Ameliorative Effects Of Natural Polyphenol On Neuro- And Nephrotoxicity Induced By Anticancer Drug

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Abstract

Cisplatin (Cis) is a common anticancer drug, particularly in breast cancer. Nevertheless, the side effects of Cis have an impact on other body systems. This study aims to investigate the therapeutic potential of natural polyphenol (ellagic acid (Ell)) to alleviate the side effects of Cis. Forty male albino rats were randomly divided into four groups: the Control group, Cis group, Ell group, and Cis+Ell group. To evaluate neurotransmitters, urea, creatinine, BUN, and brain tissue homogenates were performed. The kidney slices from all groups were processed for histopathological and immunohistochemical analyses. Our studies proved that Ell caused a significant improvement in the antioxidant enzymes, MDA level, and kidney functions and disturbance in GABA and glutamate levels in brain homogenates after the adverse effects induced by Cis. Ell also reduced the cleavage of caspase 3 and immunoreactivity of COX2 in the kidneys of Cis-treated rats and reduced cleavage of caspase 3 and the immunoreactivity of COX2 in the kidney of Cis-treated rats. Treatment with Ell can effectively mitigate the adverse effects on the kidneys and brain that the administration of Cis causes.

Keywords: Cisplatin, Ellagic acid, Hormonal assay, Nephrotoxicity

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Introduction

Cisplatin (Cis) is a potent platinum-based antineoplastic agent used to treat a variety of cancers, including solid tumors that have proven resistant to other treatments. Among the most common cancers treated with it are lymphoma and stomach, esophageal, pancreatic, bladder, head and neck, lung, ovarian, and testicular cancers [1]. Cis causes hepatic injury by activating inflammatory and oxidative stress pathways, as well as causing apoptosis and structural and functional abnormalities in the liver [2]. Because Cis is primarily processed by the kidneys and liver, it has been linked to hepatotoxicity and nephrotoxicity [3]. Cis-induced nephrotoxicity can range from mild, reversible structural changes in tubular epithelial cells that cause a variety of renal dysfunction (acute nephrotoxicity) to potentially irreversible renal failure that leads to chronic and progressive renal insufficiency (chronic and progressive renal insufficiency) (chronic nephrotoxicity) [4].

Ellagic acid (Ell) is a polyphenolic substance found in nuts, fruits, and vegetables [5]. Ell has antioxidant and anti-inflammatory activities [6, 7]. Ell protects hepatocytes from Cis-induced damage by acting as a ROS scavenger and maintaining antioxidant enzyme levels [8]. However, it is unknown if Ell effects are mediated by Nrf2 activation and/or whether this antioxidant affects mitochondrial dysfunction, which is linked to Cis-induced hepatotoxicity [9]. Ell is a polyphenol found in plants that have antioxidative properties *in vivo* [10] and *in vitro* [11]. Ell is an effective scavenger

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of both ROS and lipid peroxidation [12]. Furthermore, Ell therapy protects against Cis-induced nephrotoxicity while also lowering plasma creatinine and urea levels [13].

Materials and Methods Chemicals:

Acros organics in the USA provided cisplatin (250 mg, trace metal basis, code193762500).
Acros organics in the USA also supplied 97% ellagic acid (2.5GR, code 117740025).

Animals and experimental design

In this study, 40 healthy male adult albino rats weighing 150–180 g were used. The animals were obtained from Theodor Bilharz Research Institute, Cairo, Egypt and kept under normal conditions with free access to food and water. All animals were housed in hygienic plastic cages in well-ventilated rooms with exhaust fans; they were fed a standard pellet diet and had free access to water daily. All animal procedures were carried out with the approval of the Ethics Committee of the National Research Center, Egypt, and following recommenddations of the institutional animal care and use committee of the Zagazig University **(ZU-IACUC/3/f/21/2021)**

The rats were randomly divided into four groups, with each group including ten rats:

1— Control group: No therapy was given to the rats (five rats received saline and five rats received corn oil).
2—Ell group: for ten days, rats were given ellagic acid (10 mg/kg/day).
3—Cis group: rats were given a single intraperitoneal (IP) injection of cisplatin (7 mg/kg).
4—Cis + Ell group: rats were given ellagic acid for 10 days after receiving a single dose of Cis through IP injection.

