ORIGINAL ARTICLE

NEPHROPROTECTIVE EFFECTS OF CURCUMIN AGAINST CYCLOSPORINE A-INDUCED NEPHROTOXICITY IN RAT MODEL

DOI: 10.36740/WLek202112103

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ABSTRACT

The aim: The current study was designed to examine the possible Nephroprotective effects of CMN in preventing nephrotoxicity and oxidative stress caused by chronic administration of CsA in rats.

Materials and methods: This study consisted of four groups and each group was made up of 8 rats. The first group was considered as a control group (received vehicle (0.9% N/S orally, and olive oil S.C), and the rest included the following: CMN group (received CMN in a dose of 30mg/kg/day orally), CsA group (received CsA in a dose of 20mg/kg/day S.C), and CMN plus CsA combination group (received CMN (30mg/kg/day, orally) plus CsA (20mg/kg/day, S.C) for 21days). For each group, the following variables were assessed: Serum urea concentration, Serum creatinine concentration, initial body weight, final body weight, Tissue MDA level, Tissue GpX1 level, Tissue CAT level, Tissue SOD level, and tissue IL-2 level, and histopathological examination.

Results: Mean levels of serum urea and creatinine, tissue MDA, tissue IL-2, and histopathological scores are significantly ($P<0.05$) increased in the CsA group compared with the control, and CMN groups (normal renal tissue). Tissue SOD, CAT, and GpX1 activities are significantly ($P<0.05$) decreased in the CsA group compared with the control, and CMN group. Concomitant administration of CMN with CsA resulted in significantly ($P<0.05$) lower elevated levels of MDA, serum urea, and creatinine, significantly higher levels of antioxidant enzymes, and normalization of the altered renal morphology compared with CsA treated rats.

Conclusions: CMN has antioxidant and anti-inflammatory properties that protect the kidney from CsA's toxicity.

KEY WORDS: Cyclosporine A (CsA), Curcumin (CMN), Nephrotoxicity

Wiad Lek. 2021;74(12):3135-3146

INTRODUCTION

Cyclosporine (CsA) (formerly named cyclosporine A), a hydrophobic cyclic polypeptide consists of 11 amino acids, with a molecular weight of 1,203 Daltons [1-2]. It is extracted from a soil fungus (the fungus Tolypocladium inflatum). Because of its potent immunosuppressive function, cyclosporine A (CsA) has aided organ transplantation greatly. CsA prevents allograft rejection and improves patient survival during transplantation [3]. CsA has approved for the treatment of autoimmune disorders such as psoriasis and rheumatoid arthritis, in addition to transplantation [4-5]. Immunosuppressive effectiveness of CsA has restricted by its side effects, which include hepatotoxicity, neurotoxicity, and nephrotoxicity [6]. Hepatotoxicity and nephrotoxicity are the most dangerous toxic effects [7]. The exact mechanism of CsA-induced nephrotoxicity are not completely defined, but several studies suggest that many mechanisms have been proposed for CsA-induced nephrotoxicity, including the following: stimulation of the renin-angiotensin system [8](increase synthesis of angiotensin II, is a vasoconstrictor factor), increased synthesis of Endothelin (vasoconstrictor factor) [9], alterations in renal prostanoids (vasodilator factor) and thromboxane (vasoconstrictor factor) production [10], Oxidative stress [11], and impaired synthesis of nitric oxide (NO) (vasodilator factor) [12]**.** CsA treatment induces renal perturbation of the vasoconstriction–vasodilation balance to cause tubular hypoxia-reoxygenation; therefore, CsA might cause hopoxia-induced ROS production, at least in the kidney [13]. Turmeric (Curcuma longa) is an herbaceous natural plant whose rhizomatous sections have long been used as a food coloring and flavoring agent [14]. CMN is a yellow pigment found in turmeric[15]. CMN is known for its ability to protect biomembranes from oxidative damage. Peroxidation of lipids is believed to be a free-radical-mediated chain reaction that damages cell membranes, and curcumin's inhibition of peroxidation is primarily due to the scavenging of reactive free radicals involved in the peroxidation process. The majority of antioxidants have either a phenolic or a-diketone functional group. Antioxidant activity of CMN is due to the phenolic hydroxyl groups in its structure [16]. CMN has shown to help reduce oxidative stress markers in the body [17]. Effect of CMN on free radicals is mediated by a number of mechanisms. It has the ability to scavenge a variety of free radicals, involving reactive oxygen plus nitrogen species (ROS and RNS, respectively) [18]. It has the ability to regulate the action of GSH, catalase, and SOD enzymes, all of which are involved in the neutraliza-

tion of free radicals [19]. It can also stop ROS-producing enzymes involving Lipoxygenases/cyclooxygenase and xanthine hydrogenase/oxidase from producing reactive oxygen species [19] by inhibiting the activation of NF-kB [20], and lowers inflammatory cytokines like TNF-alpha , IL-2, IL-6, and IL-8, IL-12, and fibroblast cytokines. All have been shown to be down regulated by CMN[21], and also inhibit INF-gamma, resulting in an anti-inflammatory [22], and antioxidant [23] effect.

THE AIM

The current study was designed to examine the possible nephroprotective effects of CMN in preventing nephrotoxicity and oxidative stress caused by chronic administration of CsA in rats.

MATERIALS AND METHODS

ANIMALS

A total of 32 adult Wister Albino rats with the optimum age of 7-8 weeks, weighing 150-250 g were obtained from Animal house at the Kufa University's College of Sciences. The study protocol was approved by the Animal Ethics Committee (AEC) of Kufa University. Animals were settled in the animal house of university of Kufa in a temperature-controlled (22+-2 C) room, with alternating 12 hr. light: 12hr dark cycles. Animals had free access to water and chow diet until the start of the experiments.

DESIGN OF STUDY

After two weeks of acclimatization, the rats were randomized into four groups (n=8) as following:

I. Control (vehicle) group: Rats received 0.9% sodium chloride (4ml/kg/day, via intragastrical route) + Olive oil (2ml/kg/day, via subcutaneous route) for 21days.

II. CMN treated group: Rats received CMN (30mg/kg/day, via intragastrical route) + Olive oil (2ml/kg/day, via subcutaneous injection) for 21days.

III. CsA- treated group: Rats received 0.9% sodium chloride (4ml/kg/day, via intragastrical route) + CsA (20mg/kg/day dissolved in Olive oil, via subcutaneous injection) for 21days.

IV. CMN + CsA-combination treated group: Rats were received CMN (30mg/kg/day suspended in N/S, via intragastrical route) + CsA (20mg/kg/day dissolved in Olive oil, via subcutaneous injection) for 21days. For each group, the following variables were assessed: Serum urea concentration, Serum creatinine concentration, initial body weight, final body weight, Tissue MDA level, Tissue GpX1 level, Tissue CAT level, Tissue SOD level, and tissue IL-2 level, and histopathological examination.

PREPARATION OF DRUGS AND AGENTS

CURCUMIN

A powder of CMN with a purity of 99.8% (obtained from Aldrich/sigma, UK) (30mg/kg/day) was suspended in 0.9% normal saline (4ml/kg/day). Then, CMN was given in a dose (30mg/kg/day) via intragastrical route for 21days [24] have also used this route for the administration of CMN to rats.

CYCLOSPORINE A

Pure powder of CsA (obtained from Cheme Scene, USA, Lot. No. 34526, Cat. No. CS-2761) was dissolved in olive oil (2ml/kg/day) [25] have also used this route for the administration of CsA to rats. Then, CsA was given a dose (20mg/kg/day) via the subcutaneous route for 21 days [26] have also used this dose of CsA to induce nephrotoxicity in rats**.** The CsA powder was stored at 4 centigrade according to the manufacturer's advice.

PREPARATION OF SAMPLES

PREPARATION OF BLOOD SAMPLES FOR RENAL FUNCTION TEST

On the 22nd day of the experiment, rats were anesthetized with I.P injections of xylazine (10 mg/kg), and ketamine (100 mg/kg) [27]**.** After that, blood samples were collected directly from the heart, placed in a gel tube at 37°C without anticoagulant and left for 30 minutes, then centrifuged at 3000rpm for 10 minutes [28]. The next serum obtained was used for the determination of serum Urea and Creatinine concentration.

