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Research article

Protective effect of Silymarin against cyclosporine nephrotoxicity in male rats

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Abstract

To evaluate the protective role of silymarin in ameliorating the nephrotoxicity induced by cyclosporine in male rats, 120 adult male rats were randomly allocated to control and three treated groups (30 per each). Control group male rats were orally supplemented with drinking water, while treatment group male rats were orally supplemented with silymarin (200 mg/kg bw), cyclosporine (5mg/kg/day) and combination of cyclosporine and silymarin. Animals were treated for 30 days and left without treatment for 15 days. Each group were allocated to three subgroups (10 per each), and sacrificed after 15, 30 and 45 days of the experiment. After each treatment period, the relative kidney weights were recorded. Blood samples were obtained for assessment of serum concentrations of creatinine and urea nitrogen. Kidney samples were obtained for histopathological examination. The results of cyclosporine treated group male rats revealed significant increase in kidney weight and the concentrations of serum creatinine and urea nitrogen among experimental groups, at all experimental periods, whereas combination treatment of silymarin and cyclosporine retained them to the control levels. Kidney tissue sections from cyclosporine treated male rats showed obvious atrophy and low cellularity of glomeruli, necrosis of Bowman capsule, dilation of renal convoluted tubules and necrosis of its lining, whereas combination of silymarin and cyclosporine treatment showed normal glomeruli, mild degeneration Bowman capsule and renal convoluted tubules lining. The present changes were duration dependent. In conclusion, silymarin treatment in combination with cyclosporine has nephrotoxic ameliorating effect against nephrotoxicity.

Keywords: Cyclosporine, Creatinine, Nephrotoxicity, Silymarin, Urea

Introduction

The exposure to toxic agents normally results in oxidative stress, which could be compromised the delicate balance between the production of reactive oxygen species and antioxidants levels. The release of reactive oxygen species could exceed the antioxidant-protective mechanisms and lead to oxidative damage such as DNA, protein, or lipid peroxidation (1). This imbalance is usually neutralized by different kinds of enzymatic and non-enzymatic antioxidants to protect the integrity of cells or tissues (2). Cyclosporine is one of the effective immunosuppressive agents (3), which has been used broadly in transplant medicine and has evidently improved implant survival rates in organ transplantation (4). (5) were the first who examined a dose of 25 mg/kg and reported unexpected significant nephrotoxicity that was not reported in the

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animal experiments. Lower dose (17 mg/kg) has been used by the same group showed improved results (6, 7). At present, it is well known that structural renal damage as an important side effect of cyclosporine therapy, which may be dose and duration dependent as well as individual susceptibility (8). Therapeutic application of cyclosporine is found to be accompanied by many side effects could be affect the functions of the liver, kidneys, heart, nervous system and reproductive system (9-14). Due to the antioxidant activity, silymarin isolated from the milk thistle *Silybum marianum* seeds, has been reported to be a potent protective agent against hepatotoxicity and nephrotoxicity induced by many different agents (15-20). The present study aims to evaluate protective potency of silymarin against nephrotoxicity in male rats induced by cyclosporine and to focuse a light on the nephrotoxicity induced by cyclosporine.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study.

Experimental animals:

In the present study, mature male Sprague-Dawley rats were fed on the standard chow and drinking water *ad libitum* throughout the experiment periods. Animals were maintained under room temperature of 22 ± 2°C, 12:12 h light-dark cycle with light on at 06:00 a.m and off at 06:00 p.m throughout the experiment period.

Experimental design:

120 adult male rats were allocated to four equal groups. Control (C) group male rats were orally supplemented with drinking water, silymarin treated group (Sil) were orally supplemented with silymarin (200 mg/kg bw/day), cyclosporine treated group (Cyc) were orally supplemented with cyclosporine (5 mg/kg bw/day) and combination of cyclosporine and silymarine treated group (Sil+Cyc) were orally supplemented with cyclosporine (5mg/kg, bw/day) and silymarin (200 mg/kg, bw/day). Male rats were treated for 30 days and left without treatment for 15 days. Each group were allocated to three subgroups (10 each) and sacrificed after 15, 30 and 45 days. After each treatment period, the relative kidneys weights were recorded. Blood samples were obtained for assessment of serum concentrations of creatinin and urea nitrogen. Kidneys samples were obtained for histopathological examination.

