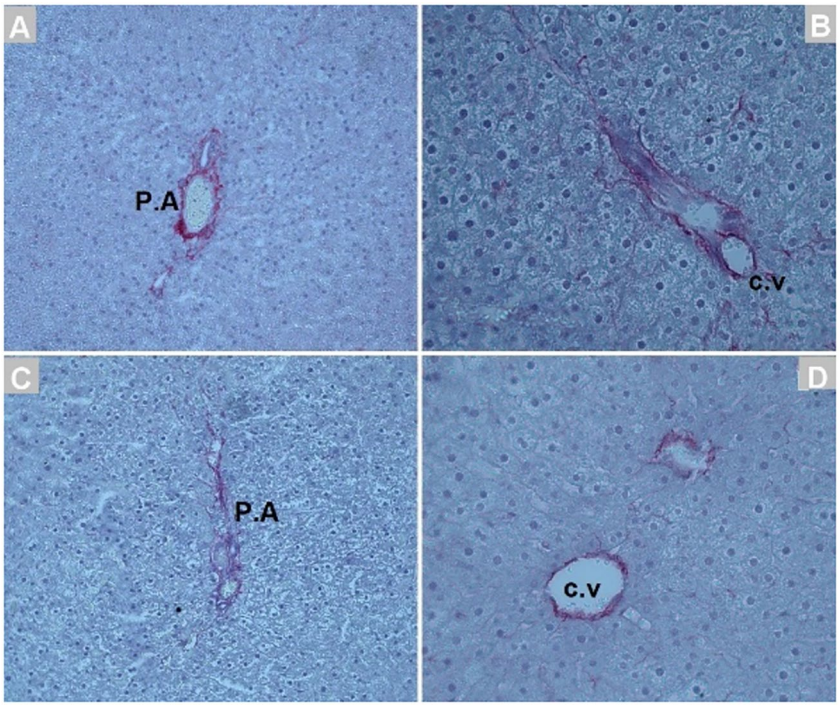

Fig. 50. The appearance of collagen amount in the liver tissue of the control group at 3 (**A**,**B**) and 4 (**C**,**D**) weeks (Picro sirius red; ×20–×40) (Portal area: P.A, Central vein: c.v).

an increase in kidney indices was particularly observed in the second week in groups treated with cisplatin or cisplatin combinations. Similarly, a study by Pandir and Kara[48] found that the kidney index was higher in the group treated with blueberry compared to the cisplatin group. Ibrahim et al.[49] stated that olive leaf extract could reduce kidney weight in rats treated with cisplatin. Abdel-Daim et al.[42] reported that the combination of ascorbic

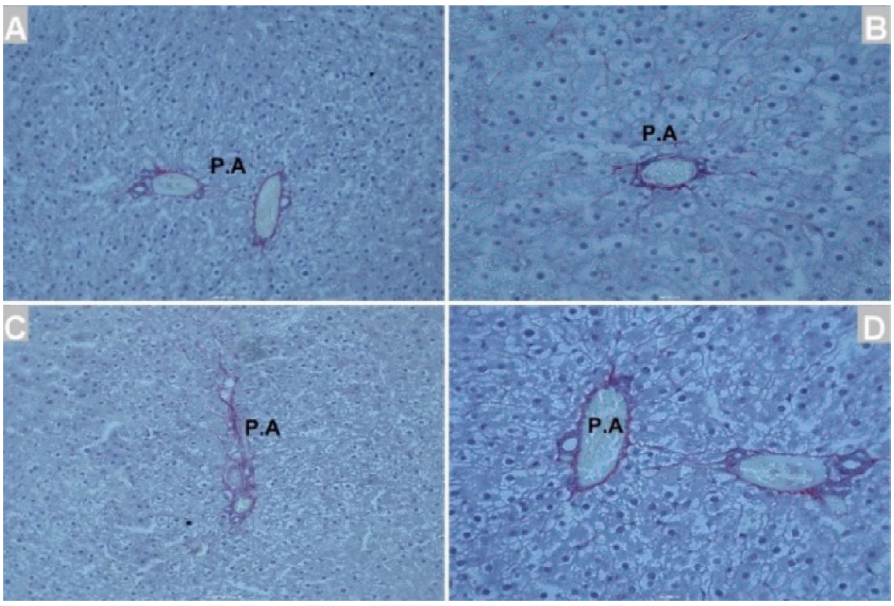

Fig. 51. The appearance of the amount of collagen in the liver tissue of the catechin group at weeks 1 (**A**,**B**) and 2 (**C**,**D**) (Picro sirius red; ×20–×40) (Portal area. P.A).

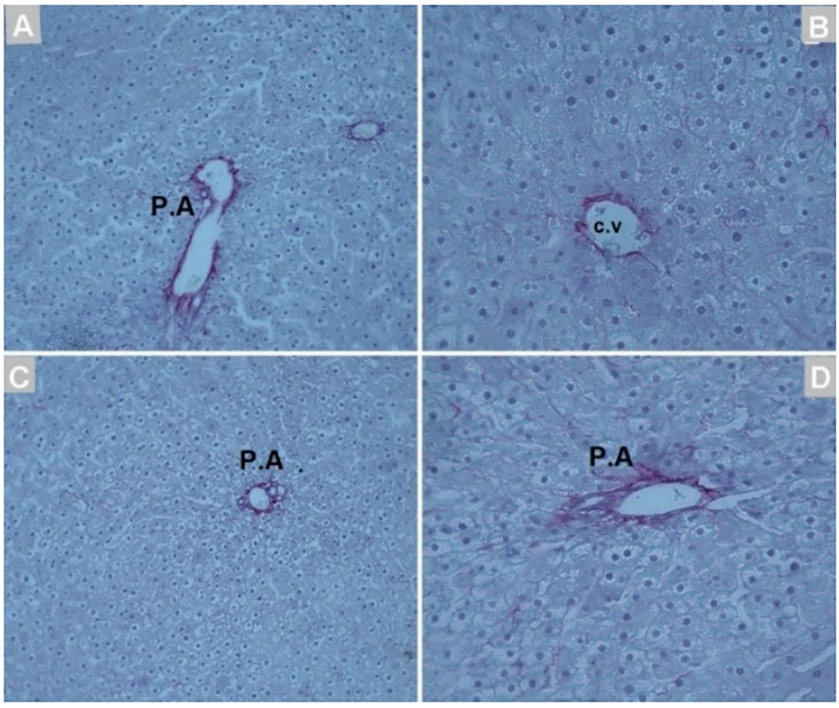

Fig. 52. The appearance of collagen amount in liver tissue of catechin group at 3 (**A**,**B**) and 4 (**C**,**D**) weeks (Picro sirius red; ×20–×40) (Portal area: P.A).

acid and allicin reduced kidney weights in cisplatin-treated groups. The increase in kidney weight and the weight loss observed in cisplatin-treated groups are associated with anorexia, gastrointestinal disorders, and tubular necrosis[25,42,50]. In our study, the liver weight in the cisplatin-treated group decreased compared to the other groups, but liver weight increased in the groups receiving catechin and gallic acid. Yasuyuki et al. and Sadzuka et al.[51] indicated that cisplatin decreases liver weight, which is attributed to body weight loss and decreased food and water intake. Pratibha et al.[52] also reported that cisplatin decreases organ weights. Additionally, Habib et al.[53] found that protocatechuic acid reduced cisplatin-induced liver weight increase. Cisplatin causes weight loss by inducing necrosis in liver cells, and increased catabolism, acidosis, and anorexia contribute to this weight

