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Original article

Comparative evaluation of single and combined efficacy of dipyridamole, ketotifen and quercetin on cyclosporine induced hepatorenal toxicity

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Abstract

Cyclosporine is an immunosuppressive drug that is used to prevent tissue rejection in organ transplants and to treat autoimmune diseases such as psoriasis and rheumatoid arthritis. It has important toxic effects in many organs such as the liver and kidney. The aim of this study was to determine and compare the effectiveness of the single and combined treatment of dipyridamole, which is a vasodilator and has an antioxidant effect, ketotifen which is toll-like receptor-4 inhibitory and has an antioxidant effect, quercetin which is an antioxidant and has an anti-inflammatory effect in cyclosporine-induced hepatorenal toxicity. Forty-eight Wistar Albino rats were divided into 7 groups. The research period was 21 days. The cyclosporine increased serum ALT and AST levels, in contrast to their increased levels prevented by all the treatments. The serum creatinine level decreased significantly with ketotifen and combined treatment, while cyclosporine partially increased serum creatinine and urea levels. The urine microalbumin and protein levels were increased significantly by cyclosporine, whereas they decreased with dipyridamole treatment. The protein levels decreased by quercetin and combined treatments. The kidney injury molecule-1 and retinol-binding protein levels were increased by the cyclosporine, while ketotifen treatment partially decreased them. In conclusion, ketotifen and dipyridamole can prevent cyclosporine-induced hepatorenal toxicity and quercetin can increase the effectiveness of this treatment.

Keywords: hepatorenal toxicity, cyclosporine, ketotifen, dipyridamole, quercetin

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Introduction

Cyclosporine (CS) is a calcineurin inhibitor drug used in organ transplantations, first used in 1980 (Burdmann et al. 2003). CS prevents acute tissue rejection after organ transplantations. In addition, it is used in the treatment of autoimmune diseases such as psoriasis and rheumatoid arthritis (Burdmann et al. 2003, Wu et al. 2018). It regulates many cytokines and the transcription of the cytosolic activating nuclear factor of T lymphocytes (Burdmann et al. 2003).

CS has some hepatorenal side effects (Vangaveti et al. 2021). It inhibits nitric oxide synthesis, dilatation of renal arteries and causes oxidative stress and autophagy (Wu et al. 2018, Vangaveti et al. 2021). The direct effect of CS leads to tubular cell apoptosis, DNA damage and an increased intracellular calcium level in renal tubular epithelial cells (Wu et al. 2018). Acute CS nephrotoxicity is a functional and reversible disorder due to renal imbalance of endogenous vasoconstrictors and vasodilators. The main cause of the nephrotoxicity is intense renal vasoconstriction, increased renal vascular resistance and decreased renal blood flow. Experimental or clinical changes are minimal in the initial stage of nephrotoxicity, even if renal dysfunction is evident (Burdmann et al. 2003).

Oxidative stress is among the main causes of CS-induced hepatorenal toxicity. It induces endoplasmic reticulum stress and mitochondrial reactive oxygen species production and causes lipid peroxidation. In addition, CS induces prostaglandin E2 (PGE2), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in hepatotoxicity, while it induces endothelin, thromboxane, and tumor necrosis factor-α (TNF-α) in nephrotoxicity. In addition, the levels of PGE2, iNOS and COX-2 in the kidney decrease due to vasoconstriction (Vangaveti et al. 2021). However, the variation in the levels of some of these parameters is contradictory in research (Josephine et al. 2007).

Ketotifen is an effective mast cell stabilizer drug (Abdelzaher et al. 2020). TLR4 is reported to be a potential drug target because its activation causes impaired kidney microcirculation (Carlos et al. 2014, Wu et al. 2018). In silico research, ketotifen has modaretly inhibited the toll-like receptor (TLR)-4 receptor (Hutchinson et al. 2010). Antioxidant treatment can contribute to the prevention of CS-induced renal and hepatic damage (Wu et al. 2018). In a study, oral ketotifen at a dose of 10 mg/kg regressed hepatotoxicity with its antioxidant, anti-inflammatory and anti-apoptotic effects in the cyclophosphamide-induced hepatotoxicity model (Abdelzaher et al. 2020). Induction of mast cells is one of the main causes of inflammation in renal vessels in experimental nephropathies. Ketotifen (1 and 10 mg/kg, orally) can prevent nephrotoxicity by increasing mast cell resistance and antioxidant and anti-inflammatory effects (Refaie et al. 2017).

