Ameliorative Effect of Melatonin against Cisplatin Induced Renal Toxicity in Albino Rats; Histological Study

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Original Article

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Abstract

Nephrotoxicity is one of the primary side effects of cisplatin (CP), a powerful chemotherapeutic drug that is frequently used to treat cancer. Antioxidant and anti-inflammatory characteristics are found in the body hormone melatonin, which is secreted by the pineal gland. The purpose of the current study is to determine whether melatonin can protect rats from cisplatin-induced nephrotoxicity.

Materials and Methods: Forty rats were used in this study and divided into four groups (10 rats for each): group I(control group) in which, the animals were injected intraperitonealy with normal saline), group II (melatonin group)in which, the animals were injected intraperitonealy with melatonin (4mg/kg bw ip for 10 days)group III (cisplatin group) in which, the animals were injected intraperitonealy with (single dose of 7 mg/kg bw),and group IV (melatonin/cisplatin group) in which, the animals were injected intraperitonealy with melatonin for 10days+ cisplatin with a single dose on the 5th day of experiment at the above-said doses. At the end of experiment time, all rats were sacrificed and kidneys were dissected out for histological study.

Results: The data were revealed that cisplatin induced histopathological changes such as vaculation of renal tubules, distortion of glomeruli, interstitial congestion and inflammatory cell infiltration. Cisplatin significantly decreases PAS reaction in renal tissue stained sections. Co administration of melatonin significantly attenuated histopathological alterations in cisplatin-injected rats.

Conclusion: In conclusion, melatonin with its antioxidant and anti-inflammatory characters can be suggested as a promising drug in the treatment of cisplatin induced acute kidney injury in cancer patients.

Introduction

Cisplatin, also known as cis-diamminedichloroplatinum-II, is a potent chemotherapy agent that is frequently used to treat solid malignancies [1]. The kidney plays avery important role in elimination of endogenous and exogenous waste materials, including medications. During treatment with Cisplatin (CP), renal tissue accumulates this drug to a higher level than other tissues and organs, stores some of this substance in the proximal tubules. It was detected that the concentration of cisplatin in the epithelial cells of renal proximal tubules to be about 5 times greater than that in the serum [2]. Accumulation of the drug leads to strong toxicity in renal proximal tubular cells and finally causes tissue damage, low perfusion rate, and renal failure [3, 4]. Low renal perfusion indicates renal toxicity and necrosis of the terminal portion of the proximal tubule which ultimately determines renal tissue fate [5].

Approximately 25–35% of subjects following a single dose of cisplatin show signs of renal impairment. [6]. Acute renal failure is a considerable cause of mortality and morbidity development among cisplatin chemotherapy recipient patients. Nephrotoxicity is evidenced by tubular dilation, tubular cell vacuolization, loss of tubular brush border, and condensation of nuclear chromatin. Tubular destruction ranges from the loss of only the margin of the epithelial cell brush to complete tubular necrosis in severe cases. Cisplatin-induced renal failure is characterised by decreased glomerular filtration and increased blood urea nitrogen and plasma creatinine [6–9].

Several mechanisms are involved in cisplatin induced nephrotoxicity including the production of free radicals such as superoxide and hydroxyl radicals, mitochondrial dysfunction, and increased activity of calcium independent nitric oxide synthase and apoptosis. One of the mechanisms that have been detected to play cisplatin toxicity is its effect on DNA synthesis and repairs, which causes inhibition of the cell cycle [9, 10]. Previous studies reported that Cisplatin induces the tumor-suppressor protein p53. This affects apoptosis through the receptor-tumor necrosis factor interaction, and Caspases that leads to mitochondrial damage also affects the calcium signaling by endoplasmic reticulum stress [9, 13, 14].