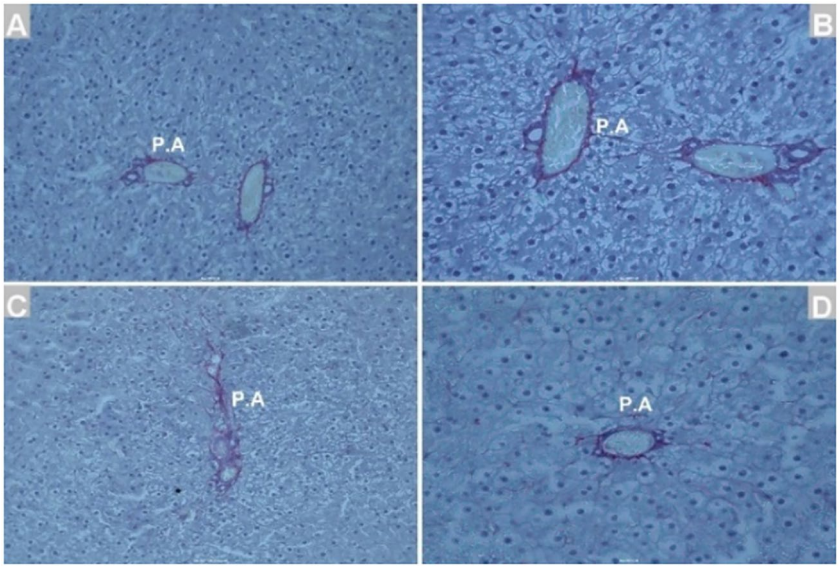

Fig. 53. The appearance of collagen amount in liver tissue of gallic acid group at 1 (**A**,**B**) and 2 (**C**,**D**) weeks (Picro sirius red; ×20–×40) (Portal area: P.A).

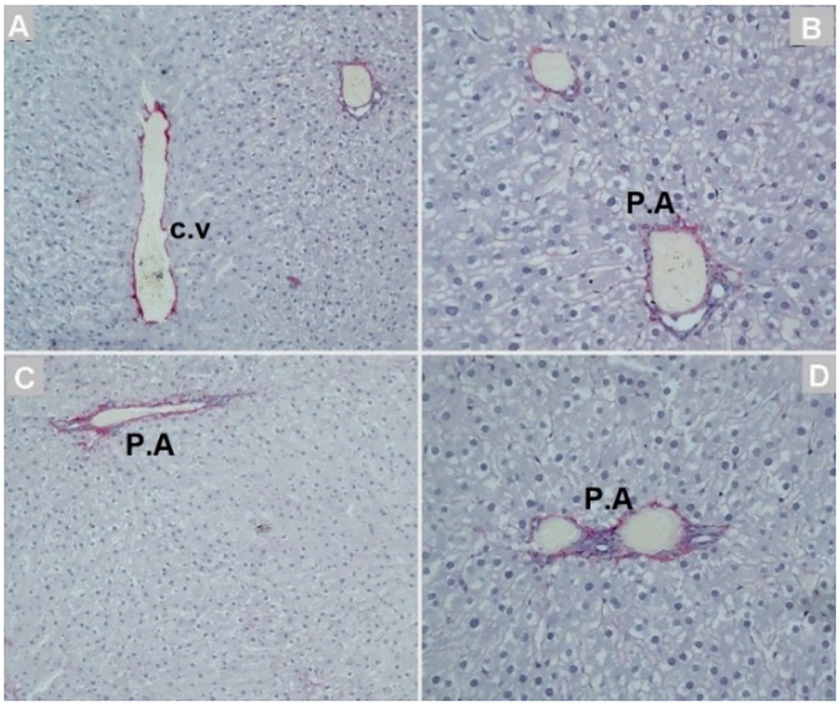

Fig. 54. The appearance of collagen amount in liver tissue of gallic acid group at 3 (**A**,**B**) and 4 (**C**,**D**) weeks (Picro sirius red; ×20–×40) (Portal area: P.A, Central vein: c.v).

loss. The liver's regenerative capacity is impaired, and this process may be hindered by cisplatin. When the histopathological results of the kidney tissue in our study were examined, more damage was observed in the cisplatin-treated groups, especially in the second week, while the damage was milder in the groups treated with cisplatin combined with catechin or gallic acid. However, it was noted that the damage in the group treated with both catechin and gallic acid alongside cisplatin increased over time. These results are consistent with the biochemical parameters. In the cisplatin-treated groups, urea, creatinine, BUN, and MDA values increased compared to the control and other treatment groups, while GPx and GR values decreased. In the treatment groups combined with cisplatin, urea, creatinine, BUN, and MDA levels decreased, while GPx and GR values increased. Our findings are in agreement with previous studies that reported antioxidant and anti-inflammatory

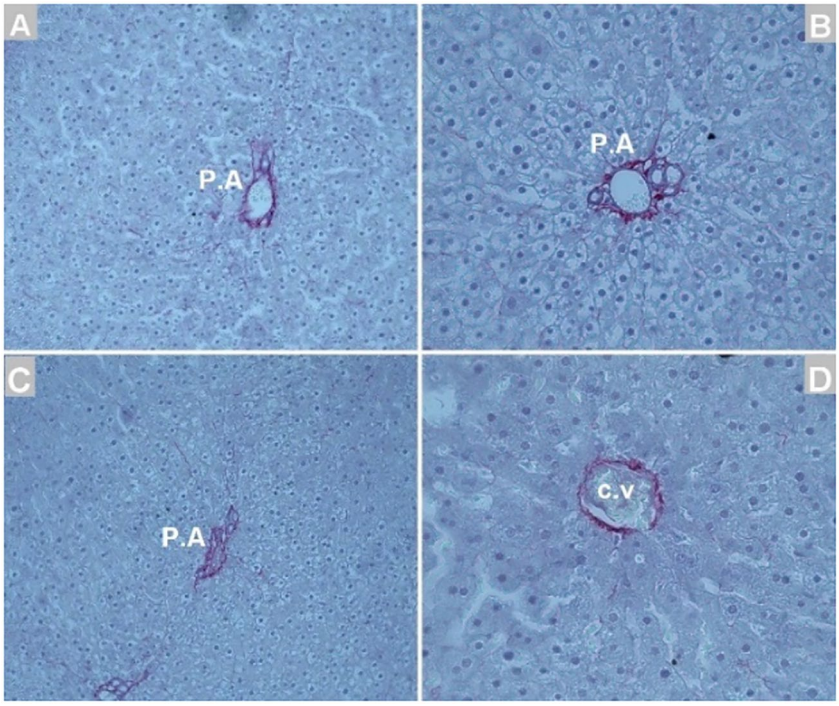

Fig. 55. The appearance of the amount of collagen in liver tissue of catechin and gallic acid group at 1 (**A**,**B**) and 2 (**C**,**D**) weeks (Picro sirius red; ×20–×40) (Portal area: P.A, Central vein: c.v).