Serum creatinin and urea nitrogen concentrations:

were assessed using special kits, according to the manufacturer instructions (Biolabo Reagents, France).

Histological study:

Histological sections from kidneys were prepared according to Luna (21) and examined under the light microscope.

Statistical Analysis:

All values were expressed as mean ± SD. Comparisons were done using one way analysis of variance (ANOVAI) and Newman- Keuls to test all groups' unpaired values. Differences were considered to be significant at the level of ($p<0.05$). The statistical analysis was carried out using the GraphPad Prism 5 (SAS Institute, Inc., USA).

Results

Kidney weight:

Kidney weight of Cyc and Sil+Cyc group male rats showed significant ($P<0.05$) increase at 15 and 30 days of treatment in comparison with control and Sil group male rats, but it declined to the control level in

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Sil+Cyc group male rats at 45 days treatment period, while still at the elevated level in Cyc group male rats at the same period. In comparison between periods for each group, control and Sil group male rats revealed no significant (P>0.05) differences among treatment periods, whereas Cyc group male rats continued in its increase at 30 and 45 days periods in comparison with 15 days period, while Sil+Cyc group male rats showed significant (P<0.05) decline at 30 and continued in its decline at 45 days periods when compared with 15 days period (Table 1).

Serum creatinin concentration:

In comparison with control group male rats, serum creatinine concentration of Sil group male rats recorded significant (p<0.05) decline among experimental groups at all treatment periods, and Cyc group male rats recorded significant (p<0.05) elevation among experimental groups at all treatment periods, whereas Sil+Cyc group male rats showed no significant (p>0.05) difference compared with control male rats but their levels were significantly (p<0.05) higher than Sil group male rats and significantly (p<0.05) lower than Sil+Cyc group male rats at all treatment periods. In comparison between periods for each group, control and Sil group male rats recorded no significant (p>0.05) differences between periods, while Cyc group male rats showed significant (P<0.05) increase at 30 days period in comparison with 15 days period but the level decreased significantly (p<0.05) at 45 days periods (Table 1).

Serum urea nitrogen concentration:

The results illustrated in table (1) recorded no significant (p>0.05) differences of serum urea nitrogen concentration between control, Sil and Sil+Cyc groups male rats, whereas Cyc group male rats showed significant (p<0.05) elevation at 15 and 30 days treatment periods and no significant (P>0.05) changes at 45 days treatment period in comparison with other experimental groups. In comparison between periods for each group, control, Sil and Sil+Cyc groups male rats showed no significant (P>0.05) difference between all treatment periods, whereas Cyc group male rats recorded significant (p<0.05) increase at 30 days period compared with 15 days period, and significantly (P<0.05) decreased at 45 days period.

Histopathological changes of kidney:

Figure (1) shows kidney sections obtained from experimental male rat groups after 15 days of treatment. Control group male rat sections (figure 1-C) showed normal glomeruli and renal convoluted tubules. Sil group male rat sections (figure 1-Sil) showed increased cellularity of glomeruli and normal renal convoluted tubules. Cyc group male rats sections (figure 1-Cyc) showed low cellularity of glomeruli, necrosis of Bowman capsule, dilation of renal convoluted tubules, and necrosis of its lining, whereas Sil+Cyc group male rats sections (figure 1-Sil+Cyc) showed normal glomeruli and mild dilation of renal convoluted tubules. At 30 and 45 day periods of treatment, kidney sections of the control group male rats (figures 2-C and 3-C) showed normal glomeruli and renal convoluted tubules. Sil group male rats sections (figures 2-Sil and 3-Sil) showed increased cellularity of glomeruli and normal renal convoluted tubules. Cyc group male rat sections (figures 2-Cyc and 3-Cyc) showed obvious atrophy of glomeruli, necrosis of Bowman capsule lining, cystic dilation of renal convoluted tubules and necrosis of their lining, whereas Sil+Cyc group male rats sections (figures 2-Sil+Cyc and 3-Sil+Cyc) revealed normal glomeruli, mild degeneration of the lining of Bowman capsule and renal convoluted tubules.