Cis was given to the animals through IP injection in a single dose of 7 mg/kg (isotonic saline was the vehicle for administering Cis).
Ell was dissolved in maize oil at a concentration of 5 mg/ml and administered to animals at a dose of 10 mg/kg/day via gavage.

Sample collection

After ten days, the rats were ether anesthetized and killed. The liver and kidney samples were removed and stored away from the light. The liver samples were washed three times in cold isotonic saline (0.9% v/w) and stored at (−20°C) until they were analyzed.

Blood was collected in test tubes from this dislocation and centrifuged to remove serum, before being stored in an Eppendorf tube in a deep freezer at −20°C.

Tissue homogenate:

Wash tissue slices in 0.01M PBS before adding the tissue protein extraction reagent in a proportion of 1G: 5–10 ml and mixing in icy water. After blending, centrifuge the mixture at 5000–10000 rpm for10 minutes. Take the supernatant immediately after testing or store it at −20°C (for 1–3 months) or −80°C (for 1–3 months).

Biochemical analysis

1. Estimation of kidney function (kidney function tests):

Urea, creatinine, and BUN were determined colorimetrically by using a Diamond kit [14].

2. Estimation of antioxidants and MDA:

2.a. Determination of GSH (glutathione):

Rat glutathione was determined using an ELISA kit [15].

2.b. Determination of SOD (superoxide dismutase):

Rat superoxide dismutase was determined using ELISA kit [16].

2.c. Determination of CAT (catalase):

Rat superoxide catalase was determined using ELISA kit [17].

2.d. Determination of MDA (malondialdehyde):

Rat malondialdehyde (MDA) was determined using ELISA kit [18].

3. Estimation of neurotransmitter:

3.a. Determination of glutamate:

Glutamate was determined using ELISA kit [19].

b. Determination of gamma-aminobutyric acid (GABA):

Rat GABA was determined using ELISA kit [20].

Light microscopy studies:

Specimens from the kidney were fixed in 10% formalin, then dehydrated in increasing degrees of alcohol (70%, 80%, 90%, 95%, and 100%), cleaned in xylene, and embedded in paraffin wax blocks. For the basic histological study, serial slices of 4–6 m thickness were stained

with hematoxylin and eosin and periodic acid Schiff (PAS) stain to indicate the glycogen content and basement membranes, and Massons trichrome [21] to detect collagen fibers.

• Immunohistochemical staining:[22]

On paraffin sections (5 m thickness), deparaffinization in xylene, rehydration in descending concentrations of ethanol, and washing in phosphate buffer solution were performed (PBS). To inactivate endogenous peroxidase, 3% hydrogen peroxide was used. The slides were then heated in a citrate buffer (pH 6.0) for 30 minutes at 95°C to extract antigens. To prevent nonspecific binding, 5% bovine serum albumin in Tris-buffered saline was added. Anti-COX2 (rabbit monoclonal antibody, ab169782) and anti-caspase 3 (rabbit monoclonal antibody, ab184787) were used on kidney slides. The slides were treated with antibodies at a 1:100 dilution for 60 minutes at room temperature before being rinsed with PBS. To obtain negative control samples, the primary antibodies were eliminated, and slices were counterstained with Mayer 's hematoxylin and mounted and viewed under a light microscope. Positive reactions COX2 showed up as brownish nuclear staining. Caspase 3 was expressed in both the nucleus and cytoplasm.

Morphometric study and the statistical analysis

The proximal and distal convoluted tubules' Feret diameter and surface area, 400× photos were used to calculate glomerular Feret (caliber) diameter, glomerular perimeter, glomerular surface area, and Bowman's (urinary) space for kidney morphometry (proximal convoluted tubules (PCT) & DCT). Measurements were taken in 20 randomly selected glomeruli and 40 tubules in each group of five rats in each group.

Morphometry was performed using ImageJ (FIJI) software. Images were captured using a LeicaICC50W light electric microscope at the Image Analysis Unit of the Anatomy and Embryology Department, Faculty of Medicine, Zagazig University.

Statistical analysis

The information was presented as a mean ± standard deviation. To find differences between groups, a one-way analysis of variance (ANOVA) was used, followed by Tukey's multiple-comparison post-hoc test. The data were statistically analyzed using GraphPad Prism version 8.0.2 (GraphPad Software, Inc., San Diego, CA). The difference was considered significant when the *P*-value was less than 0.05.