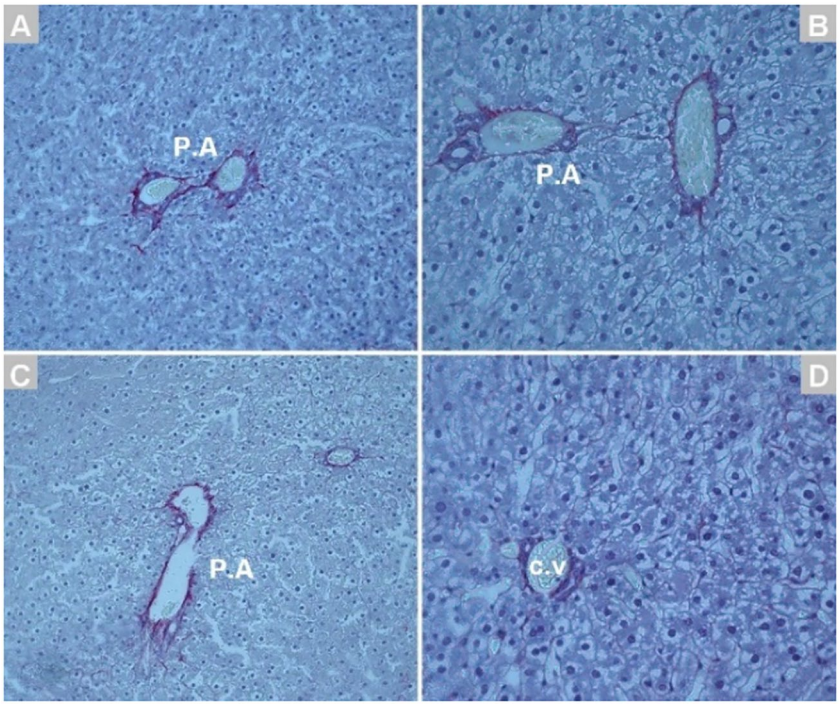

Fig. 56. The appearance of collagen amount in liver tissue of catechin and gallic acid group at 3 (**A**,**B**) and 4 (**C**,**D**) weeks (Picro sirius red; ×20–×40) (Portal area: P.A, Central vein: c.v).

agents confer protection against cisplatin-induced nephrotoxicity. For instance, Ardestani and Hajizadeh (2021) demonstrated that myrtenol administration (50 mg/kg, intraperitoneally for four consecutive days) significantly alleviated cisplatin-induced renal dysfunction in mice. Their study showed reductions in serum blood urea nitrogen (BUN) and renal malondialdehyde (MDA) levels, along with restoration of key antioxidant enzyme

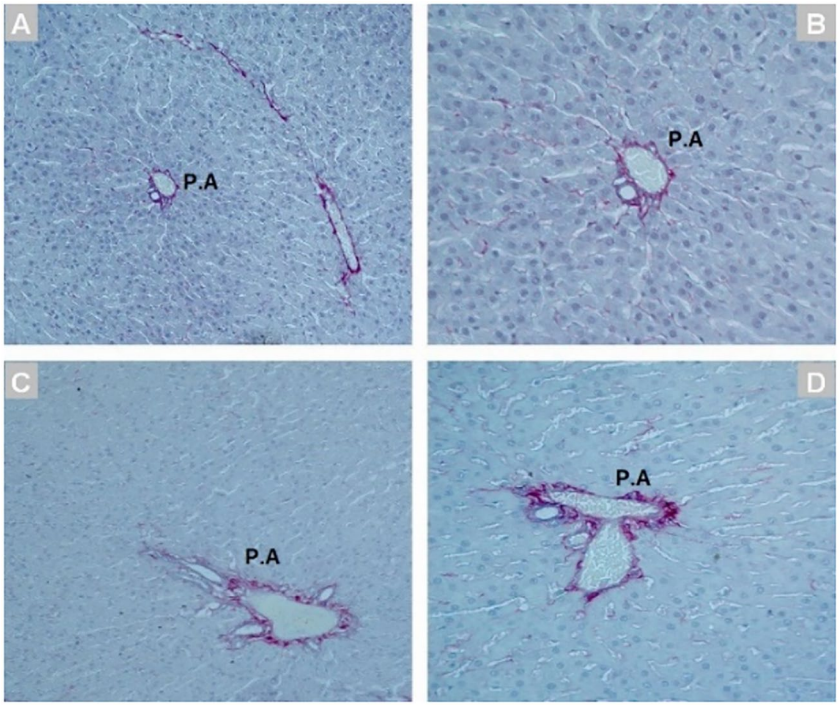

Fig. 57. Liver tissue (Picro Sirius red; ×20–×40) of the 1st (**A**,**B**) and 2nd (**C**,**D**) week of the cisplatin alone group (Portal area: P.A).

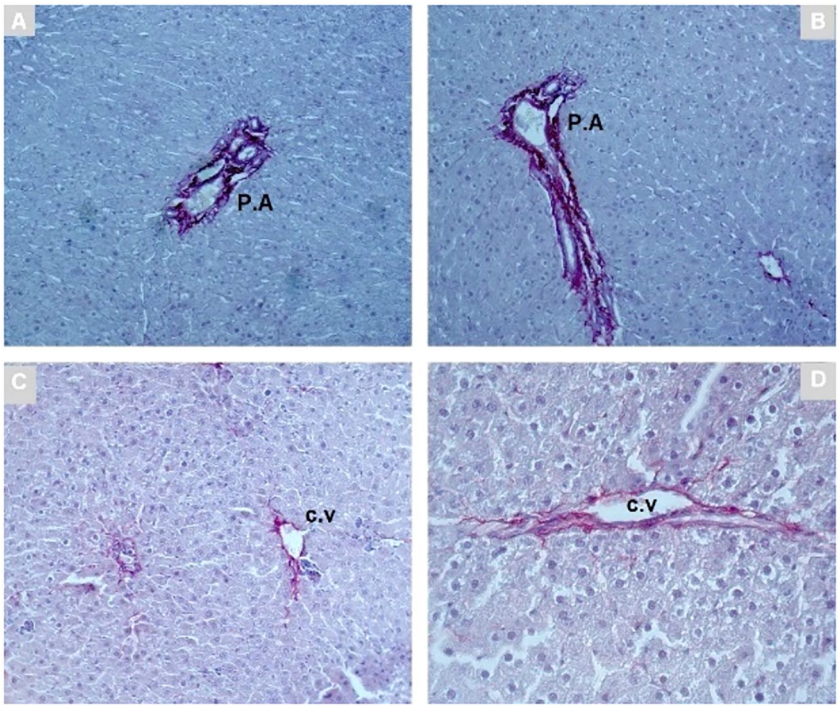

Fig. 58. Liver tissue of the 3rd (**A**,**B**) and 4th (**C**,**D**) week of the cisplatin alone group (Picro Sirius red; ×20–×40).

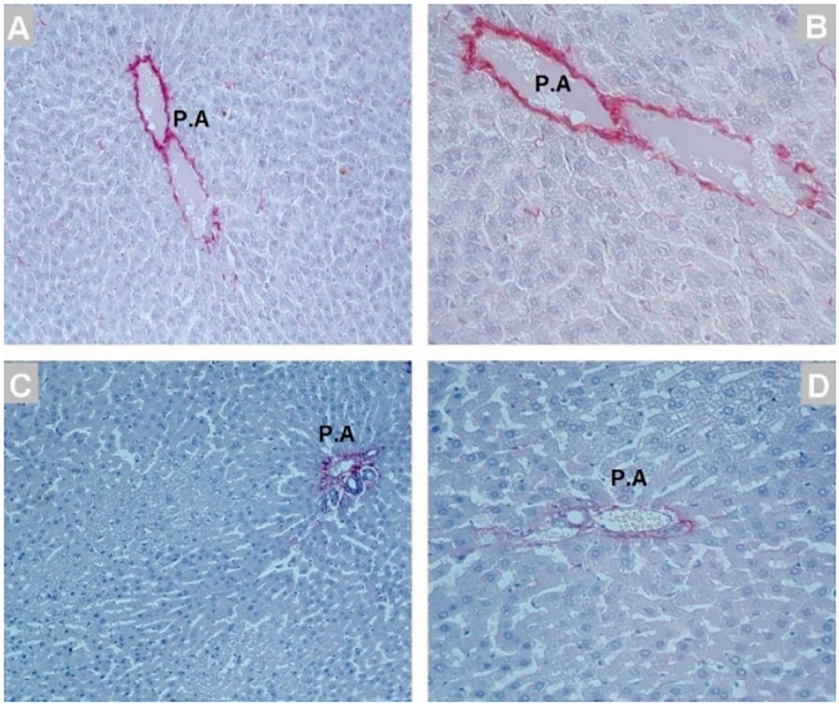

Fig. 59. Liver tissue of the 1st (**A**,**B**) and 2nd (**C**,**D**) week of the group in which cisplatin was given together with catechin (Picro Sirius red; ×20–×40).