Quercetin, which is abundant in vegetables and fruits, is a natural flavonoid. It has antioxidant and anti-inflammatory effects and has therapeutic potential in the prevention and treatment of various chronic diseases (Lesjak et al. 2018). Quercetin treatment has increased antioxidant enzymes, antioxidant capacity and prevented oxidative stress, renal ischemia, and mitochondrial damage in experimental cadmium-induced nephrotoxicity in rats (Morales et al. 2006). It has prevented renal endothelial and glomerular deterioration and oxidative damage by preventing renal mononuclear cell infiltration and inflammation in gentamicin-induced nephrotoxicity (Abdel-Raheem et al. 2009). Quercetin (15 mg/kg) has reduced free radicals, fatty liver and serum lipid levels in paracetamol-induced hepatorenal toxicity (El-Shafey et al. 2015).

Dipyridamole is an antiplatelet drug, and it has anti-inflammatory, antioxidant and vasodilator properties. Dipyridamole ensures the vitality of endothelial cells by reducing the formation of free oxygen radicals and inflammation (Chakrabarti et al. 2005, Weyrich et al. 2005). Oral administration of dipyridamole, at a dose of 20 mg/kg, has decreased inflammatory mediators, increased anti-inflammatory mediators such as interleukin (IL)-10, and provided protection against nephrotoxicity in the early period of gentamicin-induced nephrotoxicity in rats (Balakumar et al. 2017).

CS causes side effects via vasculopathy, tubulointerstitial fibrosis, increased oxidative stress and decreased antioxidant capacity. Thus, it creates hepatorenal toxicity. The research was aimed at determining hepatorenal toxicity preventive efficacy by dipyridamole, which has vasodilator, antioxidant, and anti-inflammatory effects; ketotifen which has antioxidant and anti-inflammatory effects, and quercetin which has antioxidant and antifibrotic effects. An additional aim was to compare the efficacy of these treatments for hepatorenal toxicity.

Materials and Methods

Preparation of chemical agents

CS solution (Sandimmun 50 mg/ml Concentrated Infusion Solution, Novartis, Switzerland) was purchased commercially. Ketotifen (Cat No: K0048), quercetin hydrate (Cat No: P0042) and dipyridamole (Cat No: D2274) were obtained in powder form from the Tokyo Chemical Industry with 98% purity and

HPLC standards, and dissolved with 0.9% saline, sunflower oil and 0.5% hydroxyethyl cellulose, respectively.

Animals and experimental design

In the study, 48 Wistar Albino, 8-12 weeks old (~250 gr), female rats were used. The rats were included in the study after performing health checks and they were housed in the Selcuk University Experimental Medicine Research and Application Center. The research protocol was approved by the Ethics Committee of Selcuk University Animal Experiments (Approval number: 2021-22, Date:25.03.2021). Feed and water needs were provided ad libitum. The rats were fed with standard rat chow, and they were kept under control (12/12 hours light/dark – 7:30/19.30, 22±2°C, 55±5% humidity). The experimental period was determined as 21 days and the rats were grouped as follows.

Group 1 [Healthy (control, n:6)]: Physiological saline (1 ml/rat/day, oral) was administered simultaneously with the other groups.

Group 2 [Cyclosporine (n:7)]: CS (25 mg/kg/day, oral) was administered to induce a hepatorenal toxicity model for 21 days (Vangaveti et al. 2021).

Group 3 [Cyclosporine + sham (n:7)]): CS (25 mg/kg/day, oral), 0.5% hydroxyethyl cellulose (0.5 ml/kg/day, oral), sunflower oil (0.5 ml/kg/day, oral) and 0.9% saline (0.1 ml/rat/day, oral) were administered.

Group 4 [Cyclosporine + ketotifen (n:7)]: CS (25 mg/kg/day, oral) and ketotifen (10 mg/kg/day, oral) were administered.