Melatonin (N-acetyl-5-methoxytryptamine) is a secretory product of the pineal gland. It has many important physiological functions [15]. A number of studies have reported that melatonin has important properties as anti-oxidant, anticancer, anti-inflammatory, and immunomodulatory effects [16,17]. Previous reports have been well documented its anti-apoptotic effects via mitochondrial pathways [18], so the present study investigated the protective effect of melatonin against cisplatin-induced acute kidney injury in rats

Materials and Methods

1. Animals:

The study used fourty (40) adult albino rats (average weight: 180-200 g). These rodents came from a lab at Minia University. They received water and a typical laboratory diet while being housed during the course of the experiment. All rats were kept in an air-conditioned, well-ventilated environment that was kept at a constant 22°C. Every aspect of animal care and handling adhered to the ethical standards set forth by the college of medicine at Minia University in Egypt. Approval number 701:12/2020 in compliance with world standards.

2. Experimental Work:

Our work was prepared in the Anatomy department, Faculty of Medicine, Minia University, Egypt. The animals were divided into four groups, each consisting of 10 rats.

Group I (control group): The animals didn't receive any drug.

Group II (melatonin group): The animals injected with melatonin (4mg/kg body weight i.p for 10 days).

Group III (cisplatin group): The animals received a single dose of cisplatin (7mg/kg body weight single dose i.p).

Group IV (cisplatin/melatonin group): The animals received melatonin + cisplatin (4mg/kg body weight i.p for 10 days + 7mg/kg body weight i.p single dose on the fifth day of the experiment).

The doses of cisplatin and melatonin were determined based on previous reports**[19,20]**. By the termination of experiment, animals were scarificed. The kidneys were removed and were prepared for microscopic examinations.

3. Histological Studies by light microscope:

1-Hematoxylin and eosin stain (H& E)

Following normal procedures, the kidney was removed and fixed in 10% buffered formalin for 2 days before being embedded in paraffin according to **[21].** Dehydration with series grades of ethyl alcohol, embedded in 70% alcohol for 24hours, then in 90% alcohol for two hours, and then in 100% alcohol for half of hour, clearing with xylene, three successive changes of impregnation in soft paraffin at 55°–60°C, and finally embedding in hard paraffin wax were performed after the fixation of the kidney tissue. A rotatory microtome was used to cut serial transverse sections that were 5 µm thick, which were subsequently, put on glass slides. After being deparaffinized and stained with hematoxylin and eosin, the paraffin slices were rinsed with water. The sections were cleaned in xylene and dehydrated in alcohol before mounting.

2- Periodic acid-Schiff (PAS) technique:

The PAS method works by exposing the tissue to periodic acid according to **[21]** This acts an oxidizing agent which oxidizes vicinal (neighboring) glycol groups or amino/alkylamino derivatives. This oxidation creates dialdehydes. These dialdehydes when exposed to Schiff's reagent create an insoluble magenta compound. Sections of 3 µm thicknesses were deparaffinized with xylene and stained with periodic acid for 10-15 minutes. The sections were washed in running tap water for 5-10 minutes followed by exposing to Schiff's reagent for 10- 15 minutes. The sections were washed in running tap water for 5-10 minutes, dehydrated in ascending concentrations of alcohol and then cleared by using xylol.

Result: The positive sites appeared magneta red.

4. Morphometric studies

Form each group ten random fields/section from each animal were chosen. The percentage of surface area fraction of PAS **[22]** stained sections of liver tissue of all groups were recorded using software image analysis image J program**[23]**. The mean surface area fraction was used to compare between the different groups.

5. Statistical analysis:

The analysis of the data was carried out using the IBM SPSS 28.0 statistical package software (IBM; Armonk, New York, USA). Ordinary one-way ANOVA test for non-parametric quantitative data between the four group [24]. After that an Unpaired t-test between every two groups was used to determine the statistically significant differences among groups. Values of $p \leq 0.05$ were considered statistically significant [25].

6. Image capturing:

A high-resolution colour digital camera placed on an Olympus microscope (Olympus, Japan) was used to take pictures of fields. These pictures were then uploaded to a computer to be analyzed.