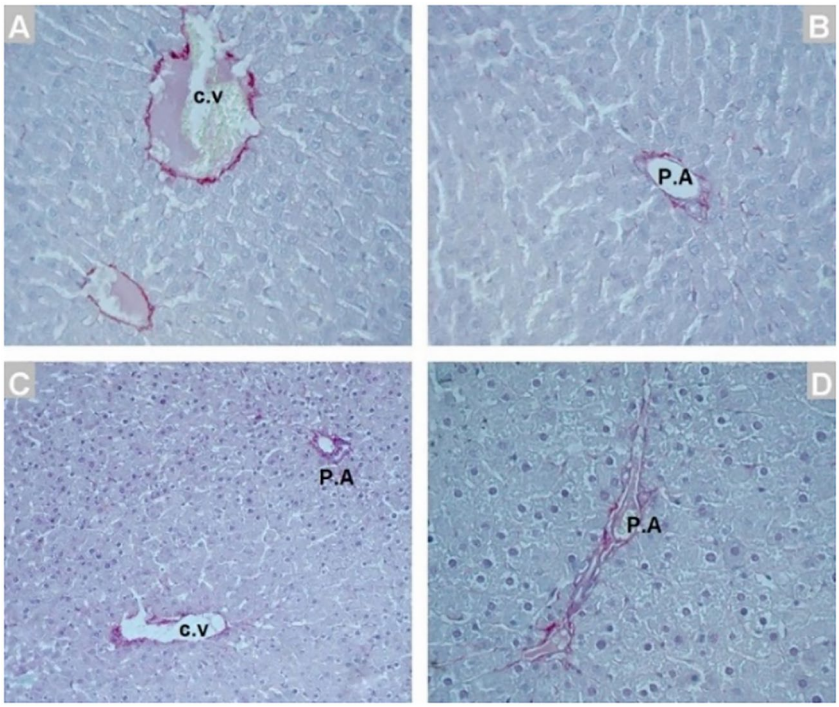

Fig. 60. Liver tissue of the 3rd (**A**,**B**) and 4th (**C**,**D**) week of the group in which cisplatin was administered with catechin (Picro Sirius red; ×20–×40).

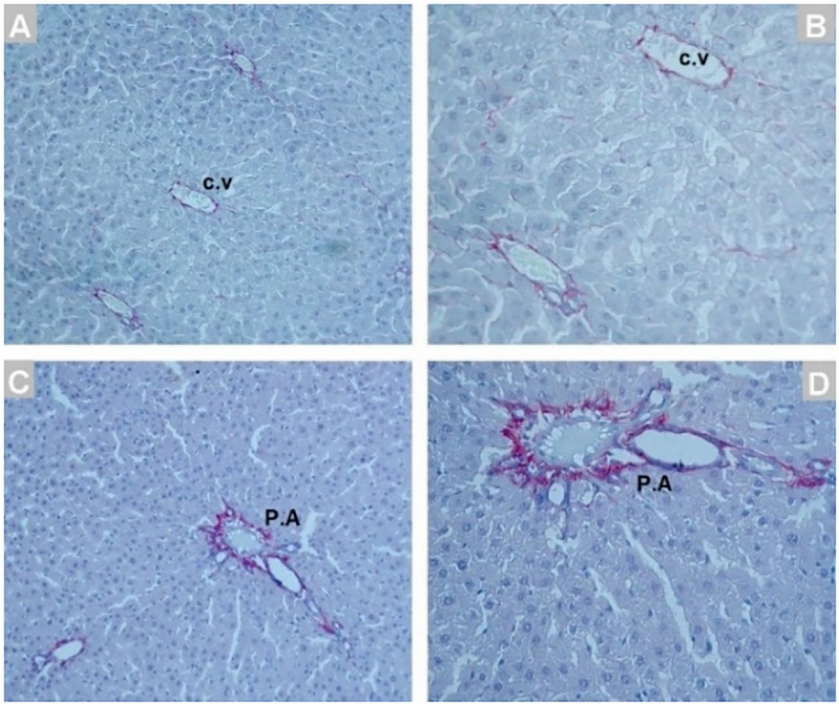

Fig. 61. Liver tissue of the 1st (**A**,**B**) and 2nd (**C**,**D**) week of the group in which cisplatin was administered together with gallic acid (Picro Sirius red; ×20–×40).

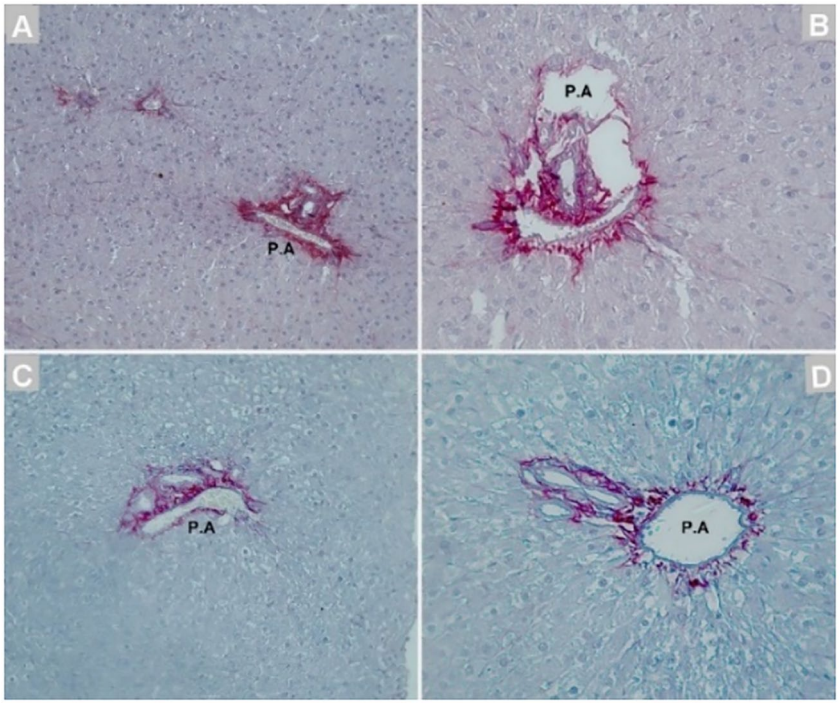

Fig. 62. Liver tissue (Picro Sirius red; ×20–×40) of the 3rd (**A**,**B**) and 4th (**C**,**D**) week of the group in which cisplatin was given together with gallic acid (Portal area: P.A).

Groups	Collagen score (Week 1)	Collagen score (Week 2)	Collagen score (Week 3)	Collagen score (Week 4)
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CP	1.00 ± 0.00	2.00 ± 0.00	1.75 ± 0.50	1.50 ± 0.57
Catechin	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Gallic acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Catechin + gallic acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Catechin + CP	0.75 ± 0.50	1.25 ± 0.50	1.00 ± 0.00	0.75 ± 0.50
Gallic acid + CP	2.50 ± 0.57	1.50 ± 0.57	1.00 ± 0.00	0.75 ± 0.50
Catechin + gallic acid + CP	0.75 ± 0.50	1.25 ± 0.50	0.75 ± 0.50	0.50 ± 0.57

Table 4. Collagen staining score change graph of liver tissue of the experimental groups.