RENAL TISSUE PREPARATION FOR ELISA MEASURING OF CAT, SOD, MDA, GPX1, AND IL-2 ACTIVITIES

On the 22nd day of the experiment, the rats were anesthetized and the right kidney of each animal was immediately excised through midline abdominal incision. After that, part of the right renal tissue was rinsed with normal saline to remove any red blood cells or clots, stored in a deep freeze at (-80C°) and kept frozen for enzymatic analysis. The next step was done by weighing renal tissue, and homogenization in a solution containing 1:10 (w/v) phosphate buffer saline (PH 7.4) that contained 1% triton X-100 and protease inhibitor cocktail by using a high-intensity liquid processor [29]. The homogenate was centrifuged with 3000rpm at 4centigrade for 20minute. The supernatant was collected for determination of CAT, SOD, MDA, GpX1, and IL-2 activities by Elisa with a commercially available Elisa kit, according to manufacturer's instructions.

PREPARATION OF RENAL TISSUE SAMPLES FOR HISTOPATHOLOGY

On the 22nd day, part of the left rat kidneys was fixed in 10% formaldehyde and processed by routine histological methods, and embedded in paraffin blocks [30]**.** The tissue

Table I. Comparisons between the mean of initial body weight (g) (mean body weight at 0 day of treatment) and the mean of final body weight (g) (mean body weight at 21 day of treatment) of the same group

Group	Mean of initial Body weight (grams)+-SD	Mean of final Body weight (grams)+-SD	P-value
Control	166.3750±7.8	188.3750±16.1	0.0001*
Curcumin	169±20.05	196±20.3	0.0001*
CsA	168±17.7	158.3±20.9	0.0001*
CsA + Curcumin	186.4±11.8	194.1±16.6	0.05*

* Statistically significant at p -value equal or less than 0.05

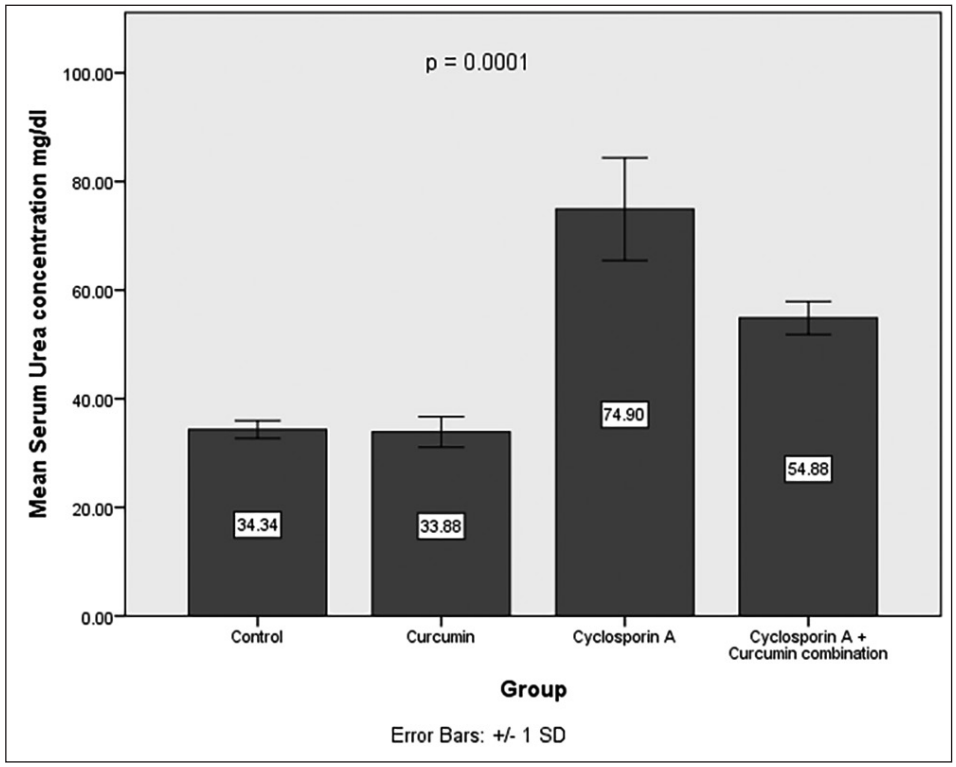

Fig 1. The Bar chart shows the effect of CMN, CsA, and CMN plus CsA combination on serum urea concentration (mg/dl) as mean ± SD for four experimental groups (Eight rats in each group)

slide sections were cut five micrometer-thick horizontal following dehydration. For histological investigation, the deparaffanized and rehydrated rat kidney tissue sections were stained with hematoxylin and 1% eosin at room temperature by the Hematoxylin and Eosin staining kit [31]. The following light microscopic features were used for assessment of the histopathological damage. The **Score 0** represents normal tissue, **Score 1** represents area of damage less than 25% of the tubules, **Score 2** represents damage involving 25-50% of the tubules, **Score 3** represents damage involving 51-75% of the tubules, **Score 4:** area of damage involves 76-100% of the tubule [32].

STATISTICAL ANALYSIS

Data were analyzed using SPSS program version 20. Categorical variables are presented as frequencies and percentages while continuous variables are presented as mean and SD. ANOVA test with post-hoc analysis (LSD) are used for comparison of the study parameters among groups. Error bars are used to demonstrate the mean of the study parameters among groups. Chi square test was used to assess the distribution of the renal damage among groups between categorical variables. Paired t-test was used to compare the initial and final body weight within each group. Statistical significance was regarded if P-value equal or less than 0.05.

RESULTS

INFLUENCE OF CMN ON CYCLOSPORINE A- INDUCED NEPHROTOXICITY IN RATS

EFFECT ON SERUM UREA AND CREATININE CONCENTRATION

The results showed a significantly (P<0.05) increased serum urea and creatinine concentration in the CsA group compared with the control, and the CMN group. There was an insignificant difference between the control and CMN group. Chronic concomitant administration of CMN plus CsA significantly lowers serum urea and creatinine concentrations when compared to the CsA group, as shown in figures (1-2); respectively.

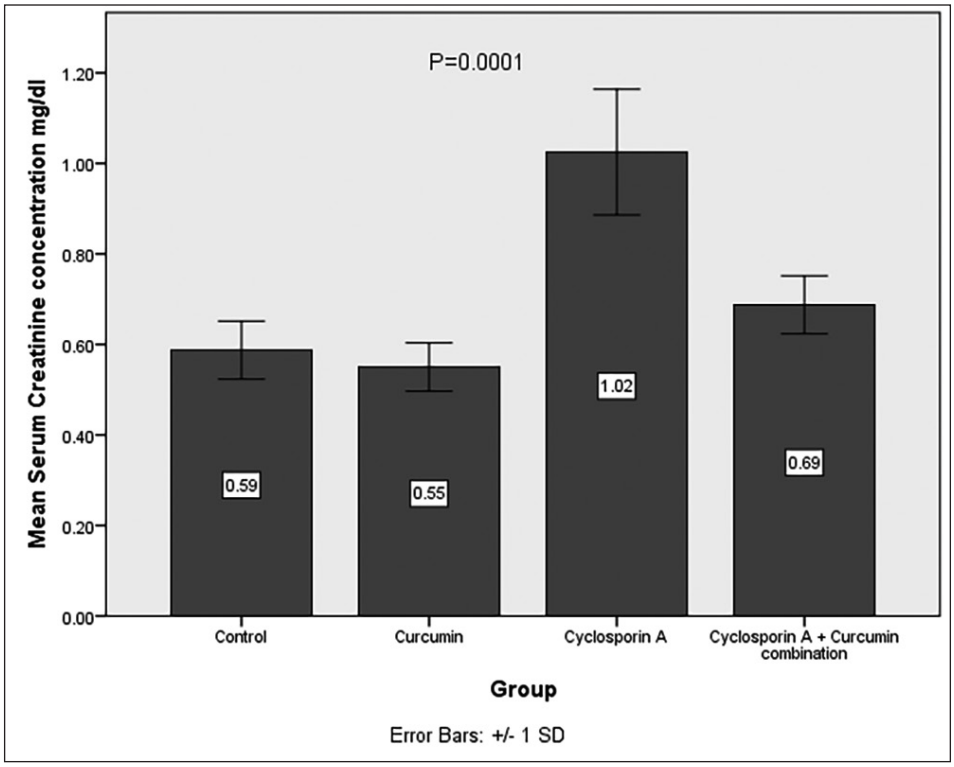

Fig 2. The Bar chart shows the effect of CMN, CsA, and CMN plus CsA combination on serum creatinine concentration (mg/dl) as mean ± SD for four experimental groups (Eight rats in each group)