Group 5 [Cyclosporine + quercetin (n:7)]: CS (25 mg/kg/day, oral) and quercetin (50 mg/kg, oral) were administered.

Group 6 [Cyclosporine + dipyridamole (n:7)]: CS (25 mg/kg/day, oral) and dipyridamole (30 mg/kg, oral) were administered.

Group 7 [Cyclosporine + combined (n:7)]: CS (25 mg/kg/day, oral), ketotifen (10 mg/kg/day, oral), quercetin (50 mg/kg/day, oral), and dipyridamole (30 mg/kg/day, oral) were administered.

All oral treatments were administered using the gastric gavage method for 21 days.

The rats were placed in metabolic cages for 21th days and urine samples were collected via metabolic cage tubes 24 hours after the last treatment. Later, blood samples were collected from the heart under ketamine (Ketasol %10, Richter Pharma, Austria)/xylazine (Xylazinbio %2, Bioveta, Czech Republic) anesthesia (90/10 mg/kg, ip.) and euthanized by decapitation. The liver and kidney tissues were removed and stored at -80°C until analysis. The liver and kidney tissues were homogenized in phosphate buffer saline (PBS) 50 mM pH (7.4) using a tissue homogenizer (Heidolph, Silent Crusher M, Germany). The tissue homogenate was centrifuged at 5,000 rpm, for 10 minutes in a cold centrifuge, and the resultant supernatant was used.

Biochemical and oxidative status analysis

The blood samples, placed into serum-separating tubes, were centrifuged at 1600xg and the sera were separated. The serum and urine samples were analyzed for biochemical parameters (serum albumin, ALT, AST, urea, creatinine, total protein, urinary protein, creatinine) using an autoanalyzer (Abbott c8000, Chicago, USA). The hematology parameters (WBC, RBC, hemoglobin, hematocrit) were measured using a hematology analyzer (Mindray Bio-Medical Electronics, Shenzhen, China). In addition, urine microalbumin, kidney injury molecule-1 (Rat KIM-1, Cat No: E0549Ra, Bioassay Technology Laboratory, Shanghai, China), retinol-binding protein (Rat RBP, Cat No: E0555Ra, Bioassay Technology Laboratory, Shangai, China) levels were determined, following the manufacturer's protocol, using an ELISA reader (Bio-Tek Instruments Inc., MWGt Lambda Scan 200). While tumor necrosis factor (Rat TNF-α, Cat No: E0764Ra, Bioassay Technology Laboratory, Shanghai, China), transforming growth factor (Rat TGF-β1, Cat No: E0778Ra, Bioassay Technology Laboratory, Shangai, China) tissue inhibitor of metalloproteinase-1 (Rat TIMP-1, Cat No: E0322Ra), Bioassay Technology Laboratory, Shanghai, China), thiobarbituric acid reactive substances (Rat TBARS, Cat No: E1369Ra, Bioassay Technology Laboratory, Shanghai, China) and glutathione (Rat GSH, Cat No: EA0113Ra, Bioassay Technology Laboratory, Shangai, China) levels were analyzed in liver tissue, TNF-α (Cat No: E0764Ra, Bioassay Technology Laboratory, Shanghai, China), TBARS (Cat No: E1369Ra, Bioassay Technology Laboratory, Shanghai, China) and GSH (Cat No: EA0113Ra, Bioassay Technology Laboratory, Shangai, China) levels were determined using ELISA in kidney tissue.

Statistical analysis

The data were analyzed using SPSS 25.0 (SPSS, Inc., Chicago, IL, USA) software, and hematological and biochemical values were evaluated as mean ± standard error of the mean (SEM). The data were statistically analyzed using one-way ANOVA followed by a posthoc Duncan test. $p<0.05$ was statistically significant.

Table 1. Effects of ketotifen (10 mg/kg, oral), quercetin (50 mg/kg, oral), and dipyridamole (30 mg/kg, oral) treatments on kidney tissue in cyclosporine-induced hepatorenal failure rats (mean±SEM).

