ORIGINAL ARTICLE

Effect of Cyclosporine on the Cortex of the Kidney and the Ameliorative Effect of Melatonin: Microscopic and Ultrastructural Alterations

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Abstract

Background: Cyclosporine is a potent immunosuppressive medication that has been used to treat autoimmune disorders and has enhanced the quality of life and survival rate of transplant recipients. However, a number of adverse effects, chief among them nephrotoxicity, restrict its use. Melatonin is an endogenous hormone released by the pineal gland. Melatonin has antioxidant qualities. Melatonin is an antioxidant found to reduce Cyclosporine toxicity. **Aim of the work:** The study aims to discover the kidney's reaction to cyclosporine exposure and explore the protective function of melatonin. **Materials and Methods:** Thirty adult male albino rats were used in the research. They were separated into three groups, with Group A serving as the control group and not receiving any medication. Ten rats in Group B (the cyclosporine-treated group) were given a subcutaneous injection of cyclosporine at a dose of 15 mg/kg/day for 40 days. Ten rats make up Group C, the group that received melatonin treatment. They were given the same intraperitoneal injection of cyclosporine and melatonin at a dose of 1 mg/kg/day in saline. The specimens were prepared for both light and electron microscopic examination at the completion of the study. **Results:** Cyclosporine causes morphological alterations in the kidney, including lobulation of glomerular capillaries in the renal corpuscle and enlargement of the urinary space with congestion. The proximal convoluted tubules had uneven cell degeneration and brush boundary disintegration. The peritubular capillaries were clogged and extravasated, and the distal convoluted tubules degenerated, with some cells inside the lumen exfoliating. In addition, the effects of cyclosporine are lessened when melatonin is administered with it. **Conclusion:** Through oxidative stress, cyclosporine damages the kidneys, while melatonin, an antioxidant, reduces but does not completely prevent these damages.

Key words: Cyclosporine, electron microscope, kidney, melatonin, ultrastructural study

INTRODUCTION

The renal tissue in the human kidney is differentiated into an outer cortex and an inner medulla. The cortex is characterized by the presence of many nephrons (1–2 million nephrons). Each nephron consists of renal corpuscle 0–2 mm in diameter (glomerulus and Bowman's capsule), proximal tubule, loop of Henle and distal tubule. Regarding the

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Received: 20-11-2025
Revised: 23-12-2025
Accepted: 29-12-2025

medulla, it is divided into inner and outer zones, containing the collecting tubules and ducts.[1] Histologically, the cortex can be divided into outer and inner zones. The inner zone is separated from the medulla by tangential blood vessels, such as arcuate arteries and veins, which are located at their intersection. However, a thin layer of cortical tissue, known as the sub cortex, arises on the medullary side of this zone. Juxta-medullary refers to the cortex near the medulla.[2] The efficacy of cyclosporine, a significant advancement in pharmacologic immune-suppression, is currently known for solid organ transplantation and is quickly becoming apparent for a variety of autoimmune diseases. The original purpose of cyclosporine was to stop organ rejection in patients having liver, heart, or kidney transplants. Cyclosporine functions by lowering your immune system, which lowers its reaction. The objective is to reduce the activity of renal disease before the inflammation causes irreversible kidney damage.[3] Although the medication can harm the kidneys, it is unknown how common nephropathy is and what risk factors exist for people receiving cyclosporine for inflammatory or autoimmune conditions.[4] When thinking about using cyclosporine medication to treat autoimmune illnesses, one should exercise caution because renal impairment is a significant possible adverse effect. Cyclosporine-induced nephropathy has mostly been reported in patients who have received renal transplants, but it has also happened in patients who have received heart transplants and in autoimmune disease patients who did not have renal disease but who received cyclosporine at doses of at least 10 mg per kilogram of body weight (BW) per day.[5] Numerous cytokines, including interleukins 1a and other lymphokines, also control the immunological and inflammatory responses. Cyclosporine can suppress these cytokines to reduce graft rejection.[6] However, cyclosporine use is associated with a number of negative side effects, including hypertension, dyslipidemia, gingival hyperplasia, hypertrichosis, nephrotoxicity, hepatotoxicity, and cancers.[7] Cyclosporine both acute and chronic nephrotoxicity is possible. Increased release of vasoactive mediators causes renal vasoconstriction in the acute form. While another feature of chronic nephrotoxicity is the development of structural damage, such as tubulointerstitial fibrosis and arteriolopathy, these changes are irreversible and may lead to end-stage renal disease.[8] It has been discovered that melatonin protects renal tissues against oxidative damage brought on by a range of harmful substances. In addition, it has recently been discovered that melatonin shields rats from the nephrotoxicity caused by cyclosporine.[9] Nonetheless, research has linked melatonin's structural characteristics to its active function, which enables it to directly scavenge free radicals and increase antioxidant enzymes in oxidative stress. Furthermore, it has been shown that melatonin effectively eliminates the renal oxidative stress brought on by carbon tetrachloride, uranium, cadmium, mercuric chloride, and chlorpryfos-ethyl. Nonetheless, ongoing research is being done to examine melatonin's potential as a therapy for drug-induced toxicity.[10] Thus, the purpose of this study was to ascertain how cyclosporine exposure affected the kidneys and to look into the potential protective function of melatonin.