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Table (1): effect of silymarin on relative kidney weight and kidney function tests in cyclosporine treated male rats

Parameters		Periods	Groups							
			C		Sil		Cyc		Sil+Cyl	
Kidneys weight (g/100g bw)		15 d	1.56 ± 0.11	Ba	1.64 ± 0.12	Ba	2.05 ± 0.09	Aa	2.03 ± 0.14	Aa
		30 d	1.62 ± 0.10	Ca	1.66 ± 0.10	Ca	2.05 ± 0.13	Aa	1.86 ± 0.10	Bb
		45 d	1.58 ± 0.12	Ba	1.59 ± 0.12	Ba	1.97 ± 0.14	Aa	1.67 ± 0.12	Bc
Kidney function test	Creatinin conc. (mg/dL)	15 d	0.66 ± 0.05	Ba	0.58 ± 0.05	Ca	0.91 ± 0.06 Ab		0.68 ± 0.06	Ba
		30 d	0.63 ± 0.05	Ba	0.56 ± 0.05	Ca	1.01 ± 0.06	Aa	0.67 ± 0.05	Ba
		45 d	0.62 ± 0.04	Ba	0.55 ± 0.05	Ca	0.80 ± 0.05	Ac	0.65 ± 0.05	Ba
	Urea nitrogen conc. (mg/dL)	15 d	21.8 ± 1.71	Ba	22.0 ± 1.60	Ba	27.0 ± 1.70 Ab		21.7 ± 1.60	Ba
		30 d	22.1 ± 1.50	Ba	22.3 ± 1.30	Ba	29.0 ± 1.50	Aa	22.0 ± 1.50	Ba
		45 d	21.5 ± 1.60	Aa	22.6 ± 1.60	Aa	23.0 ± 1.40	Ac	22.0 ± 1.50	Aa

All male rats were treated for 15 and 30 days and were left untreated for up to 45 days.
C group: drenched with drinking water. Sil group: drenched with silymarin (200 mg/kg bw), suspended in 0.5 ml of drinking water. Cyc group: drenched with cyclosporine (5 mg/kg/day). Sil+Cyc group: drenched with cyclosporine (5 mg/kg/day) and silymarin (200 mg/kg bw).
Data were presented as Mean ±SD of 10 observations (n=10). Different capital letters denote significant difference ($p<0.05$) between groups for each period. Different small letters denote significant difference ($p<0.05$) between periods for each group.