Groups/Parameters	TNF-α (ng/L)	TBARS (mmol/mL)	GSH (mg/L)
Control	1.38±0.04^{ab}	18.20±0.84^{a}	2.82±0.07^{b}
Cyclosporine	1.75±0.14^{a}	21.33±1.49^{a}	2.68±0.04^{b}
Cyclosporine+Sham	1.67±0.03^{ab}	18.78±1.31^{a}	2.77±0.03^{b}
Cyclosporine+Ketotifen	1.31±0.24^{b}	20.14±1.39^{a}	3.12±0.09^{a}
Cyclosporine+Quercetin	1.44±0.06^{ab}	21.80±0.59^{a}	2.92±0.10^{ab}
Cyclosporine+Dipyridamole	1.46±0.08^{ab}	22.37±1.84^{a}	2.68±0.05^{b}
Cyclosporine+Combined	1.62±0.05^{ab}	19.37±1.15^{a}	2.70±0.01^{b}

a, b, c – Different letters in the same column are statistically significant ($p<0.05$).
TNF-α – Tumor necrosis factor-α, TBARS – Thiobarbituric acid reactive substances, GSH – Glutathione.

Table 2. Effects of ketotifen (10 mg/kg, oral), quercetin (50 mg/kg, oral), and dipyridamole (30 mg/kg, oral) treatments on liver tissue in cyclosporine-induced hepatorenal failure rats (mean±SEM).

Groups/Parameters	TNF-α (ng/L)	TIMP (ng/mL)	TGF-β (ng/L)	TBARS (mmol/mL)	GSH (mg/L)
Control	1.40±0.01^{bc}	4.52±0.19^{ab}	360.81±10.26^{ab}	18.02±1.47^{bc}	3.31±0.13^{a}
Cyclosporine	1.64±0.14^{ab}	4.85±0.24^{a}	391.23±8.40^{a}	21.49±1.02^{a}	2.95±0.16^{b}
Cyclosporine+Sham	1.69±0.13^{a}	4.82±0.34^{a}	394.71±25.43^{a}	20.69±0.76^{ab}	2.78±0.16^{b}
Cyclosporine+Ketotifen	1.40±0.04^{bc}	4.12±0.11^{b}	383.20±10.53^{a}	14.23±0.87^{d}	2.91±0.02^{b}
Cyclosporine+Quercetin	1.19±0.05^{c}	4.27±0.12^{ab}	179.91±37.19^{d}	17.48±0.51^{c}	2.94±0.08^{b}
Cyclosporine+Dipyridamole	1.25±0.07^{c}	4.13±0.22^{b}	282.56±33.91^{c}	17.91±0.85^{bc}	2.80±0.05^{b}
Cyclosporine+Combined	1.24±0.07^{c}	4.11±0.05^{b}	290.53±14.20^{bc}	17.86±0.87^{bc}	2.82±0.04^{b}

a, b, c, d – different letters in the same column are statistically significant ($p<0.05$).
TNF-α – Tumor necrosis factor-α, TIMP – Tissue inhibitor of metalloprotease-1, TGF-β – Transforming growth factor, TBARS – Thiobarbituric acid reactive substances, GSH – Glutathione.

Results

Changes in the levels of some biomarkers in the kidney tissue after ketotifen, dipyridamole, quercetin and combined treatment in rats with hepatorenal failure are presented in Table 1. The renal TNF-α level increased partially in the CS group compared to the control group. Ketotifen decreased the TNF-α level and increased the GSH level statistically compared to the CS group ($p<0.05$).

Changes in the levels of some biomarkers in the liver tissue after ketotifen, dipyridamole, quercetin and combined treatment in rats with hepatorenal failure are presented in Table 2. The liver TNF-α, TIMP, and TGF-β levels were partially increased in the CS group compared to the control group, while the liver GSH level was significantly decreased and the TBARS levels were significantly increased in the CS group compared to the control group ($p<0.05$). The TIMP and TBARS levels by ketotifen, TNF-α, TGF-β and TBARS levels by quercetin, and TNF-α, TIMP, TGF-β and TBARS levels by dipyridamole and combined treatments were statistically decreased compared to the CS group in the liver tissue ($p<0.05$).