MATERIALS AND METHODS

In this study, 30 adult albino rats were employed. They weighed between 210 and 230 g. They were split up into three groups. Every group was kept at room temperature in a different plastic cage. They were fed a balanced diet consisting of bread, veggies, and milk. Throughout the trial, every rat was housed under the same conditions. Two drugs were used in this study Cyclosporine-A and Melatonin. Cyclosporine-A (Sandimmun Neoral, Novartis) in the form of gelatinous capsules containing either 50 mg or 100 mg of the drug. One capsule of 50 mg plus another one contained 100 mg of the drug were pinched using the tip of a sterile needle, the contents were squeezed and dissolved in olive oil and were given to 20 rats every 2 days. Each rat was given 0.5 mL of olive oil containing 3 mg of the drug to equal the dose of 15 mg/kg BW per day which is equivalent to the human low therapeutic dose.[11] Melatonin(Viva max® 3) in the form of tablets containing 3 mg of the drug. Each tablet was dissolved in normal saline. The dose was calculated as 1 mg-kg-BW per day. This means that each rat was given 0.5 mL of normal saline at night that containing 0.2 mg of the drug. To reach to this concentration of the drug, each tablet (3 mg) was dissolved in 7.5 mL saline. Hence, each 0.5 mL saline contained 0.2 mg of melatonin.[11] The animals were divided into three groups. Animals in the control group A were not given any medication. Group B (Cyclosporine-treated group): Consists of ten rats. They received cyclosporine-A at a dose of 15 mg/kg/day in olive oil subcutaneous injection for 40 days. Group C (melatonin-treated group): Consists of ten rats. They received the same dose of cyclosporine-A for 40 days along with melatonin in a dose of 1 mg-kg-day in saline by intra-peritoneal injection. At the end of experiment, all rats were anesthetized using either inhalation. The abdomen was incised followed by dissection of the kidneys. The samples were prepared for examination under both light and electron microscopy. After that, the specimens were placed into a plastic capsule that had already been labeled. For 48 h, capsules were polymerized at 60° Celsius in a temperature-controlled oven. A glass knife was used to cut semi-thin portions at a thickness of 1 mm. The chosen blocks were used to create ultra-thin sections that were 50 nm thick and placed on copper grids. Grids were placed in Petri dishes and stained for 20 min with uranyl acetate and then for 10 min with lead citrate. Ultimately, electron micrographs were captured from the designated regions.

RESULTS

Light microscopy analysis of the control group's renal cortex hematoxylin and eosin staining reveals proximal and distal convoluted tubules, as well as typical renal corpuscles. Every

renal corpuscle has a rounded appearance. It is made up of Bowman's capsule and the glomerulus. Every glomerulus is made up of a network of capillary loops that hold blood cells. Bowman's space, a parietal layer, and a visceral layer covering the glomerulus make up the Bowman's capsule [Figure 1a and b].

The electron microscopic study of the control group A shows the lining epithelial cells of the proximal convoluted tubules and a basal rounded nucleus with a prominent nucleolus and microvilli. The cytoplasm contains lysosomes, vacuoles, and elongated mitochondria. The basal part of these cells is characterized by the presence of deep basal infoldings in between the elongated parallel mitochondria, which are resting on regular basal lamina. The distal convoluted tubules are also demonstrated with their lining cells having small, rounded nuclei, less elongated mitochondria resting on the basal lamina, and a wide lumen. Furthermore, the examined sections show the ultra-structural features of the glomerulus with the details of the podocytes forming the visceral layer of Bowman's capsule. The podocytes have a nucleus and a cell body from which arise several primary (major) processes resembling the feet and numerous smaller secondary processes (minor or pedicles). The filtration slits were identified as the spaces between the secondary processes. The glomerular capillaries have a fenestrated endothelium. Between the fenestrated endothelium and pedicles of podocytes is the regular basal lamina, which is clearly seen [Figure 1c and d].