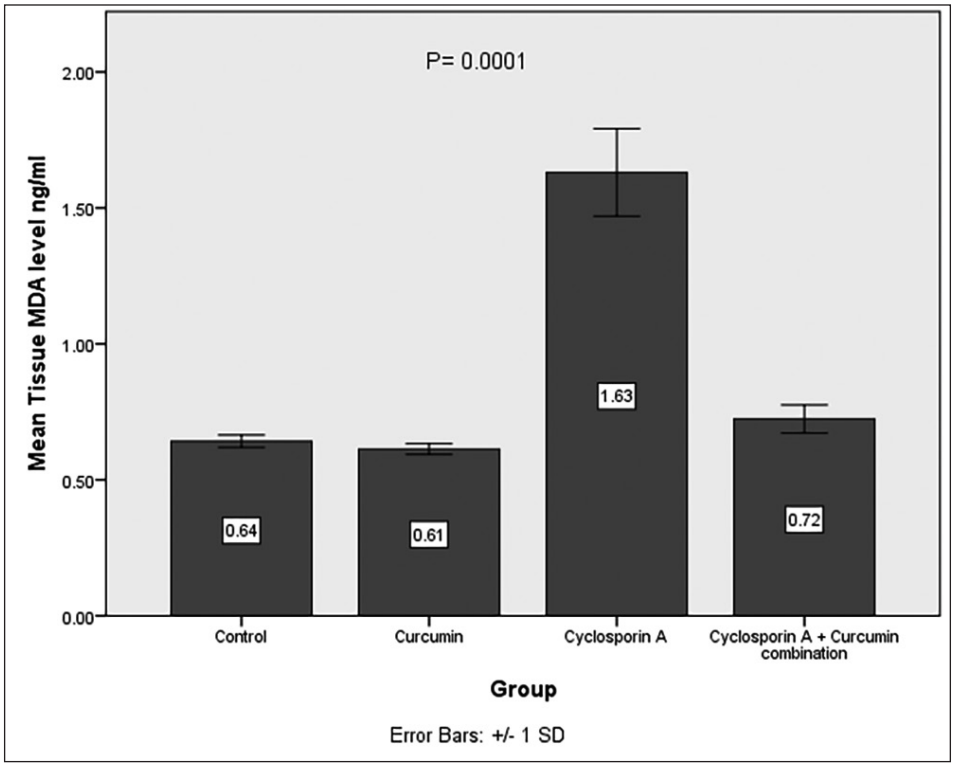

Fig 3. The Bar chart shows the effect of CMN, CsA, and CMN plus CsA combination on tissue MDA concentration (ng/ml) as mean ± SD for four experimental groups (Eight rats in each group)

EFFECT ON TISSUE MDA LEVEL

The results showed a significantly ($P<0.05$) increased in the level of renal tissue MDA in the CsA group compared with the control and CMN group. There was an insignificant difference between the control and CMN group. Chronic concomitant administration of CMN plus CsA showed a significantly lower MDA level compared to the CsA group, as shown in figure (3).

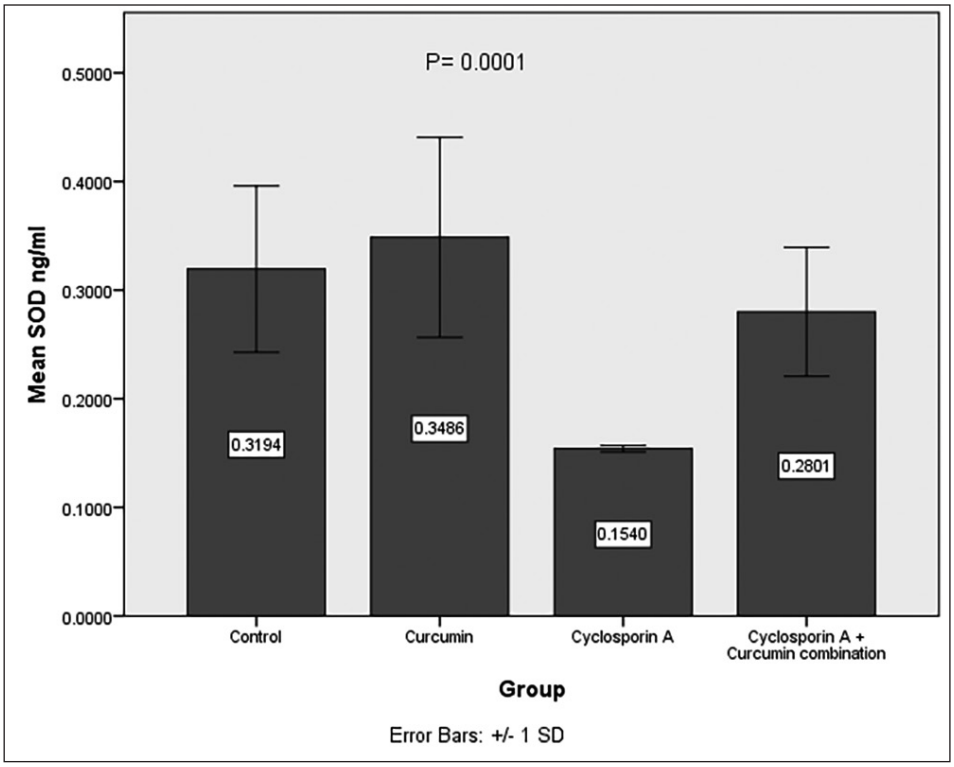

Fig 4. The Bar chart shows the effect of CMN, CsA, and CMN plus CsA combination on tissue SOD concentration (ng/ml) as mean ± SD for four experimental groups (Eight rats in each group)

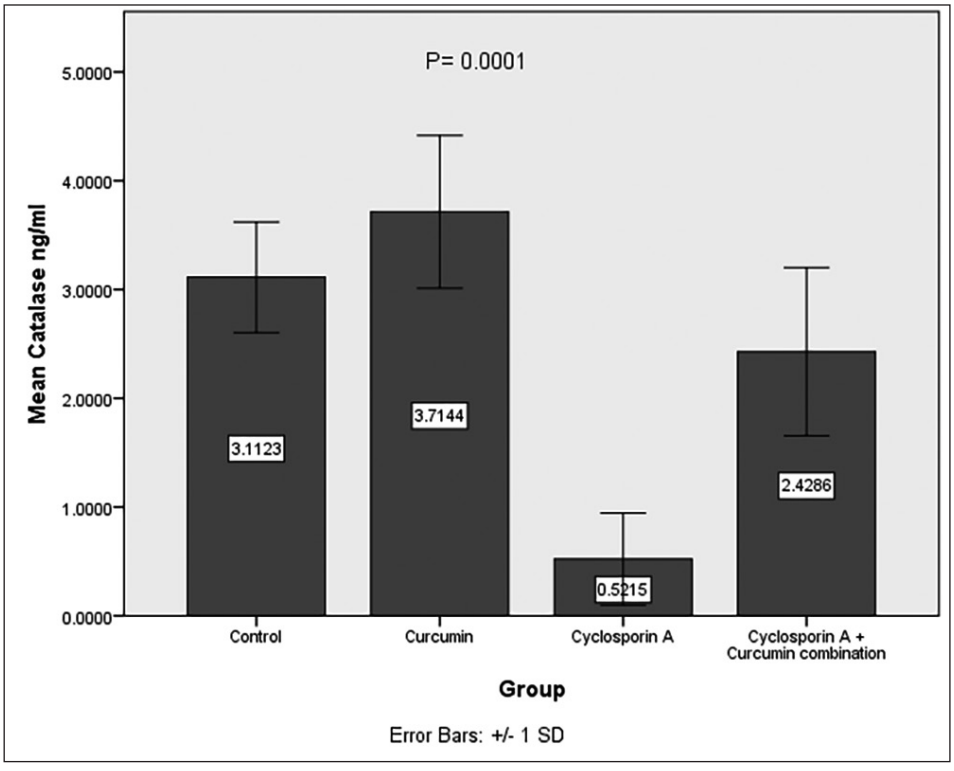

Fig 5. The Bar chart shows the effect of CMN, CsA, and CMN plus CsA combination on tissue Catalase concentration (ng/ml) as mean ± SD for four experimental groups (Eight rats in each group)

EFFECT ON TISSUE SOD LEVEL

The results showed a significantly ($P<0.05$) decreased renal tissue SOD level in the CsA group compared with the control and CMN group. There was an insignificant difference between the control and CMN group. Chronic concomitant administration of CMN plus CsA showed a significantly increased in SOD level when compared to the CsA group, as shown in figure (4).