Results

1. Histological results:

a) Hematoxylin& Eosin-stained sections results:

The renal sections of both **control and melatonin** groups presented a normal histological structure of the renal cortex demonstrating renal corpuscles, proximal (PCTs) and distal (DCTs) convoluted tubules.The renal corpuscles were formed of tufts of capillaries; the glomeruli, which were seen surrounded by Bowman's capsule which had a parietal layer and a visceral layer. Between the two layers of Bowman's

capsule, a patent urinary space (Bowman's space) was seen. Macula densa cells of the DCTs were in close proximity to the renal corpuscle that appeared tall, narrow, and crowded with closely packed nuclei viewed with the light microscope as a dense spots (Fig. 1,2). In **cisplatin** group, Examination of H&E-stained sections obtained from the cisplatin group revealed histopathological changes in the renal cortices showing significant glomerular and tubulointerstitial injury. Most renal corpuscles appeared congested, others were distorted or even were shrunken and atrophied**.** Congestion of peritubular capillaries and interstitial hemorrhage was observed in some sections. There was marked glomerular vacuolation with widening of the Bowman's space and distortion and vacuolation of tubular cells. Marked tubular distortion was observed in several renal cortical sections. Epithelial flattening with reduction of cell height and desquamation of some cells with cellular debris in the lumina were also evidenced. Some tubular lumina showed homogenous esinophilic hyaline casts (Fig.3,4)**.** In **cisplatin-melatonin** group administration of melatonin ameliorated the damaging effects of cisplatin as clearly noticed by the reduction of histopathological changes. The renal corpuscles appeared more or less normal with mild congestion of glomerular capillaries and decreased interstitial hemorrhage. Minimal intraluminal hyaline casts were observed(Fig.5).

b)Periodic Acid Schiff reaction (PAS)

PAS-stained renal sections from the **control and melatonin** groups showed positive PAS reactions in the basement membranes of intact glomerular blood capillaries, parietal layers of Bowman's capsules, basement membranes of PCT and DCT cells. It was also observed in the intact apical brush borders of PCT and DCT cells (Fig.6,7). In **cisplatin** group, PAS-stained sections showed reduced PAS reaction in the form of partial or complete loss of the brush border of most distorted tubules. The basement membranes of renal tubules and glomerular capillaries were also seen interrupted and appeared thin at some sites (Fig.8). PAS-stained sections of the renal cortex of the **cisplatin-melatonin** group showed preserved reaction of most brush borders of PCTs and DCTs. Basement membranes of glomerular blood capillaries, parietal layers of Bowman's capsules, PCTs and DCTs cells appeared continuous and intact. Only few renal tubules were seen in some sections with reduced PAS reaction (Fig.9).

2. Morphometric results

A) Surface area fraction of PAS-positive stained renal tissues

There is a normal percentage in control and melatonin groups but highly decreased in the cisplatin group while it restored its elevation in the cisplatin-melatonin group. P-value expression shows no significant difference between the control group and melatonin group, but a highly significant difference presents between control and cisplatin group, there is also a significant difference between cisplatin-melatonin group and cisplatin group **(table 1, fig.10)**

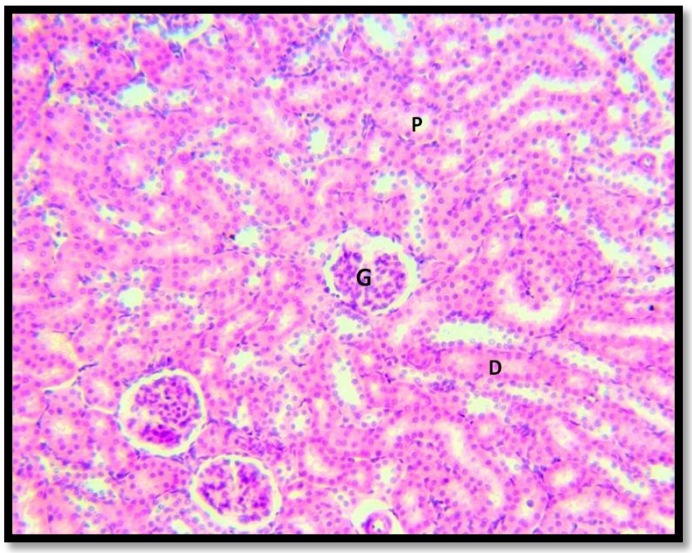

Fig. (1): A photomicrograph of renal cortex of control group (G1) showing normal organization of the renal cortex consisting of renal corpuscles (RC) with normal sized bowmans space, proximal convoluted tubules (p) and distal convoluted tubules (D). (Hx&E X 200).

