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The Effect of Cyclosporine Drug on the Function and Histological Structure of the Kidney in Albino mice *Mus musculus*

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Abstract : The current study aimed to determine the effect of cyclosporine drug on the function and histological structure of the kidney in the albino mice *Mus musculus*. The results revealed that there is no significant difference ($P < 0.05$) was seen in the level of urea in the plasma of the treated group with cyclosporine at concentration of (1.2 mg/kg) in comparison to the control group. However, this dose of cyclosporine in the treatment caused a significant increase ($P < 0.05$) in the levels of creatinine and uric acid compared to the control group.
Histologically, the kidney consists of two regions, cortex and medulla; each kidney consists of a several of the renal corpuscles (Nephrons), which is the functional unit of the kidney. Each corpuscle is composed of glomerulus that surrounded by two layers of Bowman's capsule, while the second part of glomerulus is composed of the renal tubules that divided into proximal, distal and collecting, and the endothelium of tubules is simple cuboidal to columnar epithelium.
Several histological changes have been shown in the treated mice with cyclosporine including a decrease in the size of glomeruli, increasing in the width of capsular space, enlargement and necrosis of the epithelial cells of the renal tubules. Moreover, congestion in the blood vessels of the kidney has been shown and infiltration of inflammatory cells and hemorrhage in the connective tissue.
Keywords : Kidney, Cyclosporine, Mice, Urea.

Introduction

Cyclosporine is an immunosuppressive drug that inhibits the action of lymphocytes[1,2] by formation of a complex that inhibits phosphatase, causing a decrease in the production of inflammatory cytokines; this complex inhibits the transcription of interleukin-2 (IL-2) in T-cells produced by T-lymphocytes[1]. Additionally, Cyclosporine acts as an anti-calcineurin.

Cyclosporine was isolated in 1971 from *Tolypocladium* and used as a medical drug in 1983[2,3,4,5]. Cyclosporine consists of 11 amino acids and most of peptides are produce by the ribosomes[2].

The kidney plays an important role as one of the necessary organs in the body, it filters blood and excretes the urine that pass through ureter into the urinary bladder, where the water is excreted as urine in Mammalia and as uric acid in Aves and Reptilian. The kidney is as endocrine s organ, which secretes Erythropoietin that

stimulates the production of red blood cells (Erythrocytes) in bone marrow; kidney also secretes Renin that regulates the blood pressure[6, 7, 8, 9, 10].

The histological examination observed that the kidney is composed of a large several of renal corpuscles (Nephrons) which range from one million to two million corpuscles. The renal unit is the structural and functional unit of the kidney, where it filters the substances and removes the wastes from the blood[11, 12].

Materials and Methods

Mus musculus mice were injected subcutaneously with cyclosporine (100 mg) with the dilution (1.2 mg/kg) every 48 hours for 14 days. In this study, 24 male mice were used with age (77-98 days) and weight (26-32 g); the mice were obtained from Department of Drug Control of the Iraqi Ministry of Health. Mice isolated in the Animal House of the College of Education for Pure Sciences/Ibn Al-Haitham and supplied with food and water, light and appropriate temperature. Mice were divided into two groups, treated and control; each group included 12 mice that injected with 0.1 ml of the drug in the traded group and with normal saline in the control group.

The weight of the animals was determined at the beginning of the experiment, and then the weight recorded every week until the end of the experiment. The samples of the blood plasma collected for the analysis of urea, creatinine and uric acid according to the procedure of the kit. Then, the animals were dissected by making a longitudinal incision along the ventral middle line from Cloaca slot area to thoracic griddle area; the kidneys were eradicated and its weight has been observed and then examined under the microscope. After that, the kidney washed with normal saline and fixed in formalin solution (10%) (Luna, 1968), then washed with the tap water and dried using dehydrated alcohol ethyl and cleared by xylene and infiltrated and embedded in paraffin wax; the section with seven micron in thickness collected and stained with Hematoxylin & Eosin (H & E) dye [21], and all mounted sections examined by using elected microscope with a camera filming.

Results

This study revealed that there is no significant difference ($P<0.05$) in the level of urea in the plasma in the treated group with cyclosporine at (1.2 mg / kg) at concentration in comparison to the control group (Fig. 1) (Table 1). However, cyclosporine caused increasing in the levels of creatinine and uric acid in the serum with significant differences ($P <0.05$) compared to the control group (Fig. 2) and (Fig. 3) (Table 1).