Results

When compared to the control group, the Cis-treated group had statistically significantly higher mean serum urea, creatinine, and BUN levels ($P < .05$). When compared to the control group, the Cis group showed significantly higher levels. Moreover, the Ell + Cis group had a statistically significant decrease in serum urea, creatinine, and BUN compared to the Cis group ($P < .05$). The statistical results of the groups studied are presented in **Table 1.**

Table (1): Serum creatinine, urea, BUN, and total bilirubin in various studied groups

Parameter	Control	Ell	Cis	Ell + Cis
Creatinine (mg/dl)	0.369 ± 0.075	0.348 ± 0.067	1.343 ± 0.44[a]	0.723 ± 0.036[a,b]
Urea (mg/dl)	31.82 ± 4.139	29.32 ± 4.739	69.00 ± 12.28[a]	44.48 ± 4.214[a,b]
BUN (mg/dl)	14.85 ± 1.919	14.19 ± 2.316	32.64 ± 3.946[a]	20.12 ± 2.009 [a,b]

All values are expressed as mean ± SD, *n* = 10.[a]Significant vs control group.[b]Significant vs Cis. A one-way ANOVA followed by post-hoc Tukey's multiple comparisons test between groups

When compared to the control group, the Cis-treated group had a statistically significant lower mean of GSH, CAT, and SOD ($P < .05$), whereas MDA had a significantly higher mean for the same groups. When compared to the control group, the Ell + Cis group had Significantly lower levels. Moreover, the Ell + Cis group had a statistically significant increase in GSH, CAT, and SOD compared to the Cis group ($P < .05$) but MDA explained a significant decrease in the Ell + Cis group in comparison to the Cis group. The statistical results of the various studied groups are presented in **Table 2.**

Table (2): Tissue homogenate antioxidant enzyme levels and MDA in various studied groups

Parameter	Control	Ell	Cis	Ell + Cis
GSH (ng/mg)	191.8 ± 12.66	193.1 ± 11.92	31.20 ± 5.673[a]	74.35 ± 8.152[a,b]
CAT (ng/mg)	10.09 ± 0.7418	10.26 ± 0.6949	0.6110 ± 0.09585[a]	3.435 ± 1.384[a,b]
SOD (U/mg)	203.5 ± 20.30	205.1 ± 19.53	39.58 ± 5.994[a]	93.16 ± 5.214[a,b]
MDA (nmol/mg)	3.340 ± 0.6970	2.937 ± 0.8360	30.40 ± 4.492[a]	20.28 ± 2.320[a,b]

All values are expressed as mean ±SD, *n* = 10. [a]Significant vs control group. [b]Significant vs Cis. A one-way ANOVA followed by post-hoc Tukey's multiple comparisons test between groups.

Histopathological results

1-Light microscopic

a. Hematoxylin & eosin

The histomorphology of the renal cortex in the control and Ell groups was normal (fig.2a) and (fig.2b), the majority of the cisplatin-treated cortical tubules were dilated and necrotic, with casts in the lumina of certain tubules. The periglomerular region was also enlarged, and the renal corpuscles had twisted forms and appeared smaller. The parietal epithelium and macular cells, as well as vacuolate podocytes, showed signs of degeneration. In the interstitium and lumen of DCT, a limited number of inflammatory cells were also discovered (fig. 2c-e). On the other hand, animals administered cisplatin plus ellagic acid at the same time exhibited less renal damage. In the kidney, the majority of the glomeruli were also returned to their former size and shape. The tubules in the majority of the animals were similar to those in the control group, and Bowman's capsule was constricted. Despite this, only a few tubules were found to be damaged and dilated (fig. 2f).

b. Periodic acid Schiff

Both the control group (Fig. 3a) and Ell-treated (Fig. 3b) groups of rats had thin PAS-stained basement membranes encircling both tubules and glomeruli, as well as a thick PAS-positive brush border of the PCT in the renal cortex stained with PAS. In contrast, Cis-treated rats had thickened glomerular and tubular basement membranes with a thin interrupted brush border (Fig. 3c). The Cis + Ell group had a narrow Bowman's capsule and a continuous brush boundary (Fig. 3d).

c. Masson's trichrome

The control and Ell groups had an average collagen distribution in the kidneys (Fig. 4a and b). In the Cis-treated rats, the glomerulus and the wall of renal blood vessels (Fig. 4c). Rats given both Ell and Cis had less collagen in their renal tissues (Fig. 4d)

2-Immunohistochemical results

a. Anti-COX2 immunostaining (Fig. 5):