Changes in the levels of some biomarkers in the urine are presented in Table 3. The KIM-1, RBP, microalbumin, and protein levels in the urine in the CS group were statistically increased compared to the control group ($p<0.05$). The KIM-1, RBP, microalbumin, and urinary protein levels were partially decreased by ketotifen treatment compared to the CS group. Microalbumin and urinary protein levels were statistically decreased by dipyridamole treatment compared to the CS group ($p<0.05$). The urinary protein levels were statistically decreased in the quercetin and combined groups compared to the CS group ($p<0.05$).

Both liver and kidney functions were evaluated by measuring the levels of biochemical markers in the serum and are presented in Table 4. The AST and ALT levels were statistically increased, while the ALB and TP levels were statistically decreased by CS administration compared to the control group ($p<0.05$). All treatment groups statistically prevented the increase of ALT and AST levels. Decreased ALB level was prevented

Table 3. Effects of ketotifen (10 mg/kg, oral), quercetin (50 mg/kg, orally), and dipyridamole (30 mg/kg, oral) treatments in urine biomarkers in cyclosporine-induced hepatorenal failure rats (mean±SEM).

Groups/Parameters	KIM-1 (ng/mL)	RBP (ng/mL)	µALB	Protein (mg/dL)
Control	1.88±0.31 ^{b}	243.02±47.83 ^{b}	14.00±3.27 ^{bc}	512.6±127.1 ^{bc}
Cyclosporine	2.90±0.30 ^{a}	485.91±47.51 ^{a}	28.80±3.64 ^{a}	773.3±49.25 ^{a}
Cyclosporine+Sham	2.67±0.44 ^{ab}	420.83±64.45 ^{a}	25.16±2.62 ^{ab}	584.0±87.00 ^{ab}
Cyclosporine+Ketotifen	2.09±0.08 ^{ab}	376.69±28.41 ^{ab}	25.00±1.73 ^{ab}	554.4±73.86 ^{abc}
Cyclosporine+Quercetin	2.24±0.23 ^{ab}	431.57±30.06 ^{a}	21.50±6.98 ^{abc}	426.1±63.51 ^{bc}
Cyclosporine+Dipyridamole	2.43±0.37 ^{ab}	458.07±71.50 ^{a}	9.42±2.52 ^{c}	289.1±63.51 ^{c}
Cyclosporine+Combined	2.40±0.25 ^{ab}	405.43±47.54 ^{a}	21.67±5.35 ^{abc}	368.1±37.05 ^{bc}

a, b, c – different letters in the same column are statistically significant ($p<0.05$).
KIM-1 – Kidney injury molecule-1, RBP – Retinol binding protein, µALB – Microalbumin.

Table 4. Effects of ketotifen (10 mg/kg, oral), quercetin (50 mg/kg, orally), and dipyridamole (30 mg/kg, oral) treatments on biochemical parameters in cyclosporine-induced hepatorenal failure rats (mean±SEM).

Groups/Parameter	AST (U/L)	ALT (U/L)	ALB (g/L)	CREA (mg/dL)	UREA (mg/dL)	TP (g/dL)
Control	93.00±5.74 ^{b}	39.71±2.45 ^{b}	3.28±0.08 ^{a}	0.56±0.01 ^{ab}	28.14±1.42 ^{b}	6.64±0.12 ^{a}
Cyclosporine	142.57±11.9 ^{a}	110.14±10.1 ^{a}	1.91±0.36 ^{bc}	0.60±0.06 ^{a}	39.60±4.70 ^{ab}	4.07±0.72 ^{b}
Cyclosporine+Sham	116.20±17.0 ^{ab}	92.00±17.2 ^{a}	1.79±0.26 ^{c}	0.56±0.04 ^{ab}	45.29±6.89 ^{a}	3.97±0.65 ^{b}
Cyclosporine+Ketotifen	105.80±16.2 ^{b}	53.17±11.0 ^{b}	2.87±0.36 ^{a}	0.43±0.03 ^{bc}	33.50±1.85 ^{ab}	5.78±0.81 ^{ab}
Cyclosporine+Quercetin	83.67±5.10 ^{b}	33.40±4.25 ^{b}	2.76±0.13 ^{a}	0.50±0.02 ^{abc}	32.40±2.48 ^{ab}	5.82±0.23 ^{ab}
Cyclosporine+Dipyridamole	86.00±11.4 ^{b}	39.29±5.75 ^{b}	2.70±0.31 ^{ab}	0.46±0.05 ^{abc}	36.60±3.41 ^{ab}	5.53±0.63 ^{ab}
Cyclosporine+Combined	48.75±10.0 ^{c}	35.44±3.60 ^{b}	2.97 ±0.31 ^{a}	0.35±0.06 ^{c}	29.33±4.23 ^{b}	5.95±0.63 ^{a}