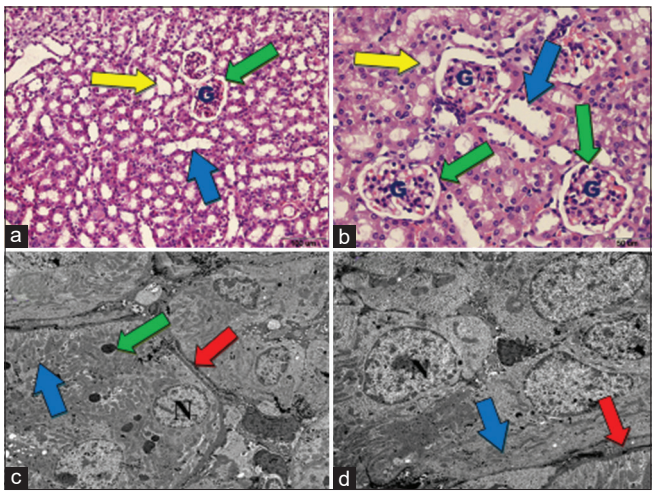

Figure 1: Different images of section of the renal cortex of a rat of group A (Control untreated) (a and b) Sections stained with Haematoxylin and Eosindemonstrating the normal architecture of the renal cortex formed of renal corpuscles (green arrow) formed of glomerular (g) tuft of capillaries and Bowman's capsule, proximal convoluted tubules(yellow arrows) and distal convoluted tubules (blue arrows). Notice straight tubules originating from the medulla toward the cortex (medullary rays) (red arrows). (a) (×200). (b) (×400). (c and d) An electron micrograph showing part of proximal tubules with their lining cells consist of basally located nuclei (n). The cells rest on basement membrane (red arrows) while apical part shows microvilli. The cytoplasm contains basal elongated mitochondria (blue arrows) and electron dense lysosomes (green arrow). (c) (TEM ×4000). (d) (TEM ×5000)

According to a light microscopic examination of the renal cortex of cyclosporine group B, the drug causes varying degrees of tubular and glomerular degeneration. The sections of renal cortex showed moderate degeneration of some tubules, while other tubules were still intact. The effect is more evident on the proximal convoluted tubules, while the distal convoluted tubules seem to be normal. Furthermore, the proximal convoluted tubules show evident dilatation and vacuolization. The epithelial lining of the proximal convoluted tubules contained many vacuoles, while other tubules were degenerated. Some nuclei are degenerated, and others are intact [Figure 2a-d].

The electron microscopic study of the cyclosporine group B shows the lining epithelial cells of the proximal convoluted tubules and partial degeneration of some nuclei, and others are intact. The apical microvilli appear intact in some areas, and in other areas, they are shortened or even completely damaged, with protrusion of cellular contents into the lumen. The lumen also contains epithelial debris and inflammatory cells. The cytoplasm contains a number of large, irregular, dense lysosomes and many vacuoles. The mitochondria are swollen, degenerated with loss of their elongated form. Few mitochondria are intact. The basal part of these cells is characterized by loss of the basal folding in in-between mitochondria, which are resting on the basal lamina. The basal lamina is relatively thickened and becomes denser than that of the control one with loss of its uniform appearance [Figure 3a and b]. There is a widening of the inter-tubular