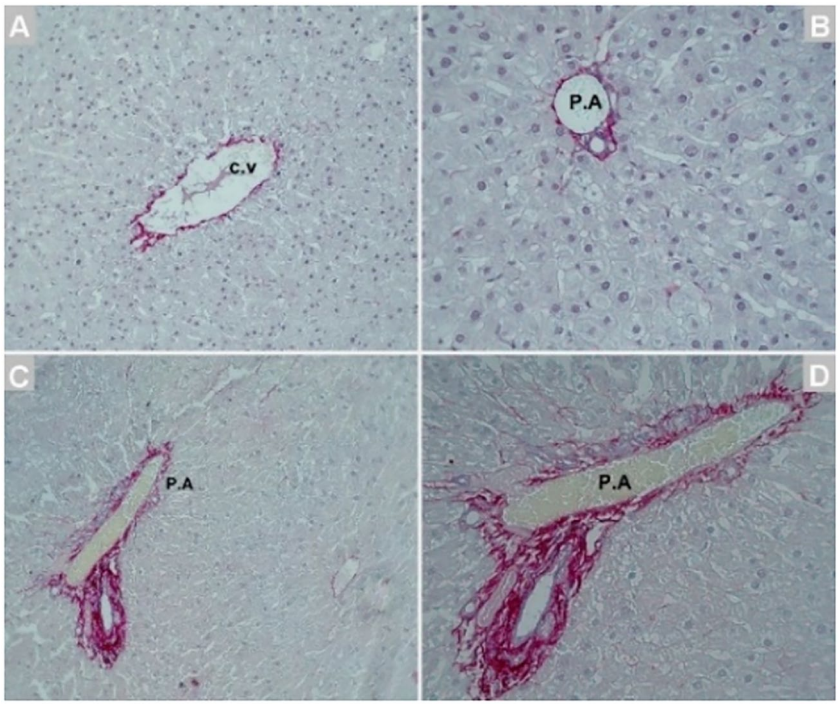

Fig. 63. Liver tissue of the 1st (**A**,**B**) and 2nd (**C**,**D**) week of the group in which cisplatin was given together with catechin and gallic acid (Picro Sirius red; ×20–×40).

activities including superoxide dismutase (SOD) and catalase (CAT). Histopathological assessments further confirmed decreased tubular degeneration and necrosis, supporting the renoprotective effects of myrtenol through oxidative stress modulation[54].

Similarly, Bazmandegan et al.[28] reported that sumatriptan (0.3 mg/kg/day, intraperitoneally for three days) exerted nephroprotective effects in cisplatin-treated mice by decreasing serum creatinine, BUN, and MDA levels, while enhancing renal antioxidant defenses (SOD, GPx). In addition, sumatriptan-treated animals showed marked improvement in renal histology, with reduced tubular injury and necrosis. These findings are consistent with the protective profile observed in our study, further emphasizing the crucial role of redox balance and inflammatory regulation in mitigating cisplatin-induced renal injury[28].

Li et al.[55] attempted to alleviate cisplatin-induced acute kidney injury by using neferin, and their study found that neferin treatment significantly reduced kidney damage. Altındağ et al.[56] aimed to improve cisplatin-induced kidney damage by reducing oxidative stress and apoptosis with sinapic acid, observing a significant reduction in kidney damage following sinapic acid treatment. The severe damage observed in the second week of our study supports the hypothesis that cisplatin begins inducing nephrotoxicity approximately from the 10th day. The highest damage observed in the 4th week is likely due to the rats' inability to consume sufficient amounts of water and food, caused by cisplatin-induced anorexia and dehydration. This situation is expected to increase the nephrotoxicity severity caused by cisplatin accumulation in the proximal tubules of the kidney[43,49,56,57]. Cisplatin-induced nephrotoxicity arises from oxidative stress, apoptosis, and inflammation[39]. ROS and vasoconstriction are major contributors to kidney damage ROS disrupt cell membranes, DNA, and proteins, impairing mitochondrial function[50]. Cisplatin affects the structure and function of kidney mitochondria[58]. Additionally, cisplatin may reduce the levels of antioxidant enzymes[39]. Antioxidant and anti-inflammatory

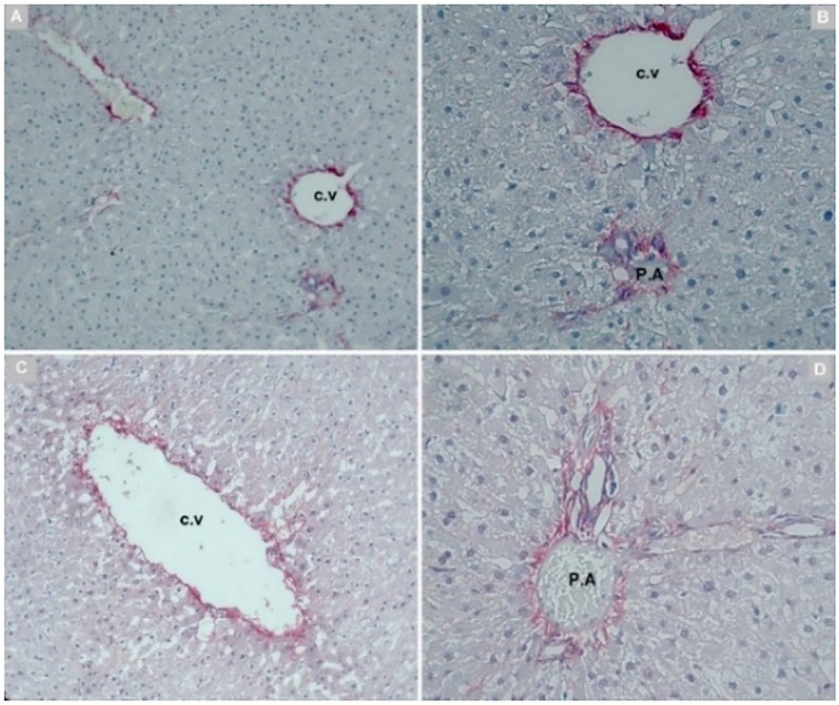

Fig. 64. Liver tissue (Picro sirius red; ×20–×40) of the 3rd (**A**,**B**) and 4th (**C**,**D**) week of the group in which cisplatin was given together with catechin and gallic acid (Portal area: P.A, Central vein: c.v).