In anti-COX2 immunostained kidney sections, both the control and Ell groups showed negative immunoreactivity. Cis elicited a strong cytoplasmic response in the epithelial cells of the cortical tubules and parietal cells of renal corpuscles. Rats were treated with Cis and Ell. Some tubular epithelial cells showed a minor reactivity.

d. Expression of caspase 3 (Fig. 6):

When stained with anti-caspase 3, the rats in the control and Ell groups showed weak immunoreactivity in their renal tissue. However, in the Cis group, there was a strong positive reaction in the nuclei of tubular, parietal, and mesangial cells, and in the Cis + ellagic group, there was only a weak positive reaction in certain tubular cells.

Morphometric results

Kidney morphometry:

In terms of glomerular diameter, glomerular perimeter, glomerular surface area, and urine space (Table 3), as well as PCT diameter, PCT surface area, DCT diameter, and DCT surface area, there was no statistical difference between the control and Ell groups (Table 3). When compared to the control group, the Cis group had significantly smaller glomerular diameter, perimeter, and surface area, while the urinary space, PCT, and the diameter of DCT in the Cis + Ell group were not significant.

Discussion:

Significant nephrotoxicity was observed following Cis treatment, as evidenced by an increase in serum creatinine, urea, and BUN levels.

Several processes were used to explain these effects including the formation of reactive oxygen and nitrogen species, inflammation, and apoptosis. The former disrupts the antioxidant defense system, causing oxidative damage to tissues as well as a reaction with thiols in proteins and glutathione, resulting in cell malfunction. Damage to the renal epithelial cells may trigger inflammation, which can aggravate renal injury and dysfunction *in vivo [23].*

Cis has a cumulative and dose-dependent nephrotoxic effect. Acute kidney injury following a single Cis injection is thought to be caused by proximal tubular cell necrosis and apoptosis caused by an impaired antioxidant system, inflammatory responses, and vascular injury [24]. Because Cis is a small molecule, it can easily pass through the glomerular basement membrane and reach the PCT. In tubular epithelial cells, Cis is converted to a highly reactive Cis-thiol compound [25].

According to the histological and morphometrical analysis of H&E-stained kidney slides, the destructive effects of Cis on renal tubules and corpuscles were similar to the previous literature. Bowman's capsule enlargement and shrinking glomeruli were observed in rats given Cis 7 mg/kg [26], 7.5 mg/kg [27], and 16 mg/kg [28]. According to Dupre *et al. [24]*, Cis stimulates fibrogenic cells in the kidney by increasing the activity of hedgehogs and other signaling pathways. TGF-1 is acquired by tubular epithelial cells to differentiate into matrix-producing myofibroblasts [29, 30]. In the Cis group, collagen deposition was visible in the mesangium and interstitium of renal tissue stained with Masson's trichrome.

Cis-induced apoptosis of tubular and mesangial cells was linked to increased levels of COX2 mRNA and protein levels. The activation of the COX2/PGE2 pathway causes inflammatory renal disease. Caspases, particularly caspase 3, are also the primary mechanism of programmed cell death. In cultured mouse proximal tubular epithelial cells, Cis promoted dosage and time-dependent caspase 3 cleavage [31]. In this study, Cis therapy increased COX2 and caspase 3 expression in the renal tissue of the Cis group compared to the control group.
There is an urgent need for clinical trials of a safe and effective renoprotective drug as an adjunct therapy for patients receiving high doses of Cis [32]. According to our findings, Ell can correct urea, creatinine, and BUN concentrations, with Ell's antioxidant mechanism being the activation of cellular antioxidant systems or direct activity as an antioxidant factor [33]. This polyphenol is sometimes used as an antioxidant supplement during Cis chemotherapy and protects against cancer [34]. Furthermore, after Ell treatment of a Cis-intoxicated kidney, the glomeruli's recovery to normal, inflammatory cells was not observed, and tubular necrosis was limited to a few tubules. Furthermore, morphometric measurements of the glomerular and distal tubules were used as controls, with significant improvements in PCT diameter and surface area.
A study by Li *et al. [35]* found that Ell reduced bleomycin-induced lung fibrosis in mice by inhibiting myofibroblast activation and increasing myofibroblast autophagy and death. In this study, the antifibrotic action of Ell was validated by improvements in the Cis + Ell group's histological and morphological data of the kidney when compared to the Cis group.