a, b, c – different letters in the same column are statistically significant ($p<0.05$).
AST – Aspartate aminotransferase, ALT – Alanine aminotransferase, ALB – Albumin, CREA – Creatinine, TP – Total protein.

Table 5. Effects of ketotifen (10 mg/kg, oral), quercetin (50 mg/kg, orally), and dipyridamole (30 mg/kg, oral) treatments on hematological parameters in cyclosporine-induced hepatorenal failure rats (mean±SEM).

Groups/ Hematological Parameters	RBC ($x10^{12}$/L)	HGB (g/dL)	HCT (%)
Control	7.99±0.24 ^{a}	13.78±0.52 ^{a}	41.64±1.52 ^{a}
Cyclosporine	5.84±0.60 ^{bc}	9.52±1.18 ^{bc}	29.10±3.59 ^{b}
Cyclosporine+Sham	6.46±0.29 ^{ab}	10.50±0.48 ^{ab}	26.28±4.00 ^{b}
Cyclosporine+Ketotifen	4.73±1.03 ^{c}	6.93±1.96 ^{c}	30.60±3.72 ^{b}
Cyclosporine+Quercetin	6.74±0.4 ^{ab}	11.57±1.02 ^{ab}	30.56±4.81 ^{b}
Cyclosporine+Dipyridamole	6.11±0.74 ^{bc}	10.70±1.55 ^{ab}	32.71±4.03 ^{ab}
Cyclosporine+Combined	6.88±0.32 ^{ab}	11.58±0.65 ^{ab}	35.17±1.81 ^{ab}

a, b, c – different letters in the same column are statistically significant ($p<0.05$).
RBC – Red blood cell, HGB – Hemoglobin, HCT – Hematocrit.

by ketotifen, quercetin and combined treatment groups ($p<0.05$). While the decrease in TP level was statistically significantly inhibited only in the combined group, it was partially prevented in the other treatment groups. The creatinine level decreased significantly in the ketotifen and combined treatment groups, compared to the CS group.

The changes in hematological parameters are presented in Table 5. The RBC, HGB and HCT levels were statistically decreased in the CS group compared to the control group ($p<0.05$). RBC, HGB levels were partially increased in the quercetin, dipyridamole and combined treatment groups compared to the CS group. However, the HCT level was partially increased in the dipyridamole and combined treatment groups compared to the CS group.

Discussion

The hepatorenal toxic/side effects of CS limits its clinical use, although CS is an effective immunosuppressive drug (Wei et al. 2021). The increased microalbuminuria, KIM-1, and TNF-α levels can be potential biomarkers in CS-induced nephrotoxicity (Wu et al. 2018).

Ketotifen treatment (10 mg/kg) has anti-inflammatory effects by decreasing TNF-α expression, and antioxidant effects by increasing GSH and SOD levels in nephrotoxicity in rats. In addition, it has prevented nephrotoxicity by partially reducing serum urea and creatinine levels (Refaie et al. 2017). In another study, ketotifen has stabilized mast cells and reduced oxidative stress and inflammation, and has prevented the development of nephrotoxicity in rats (Kaur et al. 2016). Ketotifen has increased the level of P glycoprotein and reduced the accumulation of cyclosporine in the liver; it has scavenged oxidative radicals and decreased the level of inflammatory cytokines such as IL-1β and TNF. In addition, ketotifen has reduced ALT and AST levels with these positive effects and prevented cyclosporine-caused hepatotoxicity (Abdelzaher et al. 2020).