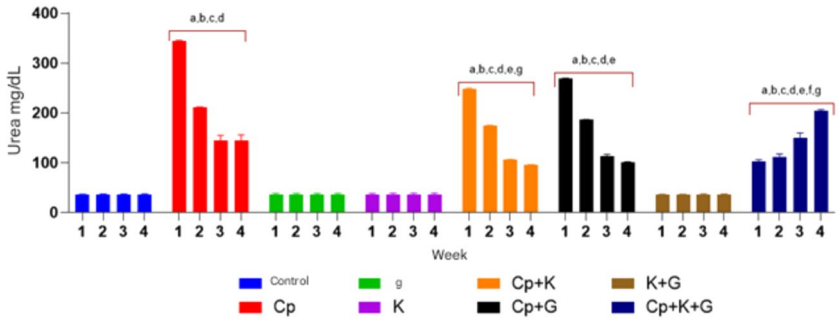

Fig. 65. Urea change graph of the experimental groups (a $p < 0.05$; compared to control group, b $p < 0.05$; compared to K group, c $p < 0.05$; compared to G group, d $p < 0.05$; compared to K + G group, e $p < 0.05$; compared to CP group, f $p < 0.05$; compared to CP + K group, g $p < 0.05$; compared to CP + G group, h; compared to CP + K + G group).

agents can prevent cell damage[59]. In our study, an increase in necrotic areas and dilatations was observed in the groups treated with catechin and gallic acid from the second week, but the damage was reduced compared to the cisplatin group. However, the kidney damage continued in the last week. This situation can be related to the effects of antioxidant therapeutic agents started before cisplatin injection, as reported in other studies. Pre-treatment with antioxidants may reduce the oxidative stress effects that could arise after cisplatin administration by enhancing the activation of antioxidant enzymes in cells in rats. Furthermore, in other studies, experiments were terminated shortly after cisplatin injection, and animals were euthanized, suggesting that the findings in our study may differ from the results of other researchers due to the study duration. When the liver tissue Figures were examined in our study, more damage was observed in the cisplatin-treated groups compared to the treatment groups. These results are consistent with the biochemical parameters. In the cisplatin-treated groups, an increase in AST, ALT, and MDA values was observed, while GPx and GR values decreased. In particular, the elevated hepatic MDA levels observed in the cisplatin group indicate increased lipid peroxidation and oxidative stress in liver tissue. This finding supports the notion that cisplatin-induced hepatotoxicity is closely associated with oxidative mechanisms. Similar increases in hepatic MDA were reported by Sherif[60] and Okkay et al.[61], confirming that cisplatin accumulation in hepatocytes leads to structural and functional hepatic damage. In our study, treatment with catechin and gallic acid led to a noticeable reduction in hepatic MDA levels, suggesting

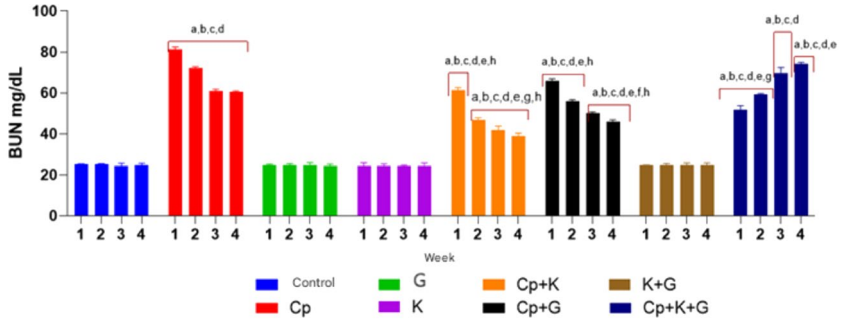

Fig. 66. BUN change graph of the experimental groups (a $p < 0.05$; compared to control group, b $p < 0.05$; compared to K group, c $p < 0.05$; compared to G group, d $p < 0.05$; compared to K + G group, e $p < 0.05$; compared to CP group, f $p < 0.05$; compared to CP + K group, g $p < 0.05$; compared to CP + G group, h; compared to CP + K + G group).

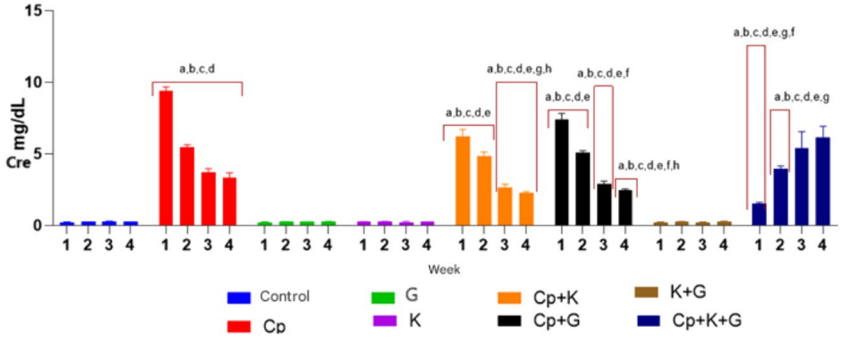

Fig. 67. Weekly serum creatinine graph of the experimental groups (a $p < 0.05$; compared to control group, b $p < 0.05$; compared to K group, c $p < 0.05$; compared to G group, d $p < 0.05$; compared to K + G group, e $p < 0.05$; compared to CP group, f $p < 0.05$; compared to CP + K group, g $p < 0.05$; compared to CP + G group, h; compared to CP + K + G group).

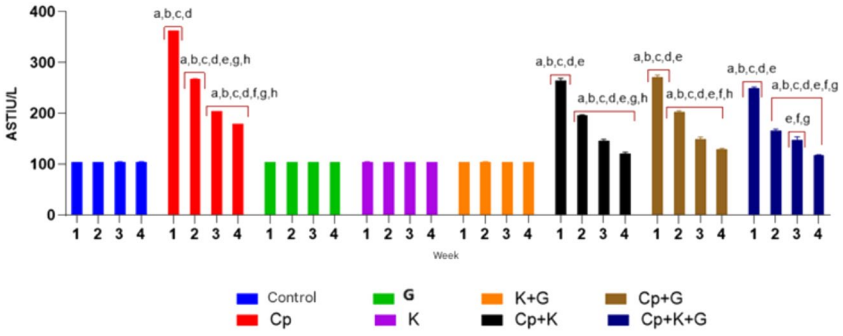

Fig. 68. AST change graph of the experimental groups (a $p < 0.05$; compared to control group, b $p < 0.05$; compared to K group, c $p < 0.05$; compared to G group, d $p < 0.05$; compared to K + G group, e $p < 0.05$; compared to CP group, f $p < 0.05$; compared to CP + K group, g $p < 0.05$; compared to CP + G group, h; compared to CP + K + G group).

their effectiveness in counteracting oxidative liver damage. The antioxidant properties of these compounds may contribute to the stabilization of cell membranes and suppression of lipid peroxidation, thus preserving liver tissue integrity. Fatima et al. also observed protective effects of EGCG and CoQ10 through similar mechanisms. These results emphasize the role of oxidative stress in cisplatin-induced hepatotoxicity and highlight the hepatoprotective capacity of antioxidant agents. Catechin and gallic acid are believed to mitigate cisplatin-induced nephrotoxicity and hepatotoxicity through multiple molecular mechanisms. These polyphenolic compounds exhibit strong antioxidant capacity by directly scavenging reactive oxygen species (ROS), thereby

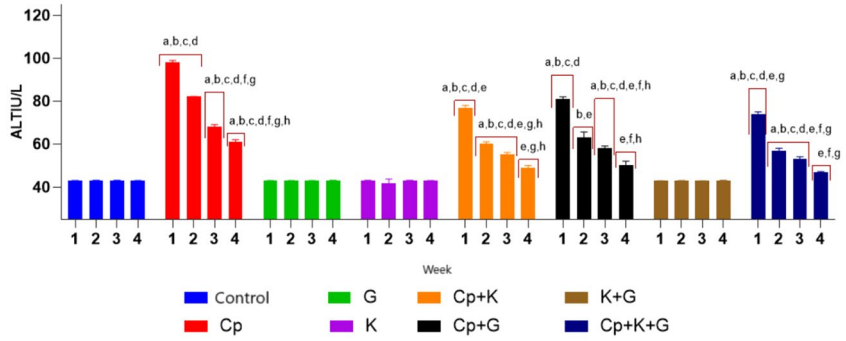

Fig. 69. ALT change graph of the experimental groups (a $p < 0.05$; compared to control group, b $p < 0.05$; compared to K group, c $p < 0.05$; compared to G group, d $p < 0.05$; compared to K + G group, e $p < 0.05$; compared to CP group, f $p < 0.05$; compared to CP + K group, g $p < 0.05$; compared to CP + G group, h; compared to CP + K + G group).