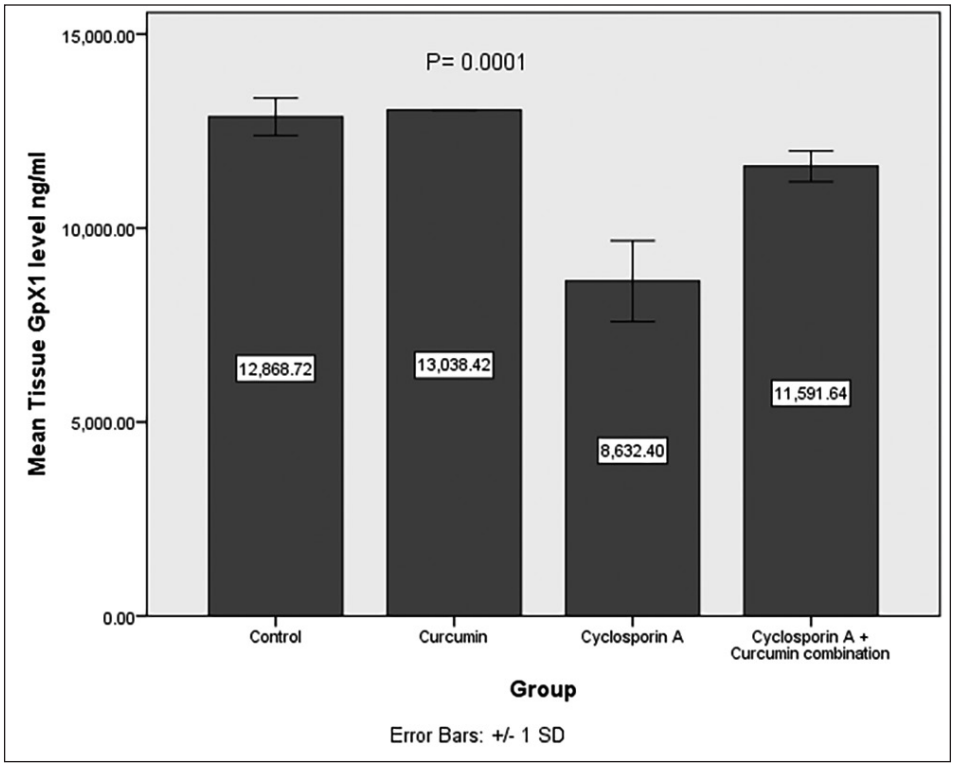

Fig 6. The Bar chart shows the effect of CMN, CsA, and CMN plus CsA combination on tissue GpX1 concentration (ng/ml) as mean ± SD for four experimental groups (Eight rats in each group)

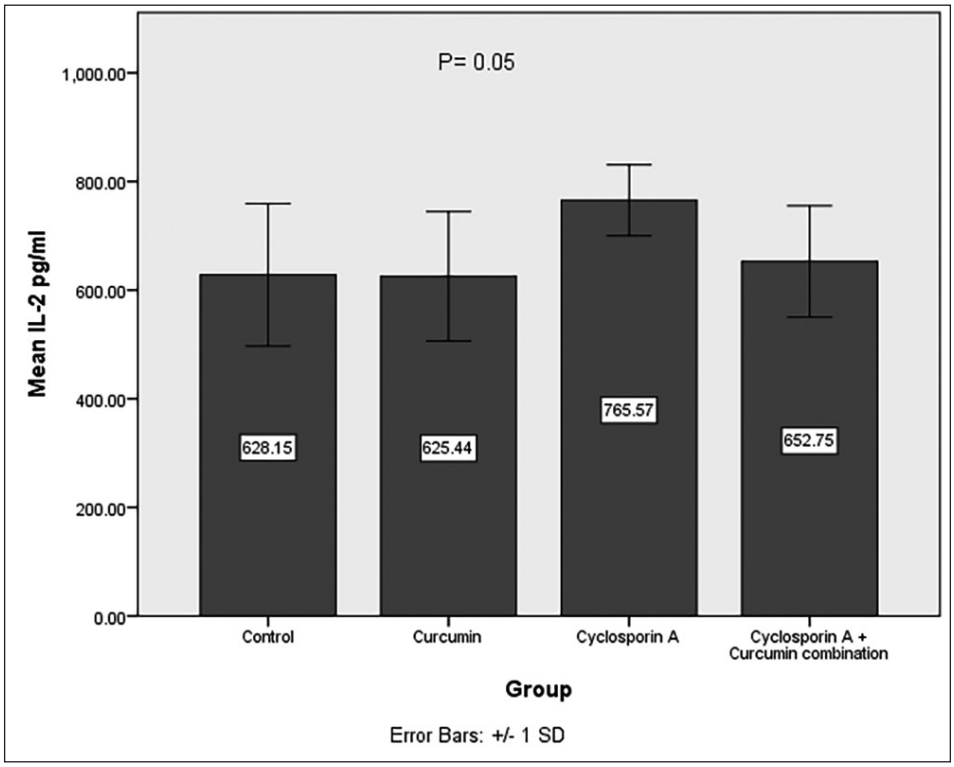

Fig 7. The Bar chart shows the effect of CMN, CsA, and CMN plus CsA combination on tissue IL-2 concentration (pg/ml) as mean ± SD for four experimental groups (Eight rats in each group)

EFFECT ON CAT LEVEL

The results showed a significantly (P<0.05) decreased renal tissue catalase level in the CsA group compared with the control and CMN group. There was an insignificant difference between the control and CMN group. Chronic concomitant administration of CMN plus CsA showed a significantly increased catalase level when compared to the CsA group, as shown in figure (5).

EFFECT ON TISSUE GPX1 LEVEL

The results showed a significantly (P<0.05) decreased renal tissue GpX1 level in the CsA group compared with the control and CMN group. There was an insignificant difference between the control and CMN group. Chronic concomitant administration of CMN plus CsA showed a significantly increased GpX1 level when compared to the CsA group, as shown in figure (6).