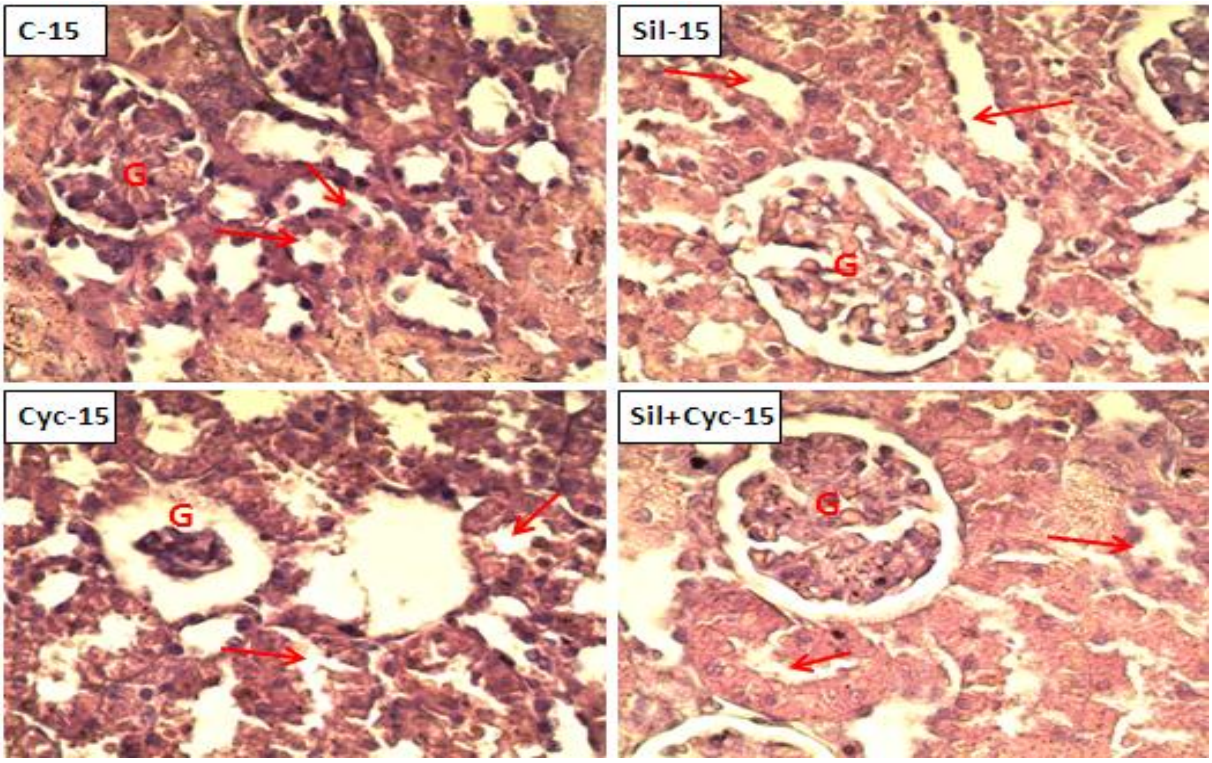

Figure (1): Kidney sections obtained from experimental male rat groups after 15 days of treatment. Control male rat section (C-15) shows normal glomeruli and renal convoluted tubules. Silymarin treated male rat section (Sil-15) shows increased cellularity of glomeruli and normal renal convoluted tubules. Cyclosporine treated male rat section (Cyc-15) shows low cellularity of glomeruli, atrophy of Bowman capsule, dilation of renal convoluted tubules and degeneration of their lining. Combination of silymarin and cyclosporine treated male rat section (Sil+Cyc-15) shows normal glomeruli (G), mild dilation of renal convoluted tubules (arrows). H&E (400x).

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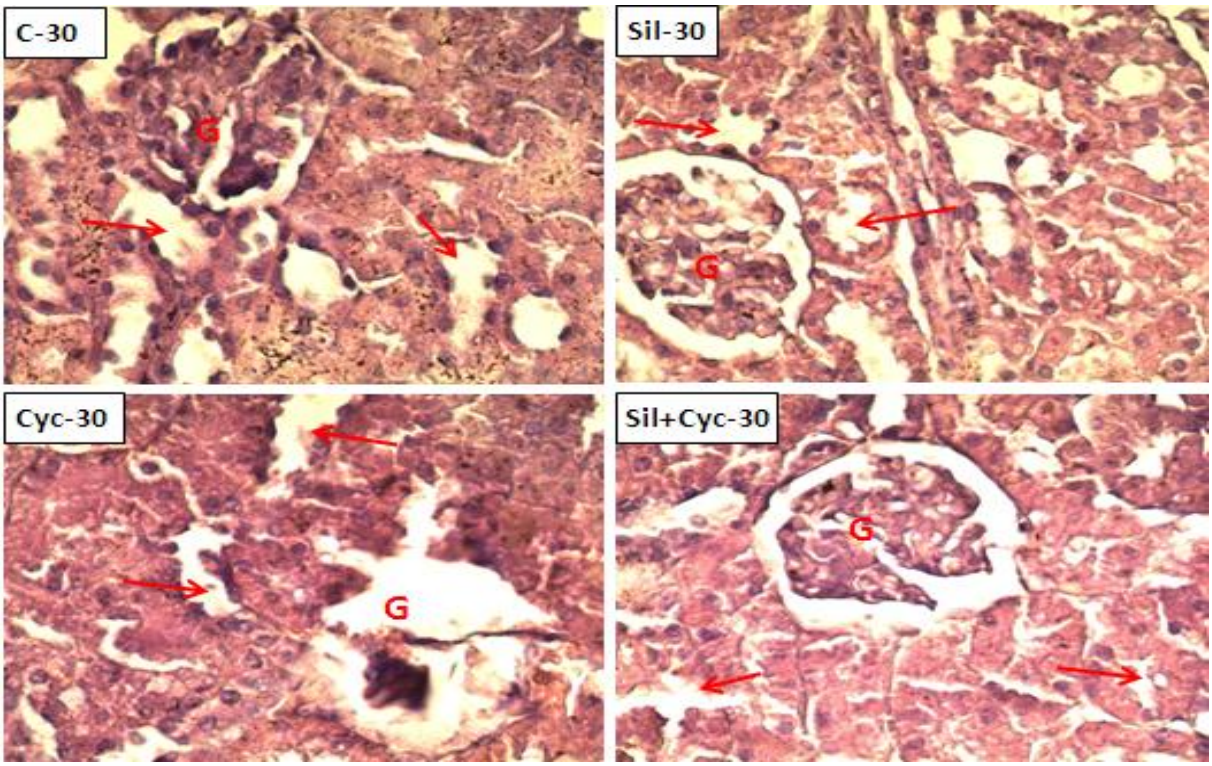