The antioxidant and anti-inflammatory effects of the metabolites and glycosides of quercetin are significantly different. Its antioxidant potential is directly proportional to the number of free hydroxyl groups; however, the hydroxyl groups are not important in anti-inflammatory activity (Lesjak et al. 2018). Quercetin prevents hepatotoxicity by protecting the integrity of the cell membrane (Aydın 2011). In addition, it has prevented CS induced hepatotoxicity by increasing CAT and GPx levels, while it did not change the liver SOD level in rats (Mostafavi-Pour et al. 2008). Quercetin has prevented free oxygen radical formation in cell lipids and nucleic acids in nephrotoxicity and reduced plasma creatinine and MDA levels (Behling et al. 2006, Yuksel et al. 2017).

Dipyridamole has decreased AST and MDA levels in hepatotoxicity by providing vasodilation of the hepatic arteries and scavenging oxidative radicals (Mokbel 2000). It has decreased serum urea, creatinine levels and tubular necrosis in gentamicin-induced nephrotoxicity in rats; however, it has not caused change in the infiltration of inflammatory cells (Balakumar et al. 2017). Moreover, the low doses of dipyridamole treatment have prevented nephrotoxicity by reducing microalbuminuria, while it has not changed the serum creatinine level (Aizawa et al. 1990).

CS-induced nephrotoxicity is dependent on time and dose (Sereno et al. 2015). CS causes renal dysfunction in the early period, while it causes macrophage infiltration and tubulointerstitial fibrosis in the late period (Carlos et al. 2014). CS induces oxidative stress and lipid peroxidation. The urinary microalbuminuria, TNF-α, and KIM-1 levels increase in the early phase (within 7 days) of CS-induced nephrotoxicity. However, the KIM-1 level is lower in the late phase than in the early phase (Carlos et al. 2014, Wu et al. 2018). In another study, the expression of KIM-1 increased in kidney damage in the 3rd week and was not different from the control group due to renal regeneration in the following weeks in the nephrotoxicity of CS (Sereno et al. 2015). The level of KIM-1 increases in the urine with renal proximal tubular damage (Perez-Rojas et al. 2007).

RBP is an important marker of tubular damage in nephrotoxicity and urinary excretion of RBP indicates impaired glomerular filtration and reabsorption (Andreucci et al. 2017). Glomerular filtration is damaged and RBP level increases when CS is used for a long time (Chinen et al. 2006). The vasoconstrictive effect of CS causes ischemic damage in the proximal tubules, and RBP reabsorption is limited and urinary excretion of RBP increases (Marchewka et al. 2007).

In the present research, CS may have caused a renal toxicity/dysfunction that proceeded from acute to chronic. Although CS treatment increased microalbumin and protein levels in the urine, the serum creatinine and urea levels did not significantly increase. This may be because nephrotoxicity is in the early stages. In addition, CS increased the levels of KIM-1 and RBP, but the level of KIM-1 and RBP may tend to descend from the maximum. The effect of treatments on nephrotoxic markers may have not clearly been seen because renal regeneration may have started.

All treatments (ketotifen, dipyridamole, quercetin and combined) inhibited the urinary excretion of microalbumin and protein. Dipyridamole treatment has a stronger effect on these parameters due to its powerful antioxidant and vasodilator effects. Ketotifen caused partial improvements in the urinary protein and RBP levels, and significantly decreased the creatinine level via the antioxidant and anti-inflammatory effects. Quercetin has significantly reduced only the urinary protein level. Although quercetin metabolites have been shown to have antioxidant and anti-inflammatory effects in many studies, this activity had a limited effect on CS-induced liver toxicity in this study. Quercetin may not have been adequately converted to its active metabolites due to liver toxicity. Therefore, it may not have shown a sufficient antioxidant effect.