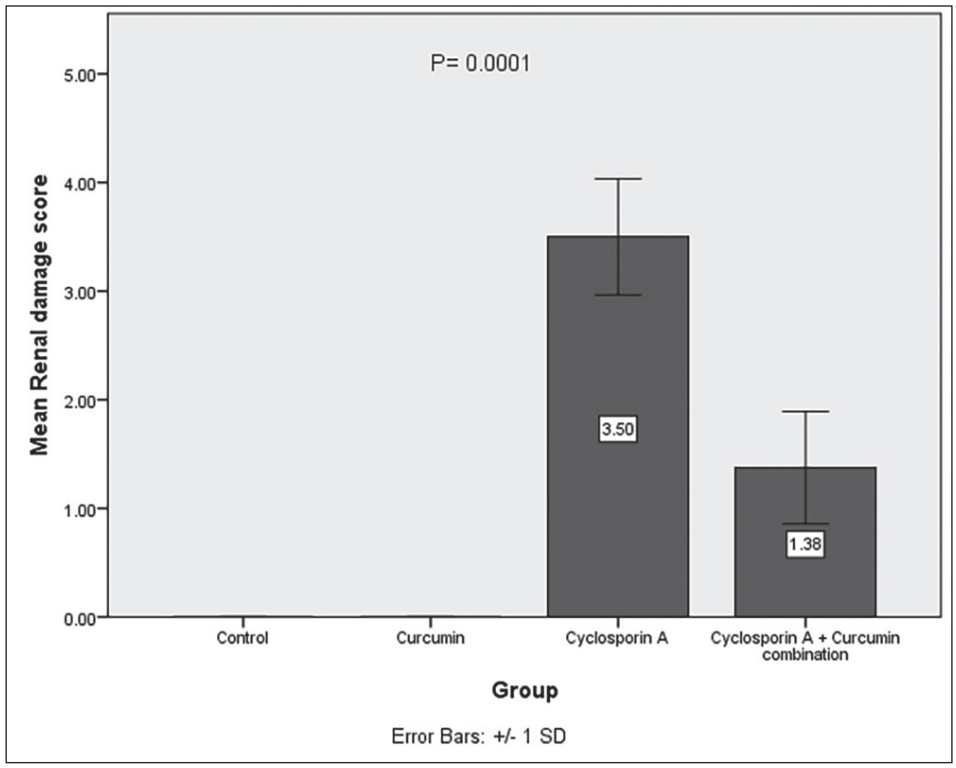

Fig 8. The Bar chart shows the effect of CMN, CsA, CMN plus CsA combination on renal histology as mean ± SD for four experimental groups (Eight rats in each group)

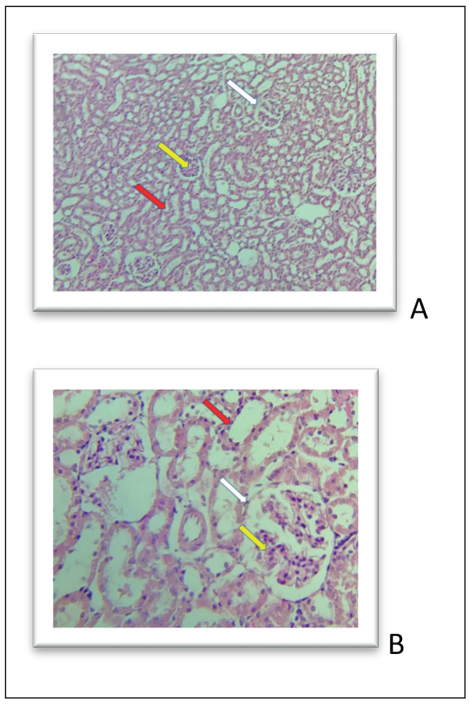

Fig 9. The cross sections of the control (vehicle) group showed normal renal tubules, glomeruli, and capillary tuft A H&E 10x10 B H & E 10x40

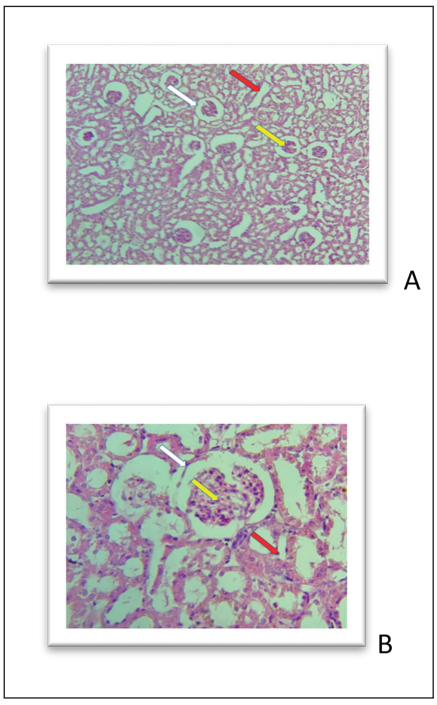

Fig 10. The cross sections of the CMN treated group showed normal renal tubules, glomeruli, and capillary tuft A H&E 10x10 B H & E 10x40

EFFECT ON TISSUE IL-2 LEVEL

The results showed a significantly (P<0.05) increased renal tissue IL-2 level in the CsA group compared with the control and CMN group. There was an insignificant difference between the control and CMN group. Chronic concomitant administration of CMN plus CsA showed a significantly decreased IL-2 level when compared to the CsA group, as shown in figure (7).

EFFECT ON BODY WEIGHT (GRAM)

The result showed a significantly (P<0.05) decreased body weight of rats in the CsA group compared with the initial body weight of the CsA treated group. Chronic concomitant administration of CMN plus CsA showed a significantly increased body weight of rats compared with the initial body weight of the CMN plus CsA combination treated group, as shown in table (I).

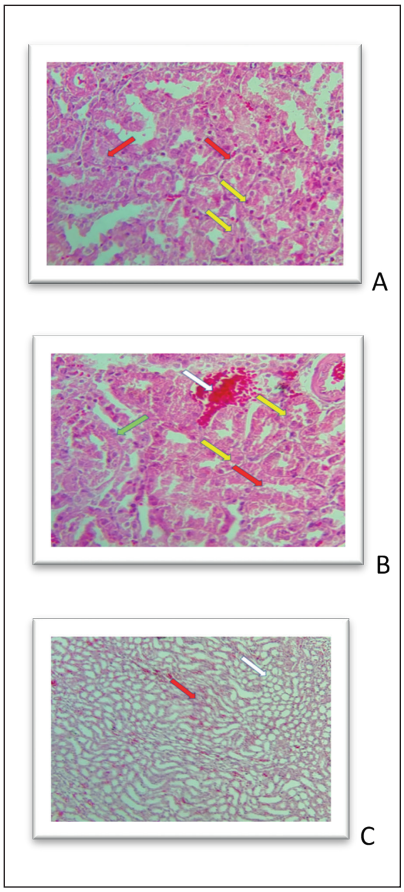

Fig 11. A: The renal cross sections of CsA treated group showed marked diffuse damage of renal tubules H & E 10x10; B: The renal cross sections of CsA treated group showed marked cellular swelling ,cytoplasmic eosinophilic, tubular dilation, loss of brush borders H &E 10x40; C: The renal cross sections of CsA treated group showed marked cellular swelling, cytoplasmic eosinophilic, tubular dilation, loss of brush borders extravasations of RBCs (hemorrhage), glomerular basement thickening, and necrosis H & E 10x40

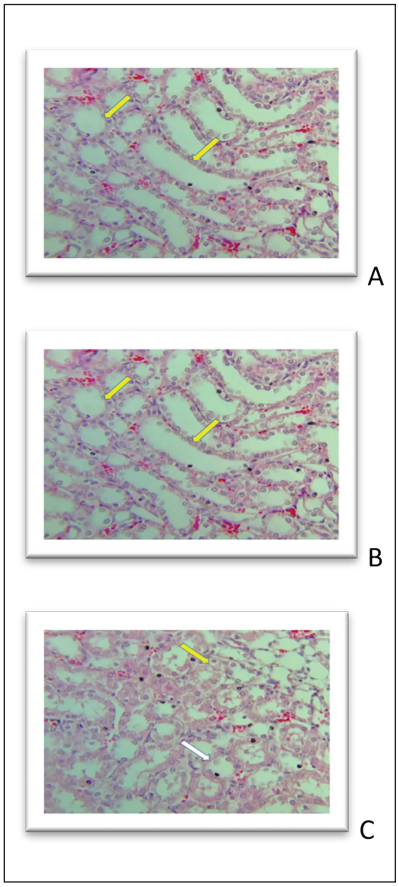

Fig 12. A: The cross sections of CMN plus CsA treated group showed marked decrease in the renal histological damage characterized by normal morphology of the kidney , normal glomeruli, no hemorrhage, slight edema of the tubular cells, and renal tubules with 15% damage H & E 10x10; B: The cross sections of CMN plus CsA treated group showed marked decrease in the renal histological damage characterized by normal morphology of the kidney, and normal renal tubules H & E 10x40; C: The cross sections of CMN plus CsA treated group showed marked decrease in the renal histological damage characterized by normal morphology of the kidney , normal glomeruli, no hemorrhage, and slight edema of the tubular cells (renal tubules with 15% damage) H & E 10x40

HISTOPATHOLOGICAL EXAMINATION

The mean percent of renal histopathological damage were showed in figure (8). The cross sections of the control (vehicle) group, and CMN group showed normal renal tissue, as shown in figure (9) (A, B), and (10) (A, B) respectively. There was insignificant difference between control and CMN group. By contrast, the renal of rats treated with CsA showed noticeable histological damage in the renal tubules, glomeruli, and capillary tuft, as shown in figure (11) (A, B and C).There was a significant($P<0.05$) difference between control and CsA group. Also, there was a significant ($P<0.05$) difference between CMN and CsA group. Chronic concomitant administration of CMN with CsA protects the kidney from the toxic effect of CsA. The cross sections of CMN plus CsA treated group showed marked a significant ($P<0.05$) decreased in the renal histological damage, as shown in figure (12) (A, B, and C).