The results of the histological study observed from cross sections of the kidney in treated group that it is surrounded by a thin capsule of connective tissue and it is composed of two major regions, the cortex and medulla (Fig. 4). In the cortex, nephrons appeared as proximal convoluted tubules and distal convoluted tubules (Figure 4).

The endothelium of proximal convoluted tubule consists of short columnar to cuboidal epithelial cells and the nucleus is dark. Moreover, the cells also have a brush border in the free surface of the cells. While the endothelium of the distant convoluted tubule consists of simple cuboidal epithelium; the cells are light-colored and contain central dark nucleus, and the free surface of the cells is not brushed (Fig. 4).

Another part of corpuscle is glomerulus. It is small ball of blood vessels that surrounded with Bowman's capsule which is consist of double layers of parietal layer consisting of simple squamous epithelium and visceral layer, and between them the capsular space (Fig. 4).

The collecting tubules found in the medulla and consist of simple columnar epithelium containing cells with dark nuclei, and these tubules are separated by interstitial connective tissue (Figure 5).

In the present study, several changes have been shown in the kidneys of mice that treated with (1.2 mg/kg) of cyclosporine including a decrease in the size of the glomerular hypoplasia and an increase in the width capsular spaces while the glaucoma is missing or shrunken (Figure 6). Additionally, enlargement of the epithelial cells of the proximal, distal, and proximal tubules were seen and necrosis in the cells of urinary tubule (Fig. 7).

Moreover, congestion has been shown in the blood vessels of the kidney, vascular degeneration in the vascular wall, infiltration of lymphocytes and multinuclear leukocytes, and hemorrhage of blood vessels (Fig. 8).

Discussion

Cyclosporine is an immunosuppressive drug that inhibits the immune system of human body. It is act to suppress the immune system by several mechanisms including suppressing the growth of immune cells; therefore, it is used after organ transplantation such as liver, kidney and heart [2,3,9].

The current study found a significant increase in the level of creatinine and uric acid in the treated mice with cyclosporine and this result may due to the cytotoxic effect of cyclosporine, and this result is like those that found by Tootian *et al*. (2012)[13] through their studies on the kidneys treated with phenol, and similar to Monferd (2013)[14] results that studied the effect of *Carthamus* plant on the kidney functions.

The level of creatinine in the plasma is as indicator to the glomerular filtration rate in the kidney because the secretion of creatinine from the kidneys is little and any change on its level will indicate a significant change in the kidney function, and the measurement of creatinine level in the plasma is more accurate than the level of urea to estimate the kidney function because the level of urea is affected after loss of more than 50% of the renal cells function, in addition to that, there are other reasons not related to the kidney function effects on the high level of urea in the plasma. These findings agree with the results of current study that found significant increases in the level of creatinine and uric acid in the treated group, while there was no significant difference shown in the level of urea in the treated group compared to the control group.

In the histological study, several changes have been shown in the kidney after the treatment with cyclosporine; these changes included modulation in the renal tubules and interstitial connective tissue of the kidney, congestion of vessels, infiltration of lymphocytes and polymorphonuclear leukocytes from blood vessels in interstitial connective tissue of the kidneys, and these results agree with other researchers [15,16,17].

Lichtiger *et al*. (1994)[15] indicates that cyclosporine increased the blood pressure and leads to the formation of blood clots in the blood vessels of the kidney, causing the occurrence of blood urine. This result is similar to the results of this study which showed congestion in the blood vessels and hemorrhage in the interstitial connective tissue of the kidney in the treated mice.

Cyclosporine is absorbed into the gastrointestinal tract and then distributes to the various organs of human body such as liver, kidney, brain and Spleen[16] the absorbent drug is eliminated by the kidney. This study revealed that cyclosporine caused an enlargement in the epithelial cells of the proximal tubules; this is consistent with previous results[5,17,18]

Additionally, necrosis of the renal tubules, as well as atrophy of the vascular components of the glomerulus have been observed, causing a decrease in the size of the glomeruli in the treated kidney. This is consistent with Ucheya & Igweh (2006)[19] that found histological changes in the glomerular which increase the capsular spaces result in kidney damage which leading to reduced kidney efficiency in the treated mice.