The pharmacological inhibition of COX enzyme activity is an effective treatment for several medical conditions [36]. Limiting COX2 was shown to significantly reduce Cis-induced mesangial cell apoptosis [31]. The amelioration of gentamicin-induced renal injury [37] and diabetic nephropathy [38] was linked to a decrease in caspase 3 protein levels and an increase in Bcl2 protein levels in the kidney of rats given 10 mg/kg Ell. Similarly, Ell reduced COX2 and caspase 3 immunoreactivity in the cortices of rats given Cis and Ell.
Our brain homogenate study after Cis treatment revealed a significant increase in glutamate levels and a significant decrease in GABA in rat brains. These findings support the findings of Abdelkader *et al. [39]*, who discovered that Cis administration was linked to a decrease in GABA and an increase in glutamate levels in the hippocampi and cortex of rats, implying that Cis use is linked to depression. This psychological dysfunction is caused either directly by nephrotoxicity or indirectly by changes in neurotransmitter levels as well as histopathological abnormalities. There is a clear link in the brain between cognitive impairment and GABA levels. Cis increased the glutamate content of the rat hippocampus and cortex [39].

When Ell was administered, the Cis-induced decrement in brain GABA levels and glutamate increase were significantly reversed. These findings are consistent with those of Dhingra and Jangra [40], who discovered that Ell had a significant antiepileptic effect in mice, probably due to an increase in GABA levels in the brain.

According to the study, the activity of the key scavenger enzymes (SOD, CAT, and GSH) was significantly reduced in the livers of Cis-treated rats. When rats were given Cis, they developed severe kidney failure, as evidenced by a significant increase in blood BUN and uric acid concentrations, as well as increased kidney MDA and decreased GSH, SOD, and CAT activities [41]. The fact that Cis treatment is linked to an increase in free radical production, severe oxidative stress, and lipid peroxidation explains these findings. Cis has previously been shown to cause oxidative damage and lipid peroxidation in kidney tissues in previous studies [42]. The amount of MDA, an important measure of oxidative stress, increased in Cis-intoxicated rats, resulting in glutathione and other endogenous antioxidants such as SOD and CAT [43]. Ell treatment reversed the Cis results by protecting against lipid peroxidation and the formation of free radical derivatives, as evidenced by lower levels of lipid peroxidation markers [44]. Ell protects cells from lipid peroxidation and thus oxidative stress by scavenging free radicals [34].

To summarize, Ell effectively mitigated Cis-induced hepato-, nephron-, and neurotoxicity by restoring all biomarkers in the liver, kidney, and brain homogenates, as well as histomor-phological alterations in rat hepatic and kidney tissues, by modulating the COX2/caspase 3 and P53/Bcl2 pathways.

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Author Contribution:

HA, AD, and AG conceived and designed the research. HA and NG conducted experiments and biochemical analysis. HA and JA contributed new analytical tools and histopathological studies. HA and JA analyzed the data. HA, NG and JA wrote the manuscript. All authors read and approved the manuscript.

Abbreviations

Cis, Cisplatin; **Ell,** Ellagic acid*;* **ALT,** Alanine transferase**; MDA**, malondialdehyde; **SOD,** superoxide dismutase; **GSH,** glutathione; **ROS,** reactive oxygen species.

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Availability of data materials:

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

Declarations

Ethics approval and consent to participate:

All animal procedures were carried out with the approval of the Ethics Committee of the National Research Center, Egypt, and following recommenddations of the institutional animal care and use committee of the Zagazig University **(ZU-IACUC/3/f/21/2021).**

Consent for publication:

Not applicable.

Competing interests:

The authors declares that there are no conflicts of interest.

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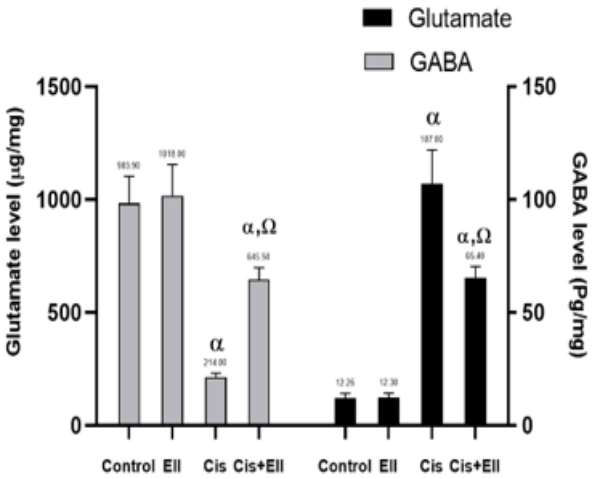

Fig. 1: Glutamate and GABA levels were measured in various study groups. One-way ANOVA was used for statistical analysis, followed by Tukey's post-hoc test. The values are shown as mean ± SE (n = 10). α—significant difference from the control group $P < 0.05$ and Ω—significant difference from the Cis group $P < 0.05$.