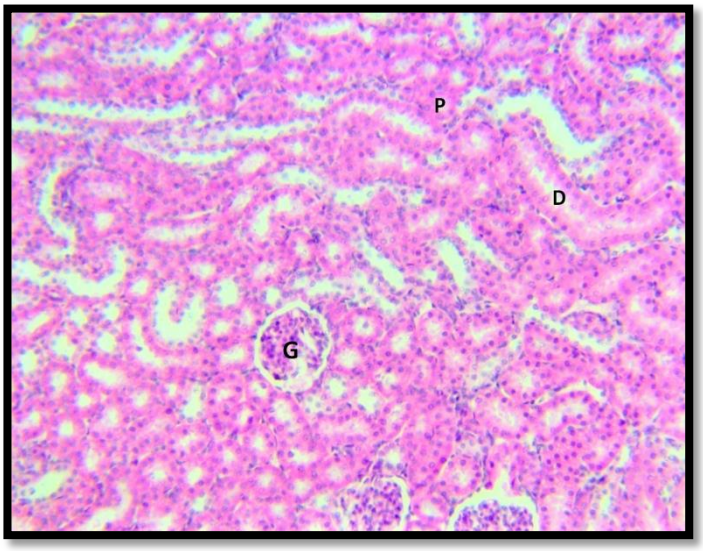

Fig. (2): A photomicrograph of renal cortex of melatonin group (G2) showing normal organization of the renal cortex consisting of multiple renal corpuscles, Proximal convoluted tubules (p) and distal convoluted tubules (D). (Hx&E X 200).

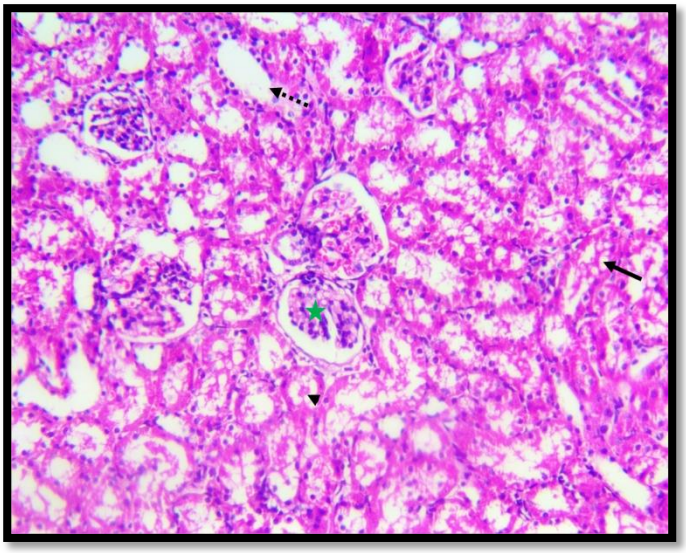

Fig. (3): A photomicrograph of renal cortex of cisplatin group (G3) showing congested, shrunked and distorted glomerulus (green star) and vacuolation of tubular cells (arrow). There is complete destruction of some tubules (dotted arrow). (Hx&E X 200)