The renal excretion of the metabolic wastes and histological changes in the kidney is determined by the health status of it [20]. Urea is the final product of the metabolic processes of proteins in the Mammalia; proteins are composed in the liver and then pass to the kidney where it excreted with urine, while creatinine is produced from creatinine phosphate after losing the phosphate group and then passes to the blood stream to the kidney to excrete with urine.

The uric acid is the final product of the purine metabolism which is essential in the synthesis of nucleic acids; it is excreted with urine that uric acid composed 80% of the urine while 20% of it excretes with bile salts.

Table (1): The levels of urea, creatinine and uric acid in plasma in mice treated with cyclosporine (1.2 mg/kg).

Parameters of kidney function	Groups			
	Control		Treatment	
	Mean (M)	Standard error (SE)	Mean (M)	Standard error (SE)
Urea	30	0.707	32.60	1.077
Creatinine	0.306	0.009	0.432	0.008
Uric acid	2.740	0.01	3.368	0.0156

Significant differences ($p \leq 0.05$)

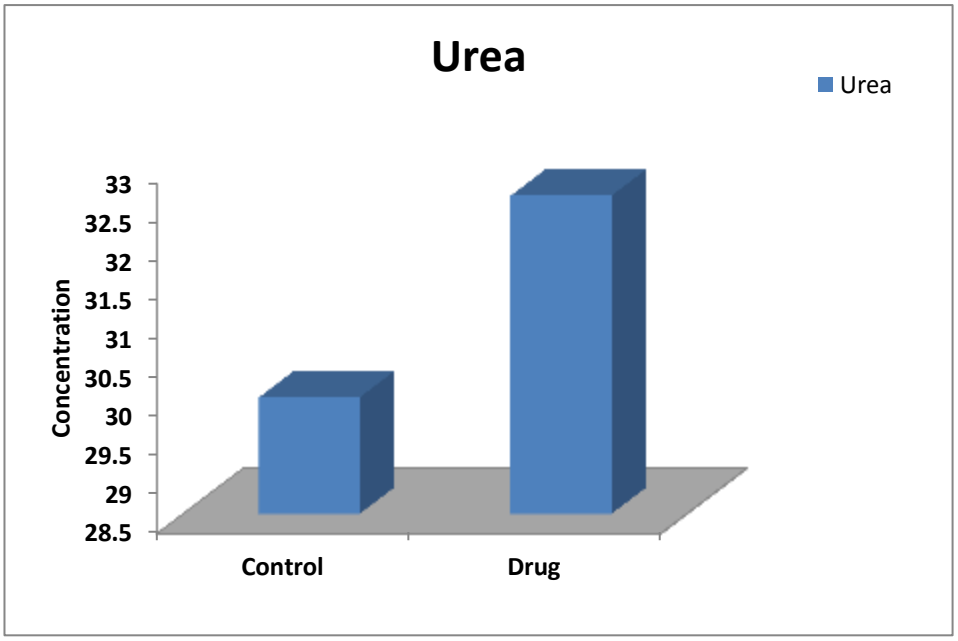

Figure (1): The level of urea in the blood plasma in mice treated mice with cyclosporine (1.2 mg/kg) and control group.