Histopathological results

1-Light microscopic

a. Hematoxylin & eosin

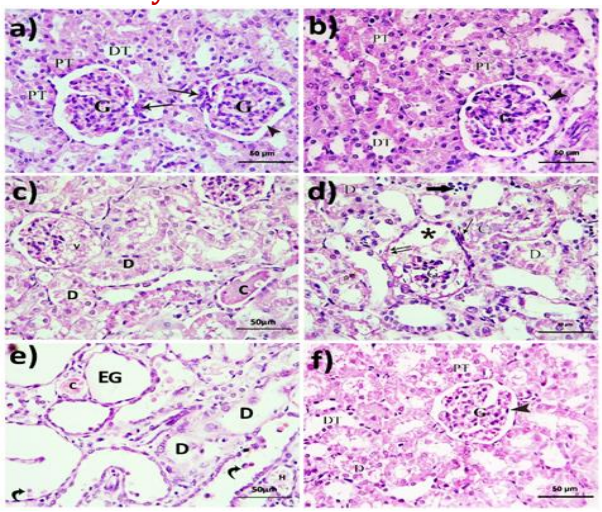

Fig. 2: Photomicrographs of histological sections of adult rats' renal cortex. Normal histological appearance of the cortex in the control **(a)** and Ell **(b)** groups: rounded regular glomeruli (G) bordered by narrow Bowman's gap (arrowhead). There is evidence of the macula densa (arrow), proximal convoluted tubules (PT), and distal convoluted tubules (DT). The cisplatin group **(c–e)** had enhanced tubular necrosis (D), tubular casts (C), intratubular hemorrhage (H), dilated tubules with white (curved arrow) within, and interstitial inflammatory cells in the cortex of the kidney (thick arrow). The glomeruli were also smaller and irregular, with a dilated urinary gap (star), podocyte vacuolization, and degeneration of parietal (double arrow) and macular cells (arrow). Occasionally, empty glomeruli were seen. The Cis + Ell rats' kidney **(f)** has a largely normal glomerulus (G) with a narrow urine space (arrowhead), PT, few dilated DT, and few degraded tubules (D). **(H&E, ×400).**

b. Periodic acid Schiff

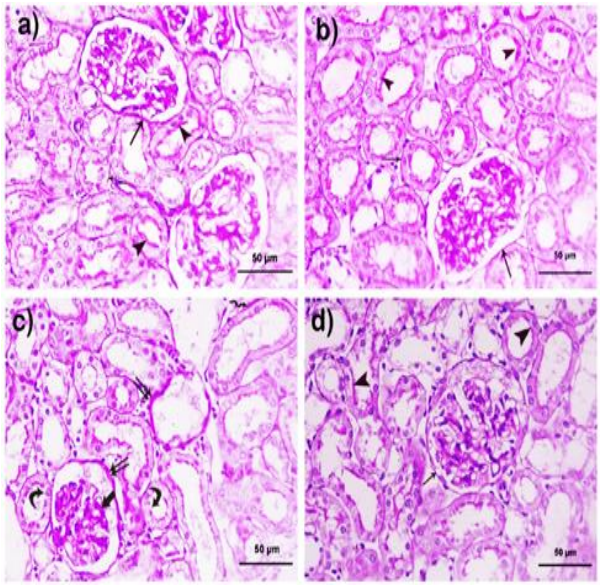

Fig.3: PAS-stained slices of an adult rat's renal cortex photographed under a microscope. Bowman's capsule and tubular basement membranes (arrow) show thin positive staining in the ellagic acid **(b)** and control **(a)** groups, as well as a positive thick reaction in the tubular brush border (arrowhead). In the cisplatin group **(c)**, the basement membranes of Browman's capsule (double arrow), glomerulus (thick arrow), and tubules (wavy arrow) thicken, and the brush border of tubular epithelium is lost (curved arrow). A thin foundation membrane (arrow) and a continuous brush boundary (arrowhead) may be seen in rats given cisplatin and ellagic acid **(d) (PAS, ×400).**