CS did not change the kidney TNF-α level in male rats while its level decreased in female rats. The difference was explained as CS showed anti-inflammatory effects by increasing the estrogen level (El-Bassossy

and Eid 2018). In addition, CS increases TNF-α levels in the early phase of nephrotoxicity (Carlos et al. 2014). In another study, TNF-α expression did not increase due to the weak stimulation of renal inflammation by CS (Lloberas et al. 2008). Moreover, TNF-α has been reported to be an important cytokine for liver regeneration, because TNF-α level increased to ensure liver regeneration by CS (Daoudaki et al. 2003). In the present study, renal and hepatic TNF-α levels were partially increased by CS. The rats used in the study were female and the analyses were performed in the middle phase of nephrotoxicity. Therefore, the renal TNF-α level may have partially increased. In addition, in vivo regeneration may have limited increased hepatic TNF-α levels in CS-induced liver damage. Ketotifen have significantly prevented in the kidneys and partially prevented in the liver the increase of TNF-α via moderate TLR4 inhibition. Dipyridamole, quercetin and combined treatments prevented inflammation via antioxidant and anti-inflammatory effects in the liver.

CS increases liver TGF-β and TIMP expression by inducing the TGF-β/Smad signaling pathway (Balah et al. 2018). In this study, the liver TIMP-1 and TGF-β levels increased partially with hepatotoxicity, but fibrosis had not yet occurred by CS. In the liver, ketotifen, dipyridamole and the combined treatments reduced the TIMP-1 level. In addition, quercetin, dipyridamole and the combined treatments reduced the TGF-β level, and the development of liver fibrosis may have been prevented. Moreover, the TGF-β level may have decreased by suppressing the TGF-β1/Smads pathway of quercetin.

CS increases lipid peroxidation by disrupting redox hemostasis and the level of TBARS increase in the liver and kidney. The change is particularly pronounced in the liver (Vangaveti et al. 2021). CS causes an increase in free oxygen radicals and depletion of the liver GSH repository (Hagar 2004, Amudha et al. 2006) and also causes an increase in the TBARS level (Hagar 2004). Although CS caused significant oxidative stress and lipid peroxidation in 4-month-old rats, it was reported that it did not show this effect in younger animals due to its strong antioxidant capacity (Palomero et al. 2001). In this study, renal lipid peroxidation by CS may not have been fully formed, and renal TBARS and GSH levels may not have changed because the animals were young. Ketotifen can have reduce the liver TBARS level due to its effects on some antioxidant enzymes such as SOD other than GSH. Dipyridamole, quercetin and combined treatments can have similarly induced other antioxidant enzymes, resulting in a decreased liver TBARS level.

The increase in AST, ALT and urea levels and the decrease in ALB and TP levels have evaluated as markers of hepatotoxicity and nephrotoxicity. Some antioxidant treatments such as vitamin E, quercetin and propolis are protective against CS-induced hepatotoxicity (Mostafavi-Pour et al. 2008, Seven et al. 2014). In the current study, serum AST and ALT levels have increased, and ALB and TP levels may have decreased with the induction of hepatotoxicity by CS. The antioxidant effects of ketotifen, dipyridamole, and quercetin have prevented liver damage and consequently the increase in AST and ALT.

CS suppresses renal erythropoietin expression and decreases stimulation of bone marrow erythropoiesis. Thus, RBC, HGB and HCT levels decrease (Lei et al. 2014, Khattab et al. 2019). In the current study, CS may have significantly lowered RBC, HGB and HCT levels by inhibiting erythropoietin synthesis. All treatments partially prevent these changes, and especially dipyridamole may have prevented induction of blood circulation.

In conclusion, CS is one of the drugs which has immunosuppressive effects, however, its use is limited due to its side effects. CS has shown different toxic effects in different organs and caused more pronounced hepatotoxicity, but fewer nephrotoxic effects. It has been seen that these side effects can be prevented by early treatment and TLR-4 may be one of the main targets for nephrotoxicity.

Ketotifen, dipyridamole, and quercetin treatments have reduced renal dysfunction and are quite successful in hepatotoxicity via their antioxidant and anti-inflammatory properties. Ketotifen and dipyridamole have provided the most effective treatment for the hepatorenal toxicity of CS. The two drugs should be investigated in more detail in the treatment of hepatorenal failure in the future. Although the treatments prevent hepatotoxicity, they can also be speculated to have preventative effects in the early stage of nephrotoxicity.

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