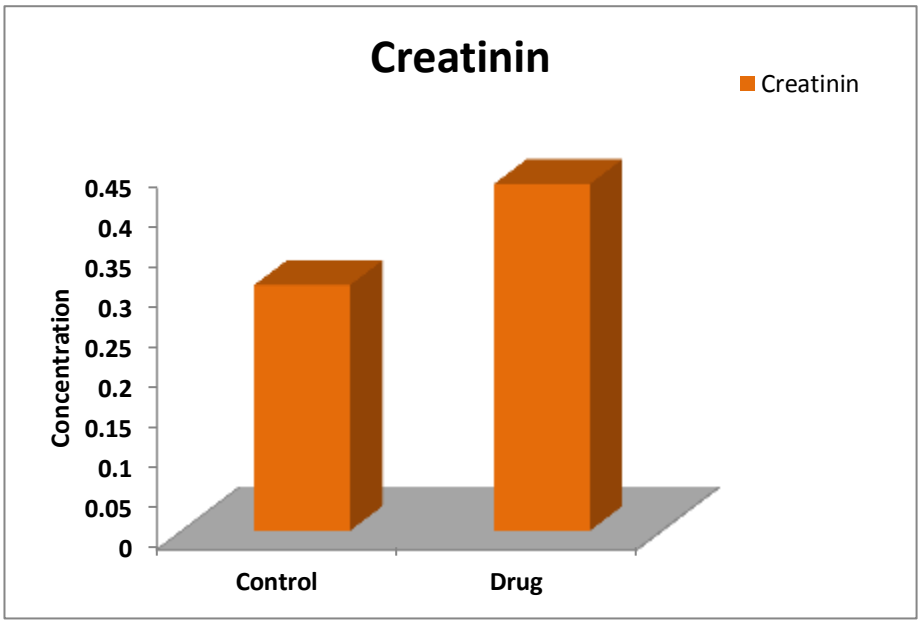

Figure (2): The level of creatinine in the blood plasma in mice treated mice with cyclosporine (1.2 mg/kg) and control group.

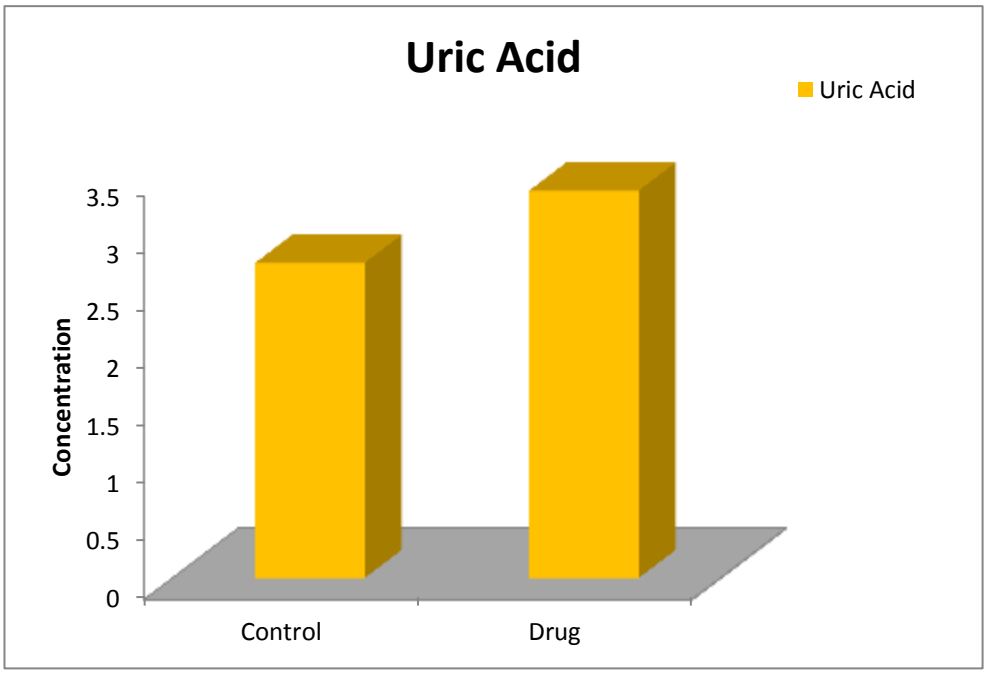

Figure (3): The level of uric acid in the blood plasma in mice treated mice with cyclosporine (1.2 mg/kg) and control group.