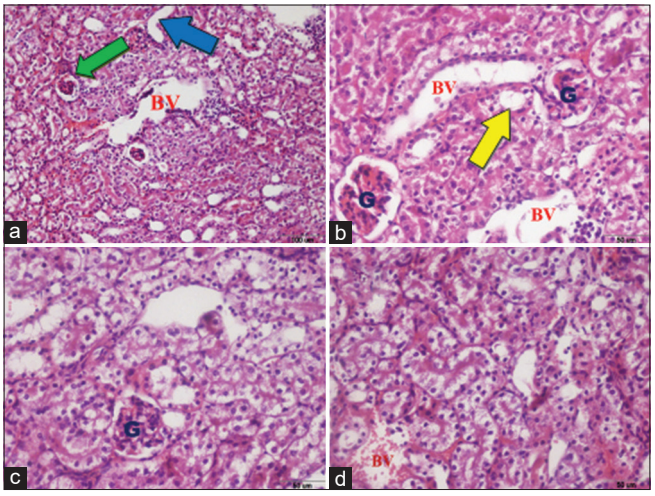

Figure 2: (a-d) Different images of section of the renal cortex of a rat of cyclosporine group B stained with Hematoxylin and Eosin demonstrating variable degrees of glomerular (g) and tubular degeneration wide interstitial spaces containing congested and dilated blood vessels, together with perivascular and peritubular inflammatory cell infiltrate (green arrow) while other tubules are still intactas distal convoluted tubules (blue arrow) and proximal convoluted tubules (yellow arrow) (a) (×200) (b-d) (×400)

space containing evident peritubular capillaries. Regarding the glomerulus, the glomerular basal lamina is thickened and amorphous. The overlying primary and secondary processes (pedicles) are distorted in shape, and some are even fused together or having an irregular form, but the endothelium seems normal. The nucleus of a podocyte can also be seen [Figure 3a and b].

The light microscopic study of the renal cortex of the cyclosporine and melatonin group C; The proximal convoluted tubules show less tubular dilatation and traces of vacuolization. Most of the glomeruli show marked regression of the effect of cyclosporine as seen before and relative return to normal findings as control. The glomerulus is surrounded by Bowman's capsule and separated from it by Bowman's space [Figure 4a and b].

The electron microscopic study of cyclosporine and melatonin group C shows that the lining epithelial cells of the proximal tubules are greatly recovered and nearly similar to the control ones. The cytoplasm is studded with mitochondria, of which few are elongated, and others are still swollen or even degenerated. There are few vacuoles and intact apical microvilli. The nucleus is normal with a prominent nucleolus. The basal lamina shows a relative return to uniform appearance when compared with the control one. The distal convoluted tubules appear in normal form, even the few mitochondria that are affected are recovered to the normal form. The overall pattern of the glomeruli shows marked regression of the effect of cyclosporine with return to the normal appearance. The podocytes on the capillary loops are apparent. Their processes are identified into primary and secondary, with their nucleus clearly seen. Few pedicles are still fused. The glomerular basal lamina becomes highly returns to the normal form with fenestrated endothelium. The filtration slits in-between pedicles are evident. Furthermore, the mesangial cell appears in normal form with pale and limited extracellular matrix [Figure 4c and d].

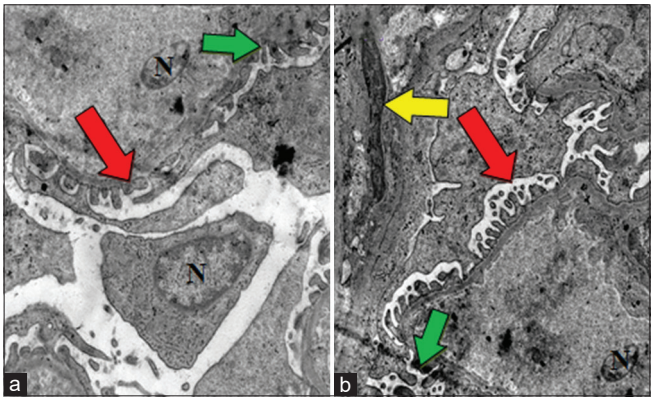

Figure 3: (a and b) Two different images of electron micrograph of section of the renal cortex of a rat of cyclosporine group shows the lining epithelial cells of the proximal convoluted tubules and partial degeneration of some nuclei (n). The mitochondria are swollen, degenerated with loss of its elongated form (green arrows). The basal lamina is relatively thickened (yellow arrows). There also a fusion of their feet processes (red arrows) at certain sites. (a) (TEM ×20000). (b) (TEM ×8000)

DISCUSSION

One organ involved in the detoxification process is the kidney. Numerous medications, chemicals, and heavy metals alter the kidney's structure and function.[12] The cyclosporine group's rat kidney showed pathological alterations in the current investigation. These alterations included lobulation of glomerular capillaries in the renal corpuscle and dilatation of the urinary area with congestion. In addition, melatonin administration in conjunction with cyclosporine resulted in fewer morphological alterations in the renal corpuscle urinary space and glomerular capillary congestion than the cyclosporine-treated group. The precise process through which cyclosporine therapy causes nephrotoxicity is not well known. Numerous medications may directly cause nephrotoxicity or increase the creation of free radicals, as a recent study thoroughly examined.[13] According to experimental data, cyclosporine nephrotoxicity may also be mediated by oxygen-free radicals. Chronic renal failure has been linked to changes in the circadian rhythm of melatonin secretion in both humans and rats, as well as a suppression of the hormone's nocturnal ascent. Our current findings