c. Masson's trichrome

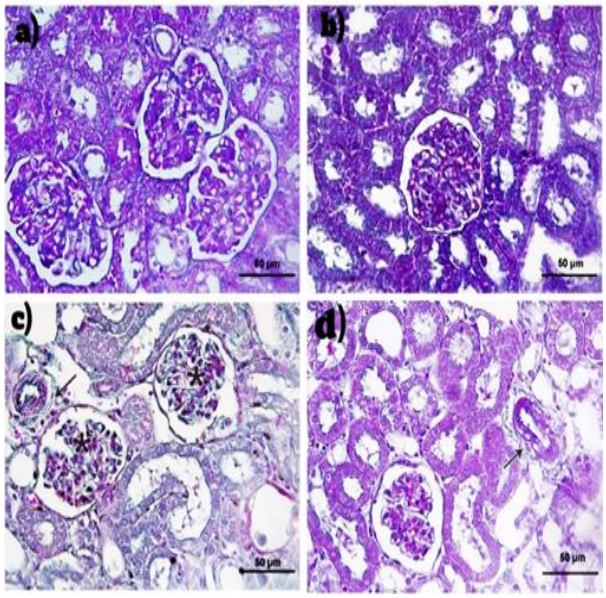

Fig.4: Adult rats' renal cortex photomicr-ographed with Masson's trichrome-stained slices. In both the control **(a)** and ellagic **(b)** groups, collagen fibers around renal corpuscles are thin. In the cisplatin group, there are dense, green-stained collagen fibers around blood vessels (arrow) and in the mesangial matrix in the cisplatin group **(c)**

(asterisks).The Cis + Ell group **(d)** (arrow) had less collagen deposition **(Masson, ×400).**

2-Immunohistochemical results

b. Anti-COX2 immunostaining (Fig. 5):

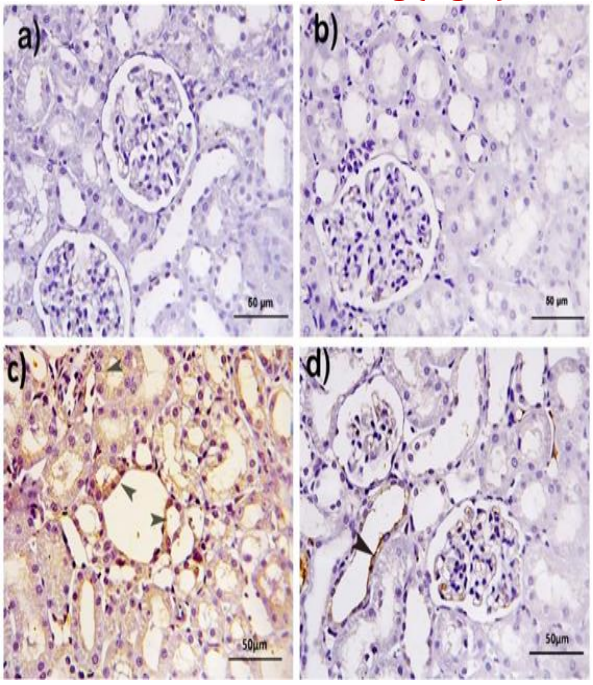

Fig.5: Anti-COX2 immunostained slices of adult rats' renal cortex are shown in photomicrographs. In both the control **(a)** and ellagic **(b)** groups, the anti-COX2 reaction in renal corpuscles and the tubular epithelium is negative. There is a high cytoplasmic immunoreaction (arrowheads) in cells lining tubules and glomerular parietal cells in the cisplatin group **(c).** Cisplatin and ellagic acid-treated rats **(d)** had moderate immunostaining in the epithelial cells of certain tubules (arrowhead) (anti-COX2, ×400).

d. Expression of caspase 3 (Fig. 6):