DISCUSSION

Cyclosporine A has significantly reduced morbidity and mortality in transplant patients over the past decade, and it has been increasingly used with significant therapeutic effect in the treatment of autoimmune illnesses [33]. But, its use is often accompanied by unfavorable side effects, such as nephrotoxicity and hypertension. Previous research has suggested that ROS generation and oxidative stress may play a role in CsA toxicity in a variety of organs [11]. The current study was planned to assess the possible beneficial effect of CMN in preventing the kidney injury, and oxidative stress triggered by administration of CsA in rats.

EFFECT OF CSA ON STUDY PARAMETERS

EFFECT OF CSA ON RENAL FUNCTION TEST

In the present study, CsA treatment for 21 days has showed to induce kidney dysfunction, which was distinguished by a significant increasing in serum creatinine and blood urea nitrogen compared with control group. This outcome was in agreement with the results reported by [34-38]. The pathogenesis is considered secondary to intense renal vasoconstriction induced by angiotensin II and other vasoactive substances [26]. Reduced GFR and renal blood flow due to renal vasoconstriction; this was related to increasing in the serum creatinine and urea levels. Chronic CsA injection for 21 days resulted in considerable oxidative stress in the kidneys, as well as a marked deterioration of renal functions [1]. Oxidative stress can encourage the formation of a number of vasoactive mediators, which can directly impair renal functions by producing renal vasoconstriction or reducing the glomerular capillary ultrafiltration coefficient; and, thus, lowering of glomerular filtration rate [39].

EFFECT OF CSA ON OXIDATIVE STRESS MARKER (TISSUE MDA LEVEL)

In the present study, CsA treatment for 21 days has showed a significant increase the level of renal tissue MDA as compared with

control group. This result is in agreement with the results reported by [34-40]. The possible mechanism of CsA-induced increasing in tissue MDA level include significant increase in lipid peroxidation [40]. Cyclosporine A therapy has demonstrated an increase of the generation of free radicals and the creation of lipid peroxides in vivo and in vitro. Cyclosporine A increased malondialdehyde a stable constituent of lipid hydroperoxide in isolated kidney microsomes [41] . Following CsA therapy, an increase in superoxide radical and hydrogen peroxide has been observed. Furthermore, CsA treatment causes an increase in local hydroxyl radical generation, which leads to lipid peroxidation and nephrotoxicity [33].

EFFECT ON ENDOGEOUS ANTIOXIDANT ENZYMES ACTIVITIES

1. EFFECT OF CSA ON ANTIOXIDANT ENZYME (TISSUE SOD LEVEL)

In the current study, CsA treatment for 21 days has showed a significantly reduced the activities of endogenous antioxidant enzyme (SOD) compared with control group. This result was agreement with the results reported by [34-36]. Superoxide dismutase is considered as the first line of defense against the deleterious effects of oxygen radicals in cells, where it scavenges ROS by catalysing the dismutation of superoxide to H_2O_2 and O_2 [35]. The decrease in renal SOD level next CsA therapy was also came in agreement with the outcomes reported by [42].

2. EFFECT OF CSA ON ANTIOXIDANT ENZYME (TISSUE GPX1 LEVEL)

In the current study, CsA treatment for 21 days has showed a significant reduction of the activities of endogenous antioxidant enzyme (GpX1) compared with control group. This result is agreed with the results reported by [34-36]. The decrease in GPx1 activity with CsA treatment indicates a decrease in GSH levels and an increase in peroxide levels. Glutathione depletion results in a proportionate reduction in H2O2 detoxification by glutathione peroxidase [41].

3. EFFECT OF CSA ON ANTIOXIDANT ENZYME (TISSUE CAT LEVEL)

In the current study, CsA treatment for 21 day has showed a significant reduce of the activities of endogenous antioxidant enzyme (CAT) compared with control group. This outcomes were in agreement with the results reported by [33-36]. The current drop in catalase activity in CsA-treated rats is related to a decrease in the availability of NADPH, which is required for catalase activity when it is converted from its inactive form. As a result, it's probable that reducing NADPH generation in CsA-treated rats reduces catalase activity [41].

4. POSSIBLE MECHANISMS OF CSA TO REDUCE THE ENDOGENOUS ANTIOXIDANT ENZYMES (TISSUE SOD, CAT, AND GPX1) ACTIVITIES

CsA therapy causes tubular hypoxia-reoxygenation by disrupting the vasoconstriction–vasodilation balance in the kidney; thus, CsA could produce hopoxia-induced ROS generation, at least in the kidneys [6, 13]. CsA inhibits mitochondrial respiration in renal tubular cells, which could lead to free radical formation [37]. CsA modified the expression and activity of numerous kidney enzymes (Glutathione peroxidase (GpX1), Catalase (CAT), and (SOD) Superoxidedismutase)[43]. It is documented that the concentration of free radicals in urine incresed significantly, following CsA therapy [36].

EFFECT OF CSA ON INFLAMMATORY MEDIATOR (IL-2 LEVEL)

In the current study, CsA treatment for 21 days has showed a significant increase of tissue IL-2 level compared to the control group (normal renal tissue). IL-2 levels in the blood are not high in healthy people, but they rise when immune cells are stimulated by numerous pathophysiological triggers [44-45]. The possible mechanisms for increment of tissue IL-2 level in CsA treated group are renal dysfunction, and oxidative stress. TGF-B production is stimulated by CSA in the kidneys and throughout the body. RAS activation appears to be linked to TGF-B overproduction produced by CSA [38]. The stimulation of cytokines by angiotensin II seems to be mediated via activation of the nuclear factor-kB (NF-kB) dependent pathway. Nuclear transcription factor activates pro-inflammatory transcription factors and, thus, encourages the synthesis of protein products such as cell adhesion molecules and chemokines [46]. Also, the RAAS may induce renal injury via stimulation of tubulointerstitial inflammation [47].

EFFECT OF CSA ON BODY WEIGHT (GR.)

In the present study, chronic CsA administration for 21 days has showed a significantly decreased body weight of rats compared with the initial body weight (body weight of rats at zero day) of CsA treated group. This results was in agreement with the outcomes reported by [33, 37]. CsA induced oxidative stress [11], may be the possible mechanism for decreased body weight.