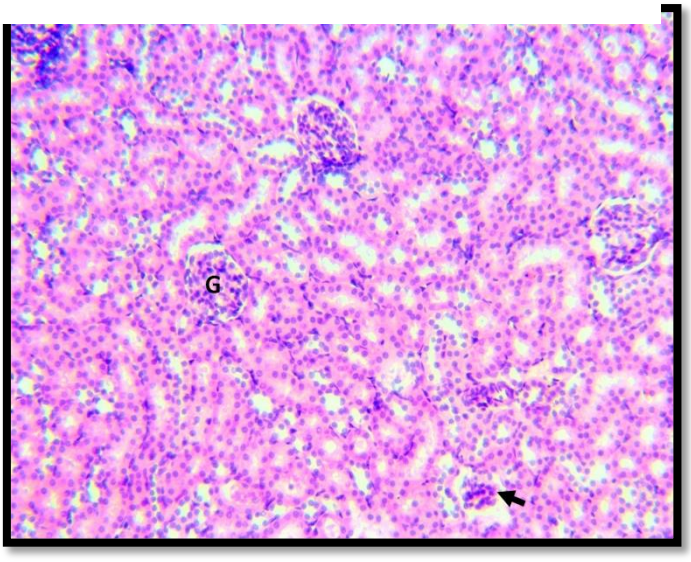

Fig. (4): A photomicrograph of renal cortex of cisplatin+melatonin group (G4)showing relative restoration of renal cortex, renal corpuscles and tubules. There is minimal distortion of renal glomeruli (arrow). (Hx&E X 200).

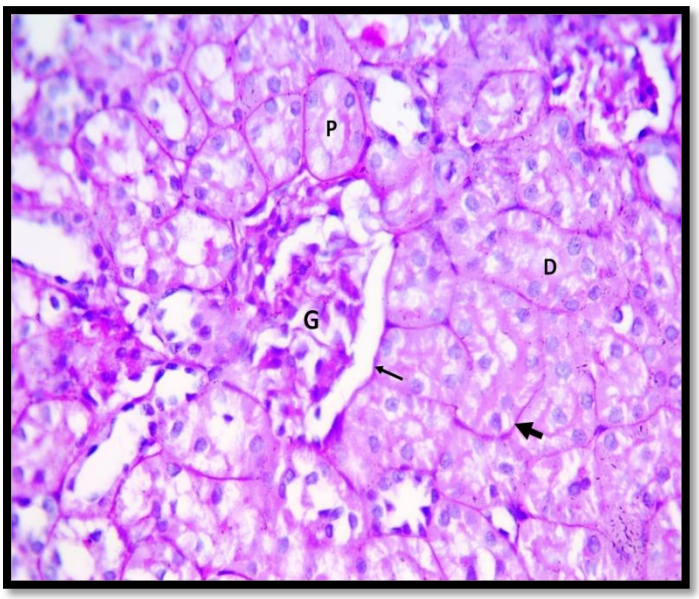

Fig. (5): A photomicrograph of renal cortex of group 1 (control), showing strong positive PAS reaction in the parietal layer of Bowman's capsule (thin arrow), basement membranes of proximal convoluted tubules (p) and distal convoluted tubules (D) (thick arrows). (PAS X 400)

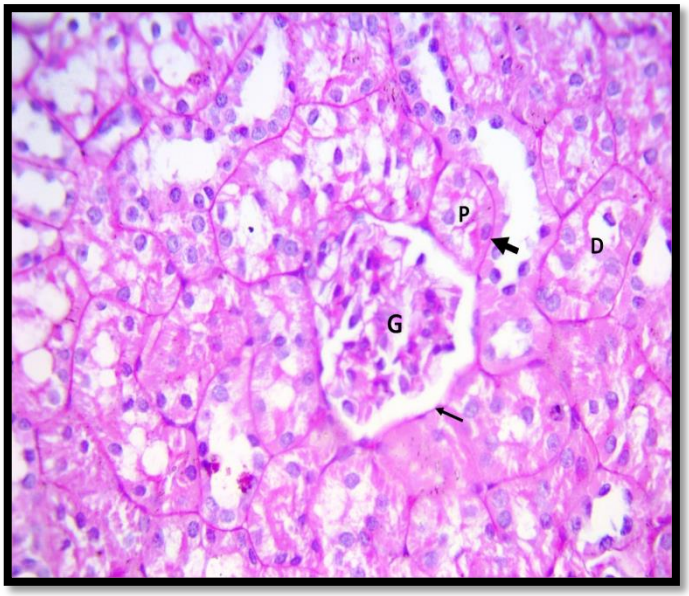

Fig. (6)*:* A photomicrograph of renal cortex of group 2 (melatonin), showing strong positive PAS reaction in the layers of Bowman's capsules (thin arrow), proximal convoluted tubules (PCTs) and distal convoluted tubules (DCTs) (thick arrow). (PAS X 400).