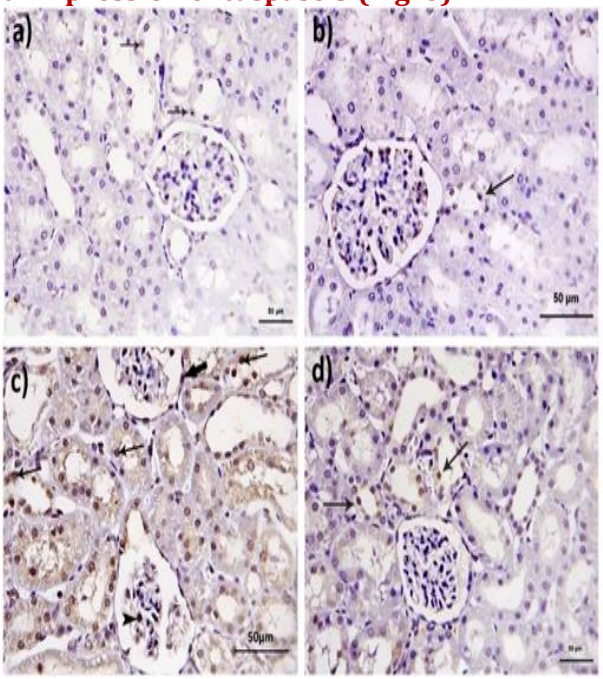

Fig.6: Caspases 3 immunostained slices in of adult rat renal cortex of adult rats photomi-crographed. Few cells in the control **(a)** and ellagic **(b)** groups exhibit modest nuclear expression (arrow). In the cisplatin group (c) mesangial cells (arrowhead), parietal cells (thick arrow), and tubule lining show a strong nuclear immunoreaction **(**arrows). Some tubular epithelial cells in rats treated with cisplatin plus ellagic acid **(d)** (arrows) show moderate reactivity **(Anti-caspase 3, ×400).**

Morphometric results

Kidney morphometry:

Table (3) Glomerular morphometry (surface area, perimeter, Glomerular diameter, and urinary space) in the experimental groups.

Experimental groups	Glomerular SA (µm²)	Glomerular Perimeter (µm)	Glomerular diameter (µm)	Urinary space (µm)
Control	12952 ± 1570	425.7 ± 27.23	142.1 ± 8.940	8.7 ± 1.3
Ell	13540 ± 3867	434.3 ± 53.85	144.2 ± 19.99	7.6 ± 1.8
Cis	5724 ± 2170 [a]	281.7 ± 46.39 [a]	96.75 ± 46.39 [a]	17.2 ± 4 [a]
Ell+ Cis	11125 ± 4914 [b]	383.4 ± 79.82 [b]	131.9 ± 31.69 [b]	8.1 ± 2.7 [b]

One-way ANOVA, Tukey's multiple comparisons test. Results are presented as mean ± SD (n = 10). [a]$P < 0.05$ vs control. [b]$P < 0.05$ compared to the cisplatin group. SA = surface area.

Table (4) Surface area (SA) and Feret diameter of proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) in the experimental groups.

Experimental groups	PCT SA (µm²)	PCT diameter (µm)	DCT SA (µm²)	DCT diameter (µm)
Control	761.4 ±136	34.85 ± 3.5	762.5 ± 92.52	36.22 ± 2.4
Ell	850.2 ± 73.8	36.86 ± 2.3	619.3 ± 97.05	32.54 ± 2.5
Cis	1903 ± 394.5[a]	56.3± 6.5[a]	2145 ± 735.9 [a]	59.2 ± 011.3[a]
Ell + Cis	1269 ± 326.7[a b]	44.75 ± 5.2[a b]	928.3 ± 171 [b]	39.72 ± 4.9[ab]

One-way ANOVA, Tukey's multiple comparisons test. Results are presented as mean ± SD (n = 10). [a]$P < 0.05$ vs control. [b]$P < 0.05$ compared to the cisplatin group.

Table (5) Area percentage of COX2, caspase 3-positive nuclei and Collagen area % in the kidney of the experimental groups.

Experimental groups	COX2 area%	Cas3-positive nuclei area%	Collagen area % In the kidney
Control	0.21 ± 0.09	1.74 ± 0.92	1.128 ± 0.61
Ell	0.27 ± 0.23	1.14 ± 2.3	0.793 ± 0.28
Cis	3.03 ± 1.39[a]	9.94± 2.28[a]	6.33 ± 1.93[a]
Ell + Cis	1.02 ± 0.59[b]	5.15 ± 2.04[a, b]	3.65 ± 1.58[a, b]

One-way ANOVA, Tukey's multiple comparisons test. Results are presented as mean ± SD (n = 10). [a]$P < 0.05$ vs control. [b]$P < 0.05$ vs cisplatin (cas = caspase).