EFFECT OF CSA ON RENAL HISTOLOGY

In the present study, CsA nephrotoxicity was confirmed by histopathological changes characterized by bynoticeable histological damage in the renal tubules, glomeruli, and capillary tuft. The renal cross sections of CsA treated group showed extremly statistically significant marked cellular swelling, cytoplasmic eosinophilia, tubular dilation, loss of brush borders, extravasation of RBCs (hemorrhage), glomerular basement thickening, and necrosis compared with control group. This results was in agreement with the results reported by [1, 40, 24]. The exact mechanism of CsA-induced nephrotoxicity are not completely defined, but several studies suggest that many mechanisms have been proposed for CsA-induced nephrotoxicity, including stimulation of the renin-angiotensin system [8] (increase synthesis of angiotensin II, is a vasoconstrictor factor), increased synthesis of Endothelin (vasoconstrictor factor) [9], variations in renal prostanoids (vasodilator factor) and thromboxane (vasoconstrictor factor) production [10],

Table II. Abbreviation

Abbreviation	Detail
ANOVA	Analysis of variance
CAT	Catalase
CMN	Curcumin
CsA	Cyclosporine A
C	Celsius degree
COX	Cyclooxygenase enzyme
DW	Distilled water
ELISA	Enzyme – linked immunosorbent assay
g	Gram
GpX1	Glutathion peroxidase 1
H&E	Hematoxylin and eosin
H2O2	Hydrogen peroxide
I.P.	Intraperitonial
IL	Interleukine
IL-2	Interleukin-2
Kg	Killogram
LOX	Lipooxygenase enzyme
MDA	Malondialdhyde
ml	Milliliter
mg/dl	Milligram /deciliter
mg/kg	Milligram /kilogram
NF-kB	Nuclear factor kappa –light –chain- enhancer of activated B cells
N/S 0.9%	Normal saline 0.9%
PBS	Phosphate buffer saline
ROS	Reactive oxygen species
rpm	Round per minute
S.C	Subcutaneous
S.cr	Serum creatinine
SOD	Sodium oxide dismutase
TNF-a	Tumour necrosis factor –alpha
NF-AT	Nuclear factor of activated T-cells
CNI	Calcineurin inhibitor
NO	Nitric oxide
TGF-B	Transforming growth factor -beta
RAAS	Renin-angiotensin-aldosterone system

Oxidative stress [11], and impaired synthesis of nitric oxide (NO) (vasodilator factor) [12]. The altered gene expression that follows calcineurin inhibition may also begin a chain of events ultimately ending in nephrotoxicity [48].

INFLUENCE OF CMN ON CSA- INDUCED RENAL INJURY IN RATS

EFFECT OF CMN ON CSA- INDUCED RENAL BIOMARKER DYSFUNCTION

The results of chronic concomitant administration of CMN with CsA showed a significant decreased serum urea and creatinine compared with CsA group. This result was in agreement with the outcomes reported by [1,24,35,38]. Renoprotective effect of CMN is mainly due suppression of pro-inflammatory enzymes such as COX2 and 5-LOX, and thus inhibition of synthesis of vasoconstrictor factors [20].

EFFECT OF CMN ON CSA- INDUCED OXIDATIVE STRESS

In current study, chronic concomitant administration of CMN in a dose 30mg/kg/day with CsA has shown extremely statistically significant reduce of the tissue level of MDA compared with CsA treated group. This result was in agreement with the outcomes reported by [1,24,34]. CMN is known for its ability to protect biomembranes from oxidative damage. Peroxidation of lipids is believed to be a free-radical-mediated chain reaction that damages cell membranes, and curcumin's inhibition of peroxidation is primarily due to the scavenging of reactive free radicals involved in the peroxidation process. The majority of antioxidants have either a phenolic or a-diketone functional group. Antioxidant activity of CMN is due to the phenolic hydroxyl groups in its structure [16].

EFFECT OF CMN ON CSA-INDUCED REDUCTION OF ENDOGENOUS ANTIOXIDANT ENZYMES (TISSUE SOD, CAT, AND GPX1) ACTIVITIES

In current study, chronic concomitant administration of CMN in a dose 30mg/kg/day with CsA has shown extremely statistically increase of the tissue level of SOD, CAT, and GpX1 compared with CsA treated group. This outcomes were in agreement with the results reported by [1, 24,34]. CMN has the ability to scavenge a variety of free radicals, involving reactive oxygen plus nitrogen species (ROS and RNS, respectively) [18]. Also it has the ability to regulate the action of GSH, catalase, and SOD enzymes, all of which are involved in the neutralization of free radicals [19, 49]. It can stop ROS-producing enzymes involving Lipoxygenases/cyclooxygenase [50], and xanthine hydrogenase/oxidase from producing reactive oxygen species [19].

EFFECT OF CMN ON CSA –INDUCED REDUCTION OF BODY WEIGHT (GR.)

In the current study, chronic concomitant administration of CMN with CsA showed a significantly increased body weight of rats compared with the initial body weight of CMN plus CsA combination treated group, and CsA group. The observed improvement of body weight by CMN may be due to increase appetite by CMN, as appetizer effect of CMN is well documented [15,51]. This together with the antioxidant effects of CMN [1], that probably prevent the toxic effect of CsA , may responsible for body weight improvement.

EFFECT OF CMN ON CSA-INDUCED RENAL HISTOPATHOLOGICAL DAMAGE

Chronic concomitant administration of CMN in a dose 30mg/kg/day with CsA protect the kidneys from the toxic effect of CsA. The cross sections of CMN plus CsA treated group showed extremely statistically significant decrease in the renal histological damage characterized by normal morphology of the renal, normal glomeruli, absence of hemorrhage, and slight edema of the tubular cells compared with CsA group. This result was in agreement with the outcomes reported by [1], and [24]. The anti-inflammatory and antioxidant effects of CMN are the possible renoprotective mechanisms against CsA-induced renal injury. CMN, according to [1], was able to alleviate oxidative stress as well as enhance renal function and tissue harm caused by chronic CsA usage.

CONCLUSIONS

CMN has antioxidant and anti-inflammatory properties that protect the kidney from CsA's toxicity.