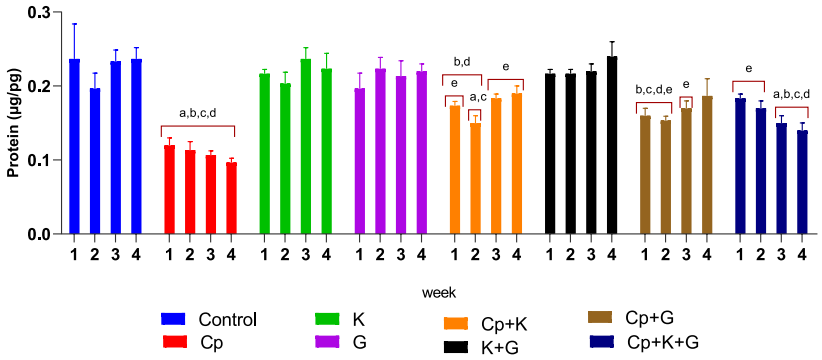

Fig. 70. GPx change graph of kidney tissue of the experimental groups (a $p < 0.05$; compared to control group, b $p < 0.05$; compared to K group, c $p < 0.05$; compared to G group, d $p < 0.05$; compared to K + G group, e $p < 0.05$; compared to CP group).

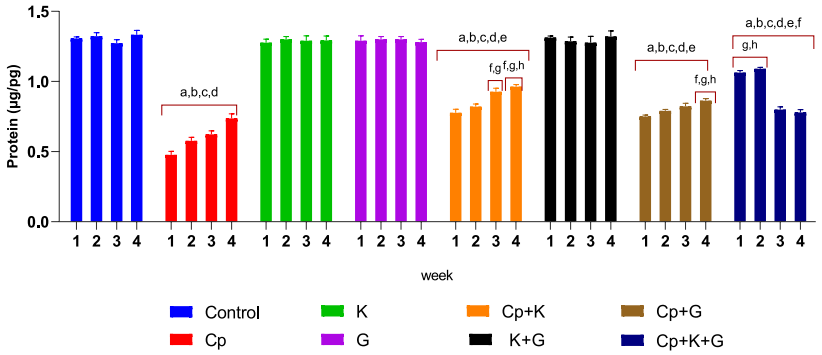

Fig. 71. GR change graph of kidney tissue of the experimental groups (a $p < 0.05$; compared to control group, b $p < 0.05$; compared to K group, c $p < 0.05$; compared to G group, d $p < 0.05$; compared to K + G group, e $p < 0.05$; compared to CP group, f $p < 0.05$; compared to CP + K group, g $p < 0.05$; compared to CP + G group, h; compared to CP + K + G group).

limiting lipid peroxidation and membrane damage[61,62]. They also enhance the cellular antioxidant defense system by increasing the activity of endogenous enzymes such as glutathione peroxidase (GPx), glutathione reductase (GR), and superoxide dismutase (SOD)[23,63]. Additionally, catechin and gallic acid may activate the Nrf2/ARE signaling pathway, which regulates antioxidant gene expression and protects tissues against oxidative insults Bai et al.[23] Their anti-inflammatory effects are associated with the inhibition of pro-inflammatory cytokines (e.g. TNF-α, IL-1β) and suppression of NF-κB signaling[23] Furthermore, they may exert anti-apoptotic effects by restoring the balance between pro- and anti-apoptotic proteins such as Bax and Bcl-2, preventing cisplatin-

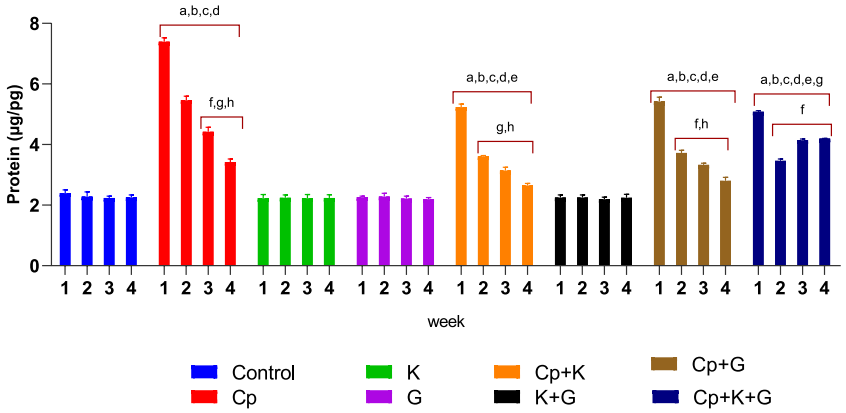

Fig. 72. MDA changes in kidney tissue of experimental groups (a $p < 0.05$; compared to the Control group, b $p < 0.05$; compared to the K group, c $p < 0.05$; compared to the G group, d $p < 0.05$; compared to the K + G group, e $p < 0.05$; compared to the CP group, f $p < 0.05$; compared to the CP + K group, g $p < 0.05$; compared to the CP + G group, h $p < 0.05$; compared to the CP + K + G group).

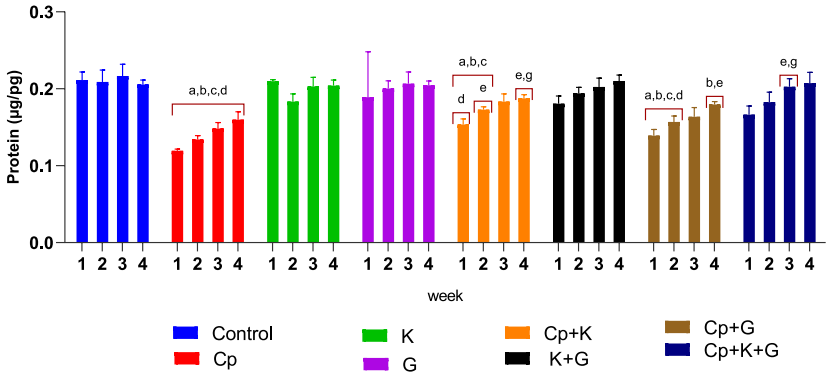

Fig. 73. GPx change graph of liver tissue of the experimental groups (a $p < 0.05$; compared to control group, b $p < 0.05$; compared to K group, c $p < 0.05$; compared to G group, d $p < 0.05$; compared to K + G group, e $p < 0.05$; compared to CP group, f $p < 0.05$; compared to CP + K group, g $p < 0.05$; compared to CP + G group, h $p < 0.05$; compared to CP + K + G group).

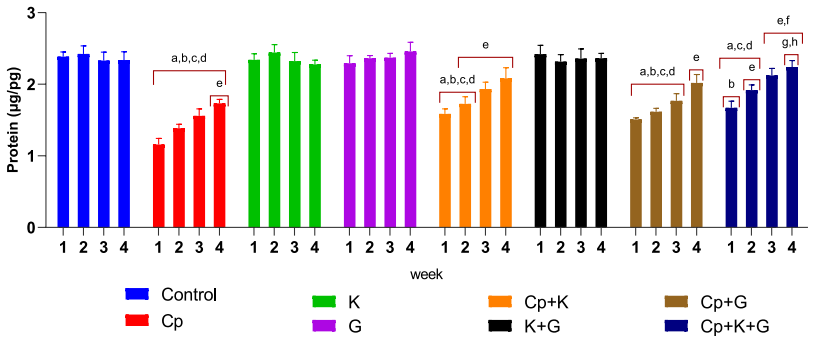

Fig. 74. GR change graph of liver tissue of the experimental groups (a $p < 0.05$; compared to control group, b $p < 0.05$; compared to K group, c $p < 0.05$; compared to G group, d $p < 0.05$; compared to K + G group, e $p < 0.05$; compared to CP group, f $p < 0.05$; compared to CP + K group, g $p < 0.05$; compared to CP + G group, h $p < 0.05$; compared to CP + K + G group).