Figure (2): Kidney sections obtained from experimental male rat groups after 30 days of treatment. Control male rat section (C-30) shows normal glomeruli and renal convoluted tubules. Silymarin treated male rat section (Sil-30) shows increased cellularity of glomeruli and normal renal convoluted tubules. Cyclosporine treated male rat section (Cyc-30) shows obvious atrophy of glomeruli (G), cystic dilation of renal convoluted tubules (arrows) and sever degeneration of their lining. Combination of silymarin and cyclosporine treated male rat section (Sil+Cyc-30) shows normal glomeruli (G), mild degeneration of the lining of Bowman capsule and renal convoluted tubules (arrows). H & E (400x).

Discussion

The present findings revealed significant decline in serum creatinine and urea nitrogen in both Sil and Sil+Cyl groups male rats than cyclosporine treated (Cyl.) group male rats. This decline could be attributed to the potency of silymarin in the treatment of nephropathy, as it has been mentioned that silymarin has an efficient effect in reducing nitrogen uria in type 2 diabetes patients with nephropathy, which could be attributed to the antioxidant and antiinflammatory roles of silymarin (22, 23). Although animal studies have not reported cyclosporine nephrotoxicity (24, 25), various side effects have been shown firstly in humans studies after renal transplantation (5, 6), which have been attributed to the functional changes of kidneys and was therefore can be reversed (26, 27). From the present results, it can be suggested that silymarin application can reverse the acute but not chronic renal damage, as it has been mentioned that chronic cyclosporine nephrotoxicity could be associated with irreversible renal histologic damage, including arterioles, glomeruli, and renal tubules (28), where nephrotoxicity is mostly caused by oxidative stress and the kidney protective activity of silymarin was reported in diabetic nephropathy (29). There are evidence proposes that silymarin has beneficial effects in diabetic nephropathy (20, 30, 32), where it concentrates in kidney cells and helps in regeneration of renal tissues by elevating protein and nucleic acid biosynthesis.The present histopathological changes were in agreement with previous studies, as it has been mentioned that acute cyclosporine nephrotoxicity was accompanied by vacuolization of the tubular cytoplasm (33, 34). These changes could be due to the ischemia caused by vasoconstriction of glomerular afferent

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arterioles, on the other hand, a direct effect of cyclosporine on tubular epithelial cells could be also exist (35). There is no study that can be compared with the present study, but our results were in agreement with that reported by Dabak and Kocaman (36) who studied the protective role of silymarin against methotrexate sodium nephrotoxicity in rats, where their results revealed a significant drop in renal damage in silymarin treated group. This protective role of silymarin could be attributable to its anti-inflammatory, antioxidant, free radical scavenging, and immunomodulatory properties (37), as well as its cytoprotective effects through inhibition of apoptosis (36).

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