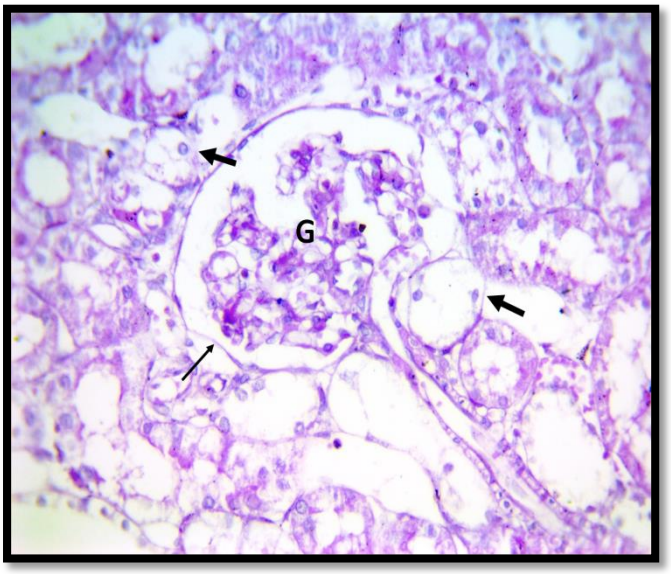

Fig. (7): A photomicrograph of renal cortex of group 3 (cisplatin), showing weak PAS reaction with interruption of the parietal layer of Bowman's capsule (thin arrow) and basement membranes of distorted renal tubules (thick arrows).(PAS X 400).

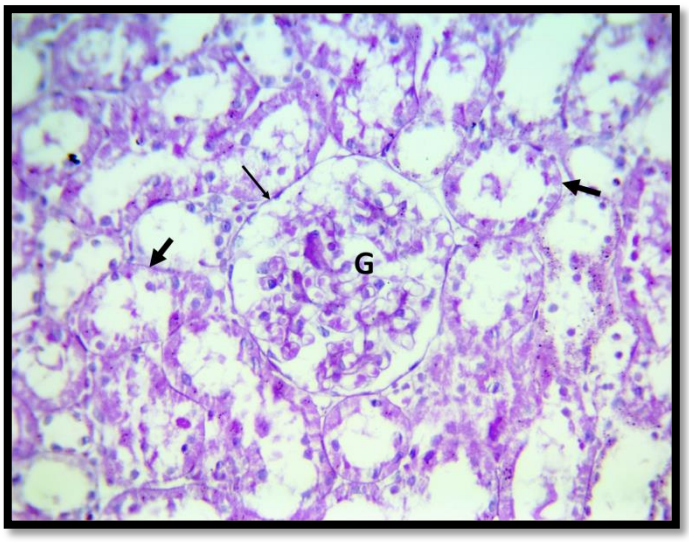

Fig. (8): A photomicrograph of renal cortex of group 4(cisplatin+melatonin), showing relatively preserved reaction in parietal layer of Bowman's capsule (thin arrow), basement membrane of renal tubules (thicks arrow). (PAS X 400).

Table 2: showing the area fraction values of PAS stain (%) among the studied groups:

		Control	Melatonin	Cisplatin	Cisplatin + Melatonin	P value
		N=10	N=10	N=10	N=10	
Kidney PAS area fraction	Range Mean ± SD	(14.3-16.5) 16.1±0.6	(14.5-16.8) 16.3±0.7	(7.1-9.1) 8.2±0.6	(10.4-12.5) 12.4±0.7	**<0.001***
P value between each two groups						
Control			0.919	**<0.001***	**<0.001***	
Melatonin				**<0.001***	**<0.001***	
Cisplatin					**<0.001***	

One Way ANOVA test for quantitative data between the four groups followed by post Hoc LSD analysis between each two groups