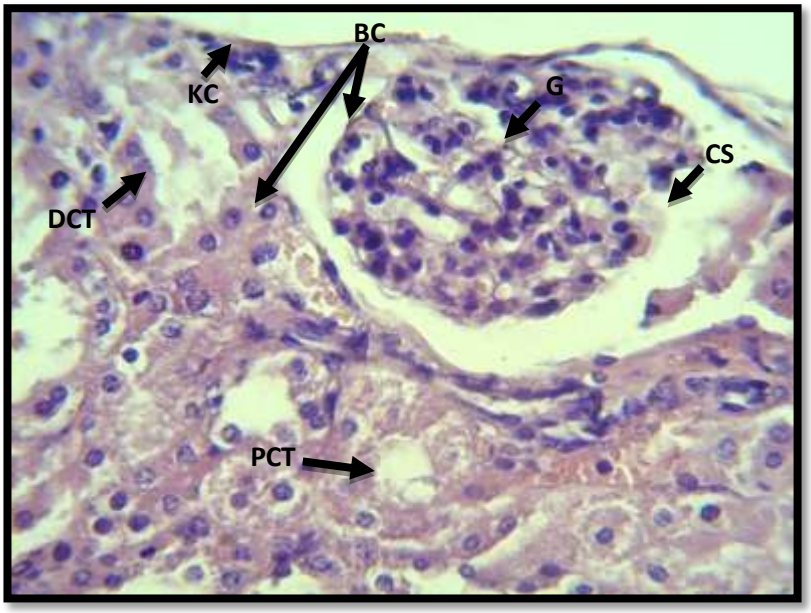

Figure (4): Cross section in the medulla of kidney's mice in control group. (G) Glomerulus, Bowman's capsule (BC), Proximal collecting tubule (PCT), Distal collecting tubule (DCT), kidney capsule (KC), capsular space (CS), (H & E, 40X).

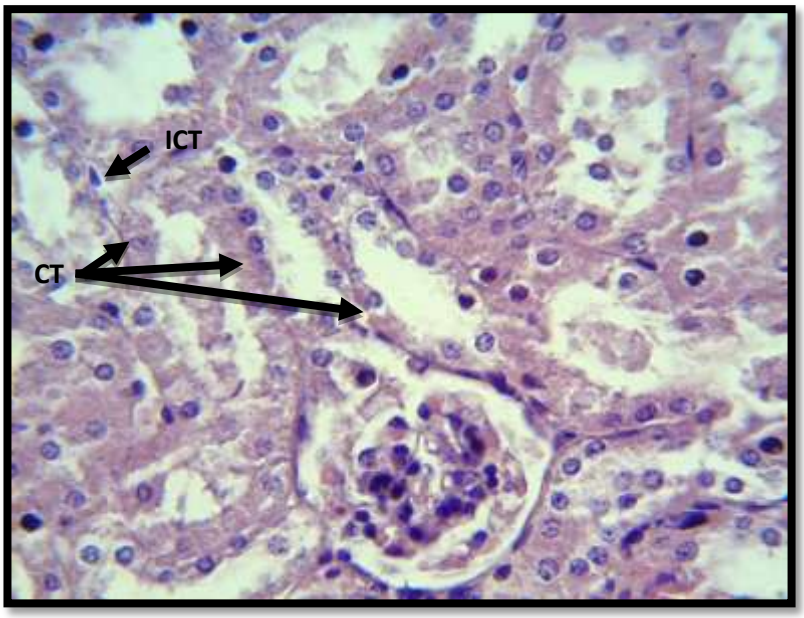

Figure (5): Cross section in the medulla of kidney's mice in control group. (CT) collecting tubule (CT), interstitial connective tissue (ICT), (H & E, 40X).

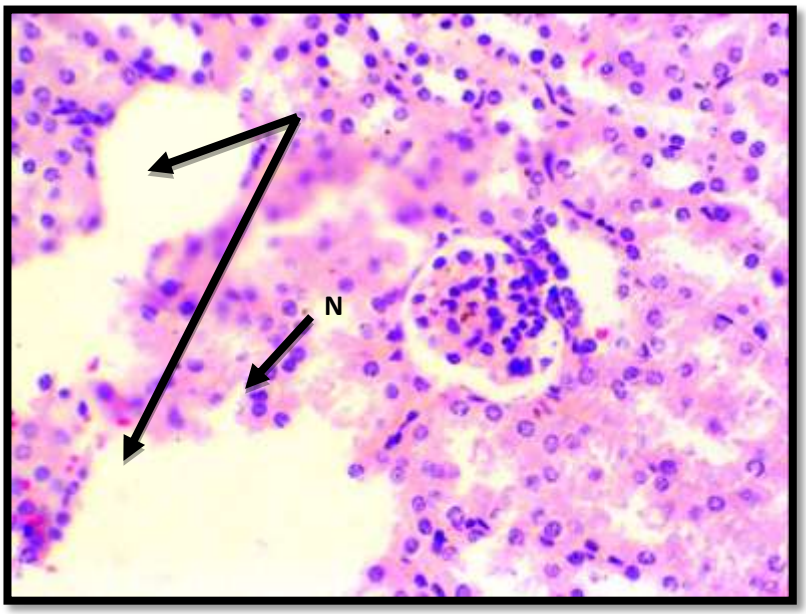

Figure (6): Cross section in treat kidney's mice with cyclosporine drug (1.2 mg/kg) in mice. Note Necrosis (N), (H & E, 40X).