REFERENCES

1. Tirkey N., Kaur G., Vij G., Chopra K. Curcumin, a diferuloylmethane, attenuates cyclosporine-induced renal dysfunction and oxidative stress in rat kidneys. BMC Pharmacol. 2005; 5(1): 1–10.
2. Kopp J.B., Klotman P.E. Cellular and molecular mechanisms of cyclosporin nephrotoxicity. J. Am. Soc. Nephrol. 1990; 1(2): 162–179.
3. Olyaei A.J., de Mattos A.M., Bennett W.M. Nephrotoxicity of immunosuppressive drugs: new insight and preventive strategies. Curr. Opin. Crit. Care. 2001; 7(6): 384–389.
4. Rezzani R. Cyclosporine A and adverse effects on organs: histochemical studies. Prog. Histochem. Cytochem. 2004; 39(2): 85–128.
5. Lawson C.A., Fraser A., Veale D.J., Emery P. Cyclosporin treatment in psoriatic arthritis: a cause of severe leg pain. Ann. Rheum. Dis. 2003; 62(5): 489.
6. Lee J. Use of antioxidants to prevent cyclosporine a toxicity. Toxicol. Res. 2010; 26(3): 163–170.
7. de Mattos D.M., Olyaei A.J., Bennett W.M. Nephrotoxicity of immunosuppressive drugs: long-term consequences and challenges for the future. Am. J. Kidney Dis. 2000; 35(2): 333–346.
8. Yang C.W. et al. Synergistic effects of mycophenolate mofetil and losartan in a model of chronic cyclosporine nephropathy. Transplantation. 2003; 75(3): 309–315.
9. Enderlein M., Gross P. Additive effects of oxidized low-density lipoproteins and cyclosporine on endothelin-1 production of renal arterial endothelial cells. Transplantation proceedings. 2003; 35(4): 1581–1583.
10. Butterly D.W., Spurney R.F., Ruiz P. et al. A role for leukotrienes in cyclosporine nephrotoxicity. Kidney Int. 2000; 57(6): 2586–2593.
11. Hagar H.H. The protective effect of taurine against cyclosporine A-induced oxidative stress and hepatotoxicity in rats. Toxicol. Lett. 2004; 151(2): 335–343.
12. Shihab F.S., Bennett W.M., Isaac J. et al. Nitric oxide modulates vascular endothelial growth factor and receptors in chronic cyclosporine nephrotoxicity. Kidney Int. 2003; 63(2): 522–533.
13. Inselmann G., Barth A., Engemann R. Cyclosporin–A–induced lipid peroxidation in human liver microsomes and its influence on cytochrome P–450. Eur. J. Clin. Invest. 1991; 21(5): 461–465.
14. Najafi H., Ashtiyani S.C., Sayedzadeh S.A. Therapeutic effects of curcumin on the functional disturbances and oxidative stress induced by renal ischemia/reperfusion in rats. Avicenna J. phytomedicine. 2015; 5(6): 576.
15. El-Zawahry B.H., Abu El Kheir E.M. The protective effect of curcumin against gentamicin-induced renal dysfunction and oxidative stress in male albino rats. Egypt. J. Hosp. Med. 2007; 29(1): 546–556.
16. Shome S., Das Talukdar A., Choudhury M.D. et al. Curcumin as potential therapeutic natural product: a nanobiotechnological perspective. J. Pharm. Pharmacol. 2016; 68(12): 1481–1500.
17. Sahebkar A., Serban M.C., Ursoniu S., Banach M. Effect of curcuminoids on oxidative stress: A systematic review and meta-analysis of randomized controlled trials. J. Funct. Foods. 2015; 18: 898–909.
18. Menon VP, Sudheer AR. Antioxidant and anti-inflammatory properties of curcumin. Adv Exp Med Biol. 2007;595:105-25. doi: 10.1007/978-0-387-46401-5_3.
19. Lin Y.G., Kunnumakkara A.B., Nair A. et al. Curcumin inhibits tumor growth and angiogenesis in ovarian carcinoma by targeting the nuclear factor-κB pathway. Clin Cancer Res. 2007; 13: 3423–3430.
20. Goel A., Kunnumakkara A.B., Aggarwal B.B. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curecumin": from kitchen to clinic. Biochem Pharmacol. 2008 Feb 15;75(4):787-809. doi: 10.1016/j.bcp.2007.08.016. Epub 2007 Aug 19.
21. Derosa G., Maffioli P., Simental-Mendia L.E. et al. Effect of curcumin on circulating interleukin-6 concentrations: a systematic review and meta-analysis of randomized controlled trials. Pharmacol. Res. 2016; 111: 394–404.
22. Kuhad A., Chopra K. Curcumin attenuates diabetic encephalopathy in rats: behavioral and biochemical evidences. Eur. J. Pharmacol. 2007; 576(1–3): 34–42.
23. Sahara H.A., MaSuda T., Takahashi T. et al B One mar-IOw Origin Of endothelial progenitor cells responsible for postnatalva Sculogenesi sinphy siological and pathologi-Cal ne OV as cularization Cir C. Res. 1999; 85: 221–228.
24. Huang J., Yao X., Weng G. et al. Protective effect of curcumin against cyclosporine A-induced rat nephrotoxicity. Mol. Med. Rep. 2018; 17(4): 6038–6044.
25. Buffoli B. et al. Provinol prevents CsA-induced nephrotoxicity by reducing reactive oxygen species, iNOS, and NF-kB expression. J. Histochem. Cytochem. 2005; 53(12): 1459–1468.
26. Mazali F.C., Johnson R.J., Mazzali M. Use of uric acid-lowering agents limits experimental cyclosporine nephropathy. Nephron Exp. Nephrol. 2012; 120(1): e12–e19.
27. Aktoz T., Aydogdu N., Alagol B. et al. The protective effects of melatonin and vitamin E against renal ischemia-reperfusion injury in rats. Ren. Fail. 2007; 29(5): 535–542.
28. Liu H.B., Meng Q.H., Huang C. et al. Nephroprotective effects of polydatin against ischemia/reperfusion injury: a role for the PI3K/Akt signal pathway. Oxid. Med. Cell. Longev. 2015; 201p.
29. Rossoni G., Gomaraschi M., Berti F. et al. Synthetic high-density lipoproteins exert cardioprotective effects in myocardial ischemia/reperfusion injury. J. Pharmacol. Exp. Ther. 2004; 308(1): 79–84.
30. Chiao H., Kohda Y., McLeroy P. et al. Alpha-melanocyte-stimulating hormone protects against renal injury after ischemia in mice and rats. J. Clin. Invest. 1997; 99(6): 1165–1172.
31. Abdallah H.N. et al. Zinc mitigates renal ischemia–reperfusion injury in rats by modulating oxidative stress, endoplasmic reticulum stress, and autophagy. J. Cell. Physiol. 2018; 233(11): 8677–8690.
32. Fu Z.J. et al. HIF-1α-BNIP3-mediated mitophagy in tubular cells protects against renal ischemia/reperfusion injury. Redox Biol. 2020; 36: 101671.

33. Hagar H.H., El Etter E., Arafa M. Taurine attenuates hypertension and renal dysfunction induced by cyclosporine A in rats. Clin. Exp. Pharmacol. Physiol. 2006; 33(3): 189–196.
34. Heibashy M., El-Nahrawy W., Zaki N. Cyclosporine-Induced Oxidative Stress and renal Dysfunction in Rat kidneys: A Possible Ameliorated Effect by Curcumin as an Antioxidant. Bull. Egypt. Soc. Physiol. Sci. 2009; 29(2): 179–194.
35. Hussein S.A., Ragab O.A., El-Eshmawy M.A. Protective effect of dietary fish oil on cyclosporine a-induced nephrotoxicity in rats. Benha Vet Med J. 2013; 25(2): 218–231.
36. Hussein S.A., Ragab O.A., El-Eshmawy M.A. Renoprotective effect of dietary fish oil on cyclosporine a: induced nephrotoxicity in rats. Asian J. Biochem. 2014; 9(2): 71–85.
37. Kasap B. et al. Protective effect of Epo on oxidative renal injury in rats with cyclosporine nephrotoxicity. Pediatr. Nephrol. 2008; 23(11): 1991–1999.
38. Burdmann E.A., Andoh T.F., Yu L., Bennett W.M. Cyclosporine nephrotoxicity. Seminars in nephrology. 2003; 23(5): 465–476.
39. Garcia-Cohen E.C., Marin J., Diez-Picazo L.D. et al. Oxidative stress induced by tert-butyl hydroperoxide causes vasoconstriction in the aorta from hypertensive and aged rats: role of cyclooxygenase-2 isoform. J. Pharmacol. Exp. Ther. 2000; 293(1): 75–81.
40. Sereno J. et al. Transition from cyclosporine-induced renal dysfunction to nephrotoxicity in an in vivo rat model. Int. J. Mol. Sci. 2014; 15(5): 8979–8997.
41. Amudha G., Josephine G., Varalakshmi P. Role of lipoic acid in reducing the oxidative stress induced by cyclosporine A. Clin. Chim. Acta. 2006; 372(1–2): 134–139.
42. Mohamadin A.M., El-Beshbishy H.A., El-Mahdy M.A. Green tea extract attenuates cyclosporine A-induced oxidative stress in rats. Pharmacol. Res. 2005; 51(1): 51–57.
43. Cid T.P., Garcıa J.R.C., Alvarez F.C., De Arriba G. Antioxidant nutrients protect against cyclosporine A nephrotoxicity. Toxicology. 2003; 189(1–2): 99–111.
44. Rubin L.A. et al. Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. J. Immunol. 1985; 135(5): 3172–3177.
45. Wang X., Rickert M., Garcia K.C. Structure of the Quaternary Complex of Interleukin-2 with Its α, ß, and γc Receptors. Science (80). 2005; 310(5751): 1159–1163.
46. Ruiz-Ortega M., Lorenzo O., Rupérez M. et al. Angiotensin II activates nuclear transcription factor κB through AT1 and AT2 in vascular smooth muscle cells: molecular mechanisms. Circ. Res. 2000; 86(12): 1266–1272.
47. Li C. et al. Long-term treatment with ramipril attenuates renal osteopontin expression in diabetic rats. Kidney Int. 2003; 63(2): 454–463.
48. Campistol J.M., Sacks S.H. Mechanisms of nephrotoxicity. Transplantation. 2000; 69(12): SS5–SS10.
49. Marchiani A., Rozzo C., Fadda A. et al. Curcumin and curcumin-like molecules: from spice to drugs. Curr. Med. Chem. 2014; 21(2): 204–222.
50. Lin J.K. Molecular targets of curcumin. Mol. targets Ther. uses curcumin Heal. Dis. 2007; 227–243.
51. Ammon H.P.T., Wahl M.A. Pharmacology of Curcuma longa. Planta Med. 1991; 57(01): 1–7.

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Conflict of interest:
The Authors declare no conflict of interest

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Received: 25.06.2021
Accepted: 29.11.2021

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article