***: Significant level at P value < 0.05**

Discussion

The anticancer drug cisplatin is still effective and useful. It is clinically related to acute renal injury in 30% of patients**[26, 27]**. It Inhibits the mitochondrial respiratory complex in renal tubular cells causing tissue damage by producing reactive oxygen species (ROS) **[28]**. Lipid peroxidation, alterations in the enzymatic and nonenzymatic antioxidant system, and changes in gene expression have been seen in renal tissue as a result of ROS production and oxidative stress **[26, 29]**. Since te cisplatin has a low molecular weight, it can easily pass through the membrane of the glomerular base and gather in the outer cortex and inner medulla of the proximal tubules. Increased oxidative stress, apoptosis, vasoconstriction of the renal vascular system, and inflammation all contributed to the pharmacological consequences of cisplatin in renal tissues, which manifested as tissue necrosis in tubules. Numerous studies have shown that cisplatin-stimulated nephrotoxicity is characterised histologically by tissue necrosis in the tubules**[8,30,31]**.

Melatonin (N-acetyl-5-methoxytryptamine), a wellknown physiological mediator that is found in most organisms ranging from bacteria to humans **[32]**. It has been reported that Exogenous melatonin treatment reduces lipid peroxidation even if melatonin levels in the elderly are declining **[33]**. Both directly and indirectly, melatonin manifests its effects against free radicals and associated products, it directly works as a scavenger **[34]**. Melatonin shows an indirect antioxidative action via activation of the cellular antioxidant defense system by increasing mRNA levels and activating several important antioxidant enzymes as, superoxide dismutase and glutathione. So, melatonin reduces the activity of the pro-oxidative enzyme nitric oxide synthase and diminishes formation of free radicals at the mitochondrial level by reducing the leakage of electrons from the electron transport chain **[32]**.

Our histopathogical results using haematoxylin and eosin staining revealed that Cisplatin causes many pathological changes demonstrating significant glomerular and tubulointerstitial injury. The majority of renal corpuscles showed signs of congestion, while some were deformed or even undersized and atrophied. In several portions, interstitial haemorrhage and peritubular capillary congestion were seen. There is marked tubular deformation, tubular cells that were vacuolated, and some cells that had apoptotic patterns (deeply acidophilic cytoplasm and dark pyknotic nuclei). Also there is reduction in cell height and flattening of the epithelium. Homogeneous esinophilic hyaline casts were visible in some tubular lumina. All these findings are in agreement with **[35,36,37]**.

Our study showed that co administration of melatonin successfully reduced the degenerative alterations caused by cisplatin. The kidney tissue from the cisplatin-melatonin relatively restored its normal structure, with decreased glomeruli congestion, interstitial haemorrhage, tubular vacuolation, and intraluminal casts. These results are in agreement with **[38,39]**.

By using PAS staining, reduced PAS reactivity in the form of partial or total removal of the brush border of the majority of deformed tubules was seen in the cisplatin group. in agreement with**[37].** At other sections, the glomerular capillaries' and renal tubules' basement membranes were also shown to be disrupted. The percentage of PAS-positive surface area fraction in the cisplatin group significantly decreased as compared to the control group, according to our morphometric analysis.

Combination of melatonin and cisplatin results in a noticeable improvement as seen by the preservation of most brush boundaries of PCTs and DCTs. Additionally, the basement membranes of glomerular blood capillaries, Bowman's capsule parietal layers, PCTs, and DCTs appeared continuous and undamaged. This was in the same line with other studies **[38.40]**.

Conclusion

Oxidative stress (increased ROS and decreased natural antioxidants), apoptosis, and altered tissue structure are all related to cisplatin-induced kidney damage. Melatonin therapy reduced oxidative stress and apoptotic activity, which reduced histopathological damage. Since melatonin is well tolerated by the body and still retains its anticancer capabilities while scavenging free oxygen radicals and reducing inflammation caused by cisplatin, it can be inferred that melatonin pretreatment will undoubtedly be a beneficial support for chemotherapy.

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