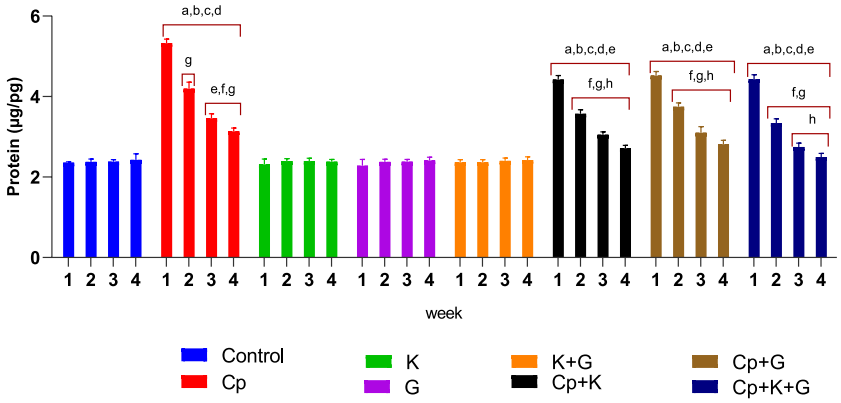

Fig. 75. MDA change graph of liver tissue of the experimental groups (a $p < 0.05$; compared to control group, b $p < 0.05$; compared to K group, c $p < 0.05$; compared to G group, d $p < 0.05$; compared to K + G group, e $p < 0.05$; compared to CP group, f $p < 0.05$; compared to CP + K group, g $p < 0.05$; compared to CP + G group, h $p < 0.05$; compared to CP + K + G group).

induced cell death[60,62]. These mechanisms are consistent with the improvements observed in biochemical and histopathological parameters in our study and support the protective role of catechin and gallic acid in cisplatin-induced organ injury.

However, in the treatment groups, AST, ALT, and MDA values decreased, while GPx and GR values increased over time. Fatima et al.[63] investigated the effects of EGCG and CoQ10 treatments against cisplatin-induced hepatotoxicity and observed that this treatment preserved the normal structure of liver tissue. Doğan et al.[62] conducted a study in which cisplatin, silymarin, and gallic acid were applied together, and they observed severe damage in the liver tissue of the cisplatin-only group, but the damage was alleviated in the silymarin and gallic acid combination treatment group. Okkay et al.[61] targeted cisplatin-induced hepatotoxicity with Centella asiatica extract and observed dose-dependent reduction in liver damage in the treatment groups. Sherif[60] examined the effect of arjunolic acid on cisplatin-induced hepatotoxicity and reported that in the treatment group, the structural changes in liver tissue were similar to those in the control group. Cisplatin also accumulates in the liver after the kidney, and liver toxicity occurs due to the increased accumulation of ROS. These free radicals cause tissue damage by disrupting normal antioxidant defense systems and lead to cell death[44,64]. Oxidative stress and mitochondrial damage play significant roles in cisplatin-induced hepatotoxicity, with ROS weakening mitochondrial function and reducing antioxidant defenses[44,58,64].

Conclusion and recommendations

The findings of this study revealed that cisplatin administration induced more severe toxicity in renal tissue compared to hepatic tissue. Catechin and gallic acid exhibited notable protective effects against cisplatin-induced toxicity, contributing to the preservation of organ integrity, enhancement of cellular function, and improvement in survival rates. These results highlight their potential as safer adjunct therapeutic agents. Although cisplatin caused considerable damage to both liver and kidney tissues, treatment with catechin and gallic acid attenuated this damage, though not entirely. Notably, catechin alone demonstrated greater efficacy than gallic acid when administered individually. However, the combination of both compounds did not produce the anticipated therapeutic benefit, suggesting a possible antagonistic interaction between the two. While each compound was effective on its own, their concurrent use did not result in a synergistic effect. Importantly, this study differs from previous research in its experimental design. Unlike many earlier studies that administered antioxidant compounds prior to cisplatin injection and terminated the experiment shortly thereafter, our study extended beyond the immediate post-injection period. This approach provides a broader and more clinically relevant understanding of the therapeutic potential of catechin and gallic acid. The findings presented herein may serve as a valuable foundation for future investigations into supportive strategies aimed at mitigating cisplatin-induced toxicity. A summary of the study is provided below (Fig. 76).

Graphical abstract

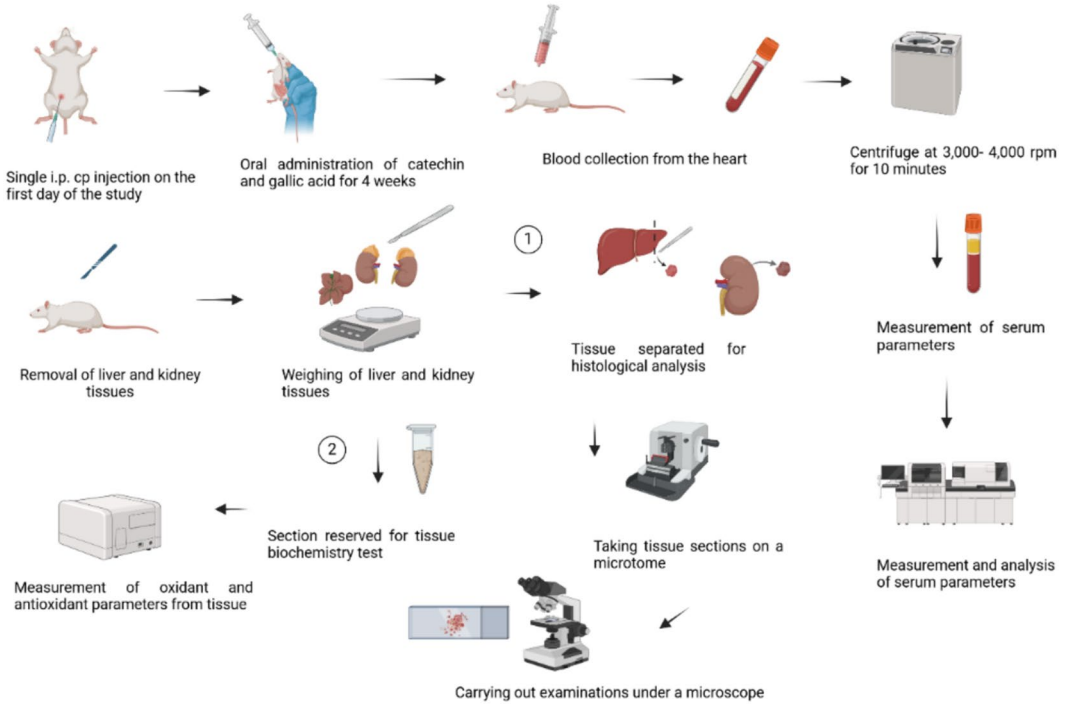

Fig. 76. Graphical summary of the study.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions
N.S.K. and A.G. contributed to the study conception and design. N.S.K. conducted the animal experiments, collected data, and performed histological and biochemical analyses. A.G. supervised the study and provided critical revisions. N.S.K. wrote the initial draft of the manuscript. A.G. edited and finalized the manuscript. All authors reviewed and approved the final version of the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Additional information
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