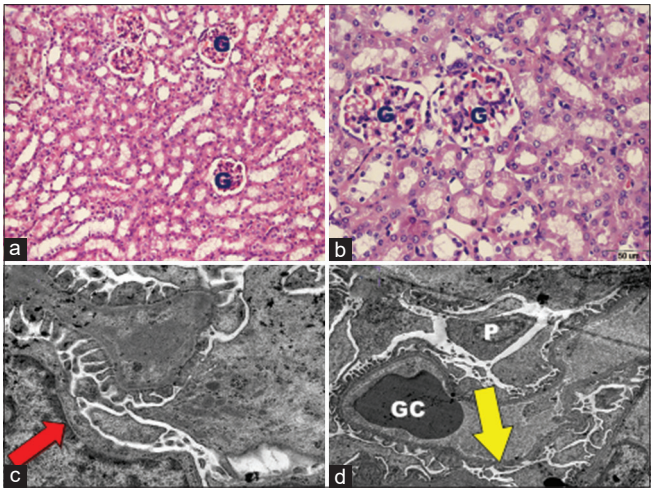

Figure 4: Different images of section of the renal cortex of a rat of group C (cyclosporine and melatonin group) (a and b) Sections stained with Hematoxylin and Eosin demonstrating most of glomeruli (g) show marked regression of the effect of cyclosporine and relative return to normal finding as control one. (a) (×200). (b) (×400). (c and d) A n electron micrograph showing the lining epithelial cells of the proximal tubules are greatly recovered. The basal lamina shows relative return to uniform appearance when compared with the control one. It shows glomerular capillary with some areas of preserved endothelial fenestrations (yellow arrow). Regular basement membrane (red arrow) is seen. Notice the podocyte (p) on the capillary loops are apparent. (c) (TEM ×15000). (d) (TEM ×12000)

clearly demonstrate that melatonin activity does indeed take place at the renal level, as they are consistent with melatonin protecting renal structure following treatment of mice. Melatonin-induced renal perfusion significantly reduced tissue damage from the production of oxygen-free radicals. Recent research has demonstrated that melatonin shields various tissues and cells against oxidative damage brought on by the production of extremely harmful reactive oxygen intermediates.[14] In addition, another study found that melatonin prevents the biochemical changes brought on by Adriamycin, acting as a very potent scavenger of free radicals in the kidney. We come to the conclusion that melatonin's positive benefits are caused by its antioxidant qualities acting on the kidney.[15] The administration of cyclosporine was also linked to either a suppression of relaxing factors such as nitric oxide, or an increase in the synthesis of vasoconstrictor factors such as endothelin and thromboxane.[16] According to earlier research, the injection of cyclosporine causes endothelial cells in culture to sustain structural damage and impairs endothelium-dependent vaso-relaxation.[17] The cyclosporine plus melatonin group exhibited less renal ultrastructural alterations than the cyclosporine group alone, which is another significant discovery discussed here. In addition, there were little histological and quantitative findings in the proximal tubules, and the glomerulus was almost identical to controls. This is consistent with another study in which melatonin significantly reduced malondialdehyde levels and improved creatinine clearance in kidneys treated with cyclosporine.[18]

CONCLUSION

Cyclosporine had negative effects on the cortical structure of the kidneys. However, melatonin demonstrated a strong nephron-protective effect in avoiding or at least lessening the renal damage brought on by cyclosporine. Administration of melatonin would therefore be very beneficial in preventing the harmful effects of cyclosporine on renal tissue.

ACKNOWLEDGMENTS

The author is grateful to the Deanship of Scientific Research, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia, for its support and encouragement in conducting the research and publishing this report.

AVAILABILITY OF DATA AND MATERIALS

The data are available upon request from the authors.

ETHICS APPROVAL

All series of steps that were implemented in this study complied with the Ethics Committee of Prince Sattam bin Abdulaziz University Institutional Review Board (SCBR-439/2025).

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Source of Support: Nil. **Conflicts of Interest:** None declared.