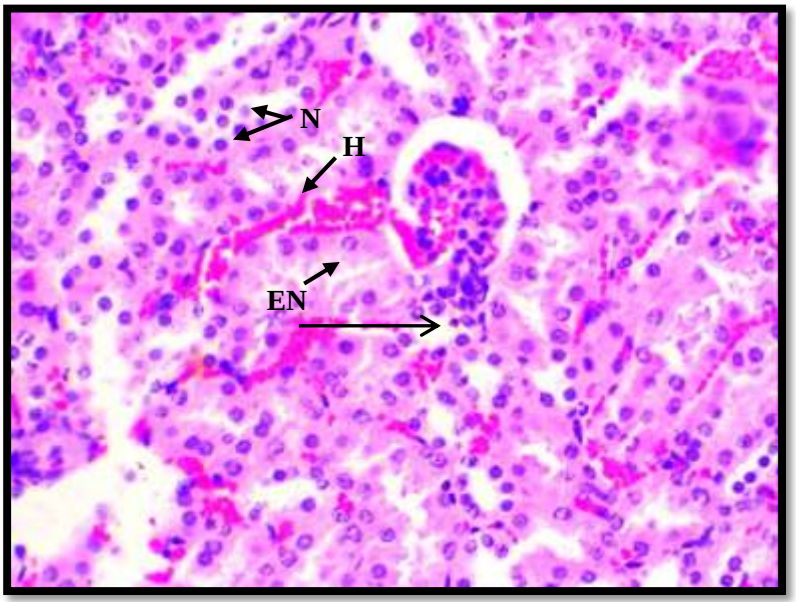

Figure (7): Cross section in treat kidney's mice with cyclosporine drug (1.2 mg/kg), noted the enlargement of endothelial cells of tubules, hemorrhage (H), necrosis of the cells (N), enlargement of e the cells (EN), (H & E, 40X).

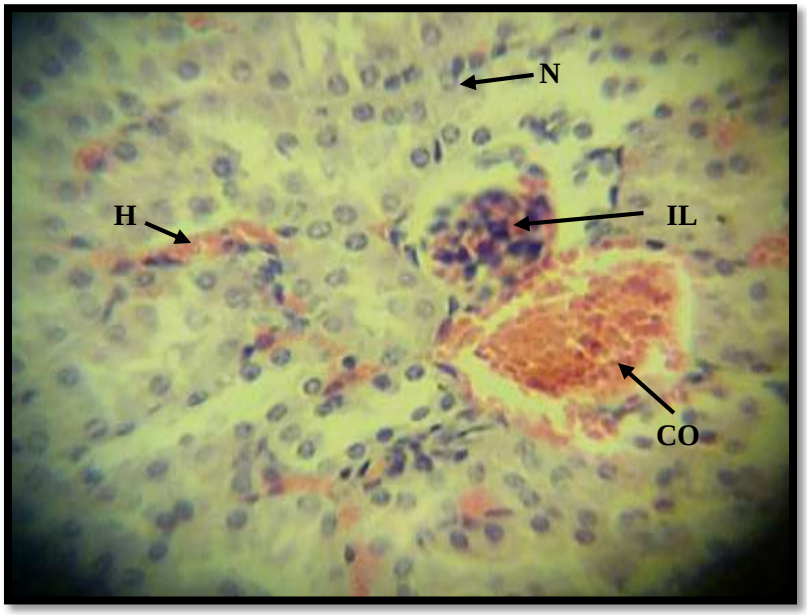

Figure (8): Cross section in treat kidney's mice with cyclosporine drug (1.2 mg/kg), noted necrosis of epithelial cells, infiltration of lymphocytes (IL), hemorrhage (H), congestion of blood vessels (CO), (H & E, 40X).

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