Original Article

Improvement of Kidney Injury Molecule-1 (KIM-1) and N-Acetylglucosamindase (NAG) in the Kidney Following Platelet Rich Plasma (PRP) Administration in the Treatment of Cyclosporine A-Induced Nephrotoxicity in Adult Male Albino Rats

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ABSTRACT

Introduction: Cyclosporin A (CsA) is a drug used to suppress the immune system thus treating rejection of transplantation and autoimmune diseases. However, adverse effects limit its use in clinical practice. PRP is an autologous growth factor-rich product that appeared useful as a product allowing regeneration of tissues by releasing growth factors that alleviate destruction of tissues.

Material and Methods: Forty adult male albino rats were involved in this work. They were classified into four equal groups; control, sham control, CsA-treated, Cyclosporine A-PRP treated. Histological (H & E and Masson's trichrome), immunohistochemical (caspase-3 and TGFβ-1), biochemical assessment (serum urea, create, tissue level of Glutathione reductase, SOD and MDA, and tissue expression of KIM-1 and NAG) were performed.

Results: In CsA treated group, renal cortex revealed shrunken segmented glomerulus with darkly stained nuclei, widened urinary space, interstitial hemorrhage, and intraluminal casts. Other sections showed hypercellular glomerulus with extremely narrow urinary space, intraglomerular hemorrhage, tubular vacuolations and karyolysis. Also, there existed too much accumulation of collagen fibers around and within glomeruli in addition to around the tubules in group III. Immunohistochemically, Sections from group III showed strong positive reaction of caspase-3 and TGFβ-1. Biochemically there was elevated serum urea, creatinine, and tissue MDA level together with decreased tissue level SOD and Glutathione reductase in group III. PRP could ameliorate histopathological nephrotoxic effects induced by CsA. As well as improving biochemical markers and gene expression values.

Conclusion: PRP appeared to be a safe product and could be used to reverse the adverse effects induced by CsA administration.

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Key Words: Cyclosporin A, kidney, KIM-1, NAG, PRP.

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INTRODUCTION

Cyclosporin A (CsA) is a drug used to suppress the immune system in order to treat rejection of transplantation and autoimmune diseases having a better overall efficacy in preventing allograft rejection[1]. However, severe undesired effects, as toxic effect on kidney, neurotoxicity, hepatotoxicity, raised blood pressure, and dyslipidemia, limit its use in clinical practice[2].

Renal function impairment and morphological damage are both signs of CsA renal toxic effect[3]. The activation of the renin-angiotensin-aldosterone system, up-regulation of the transforming growth factor-beta (TGF-β), direct activation of apoptosis genes and enhanced apoptosis in tubular and interstitial cells, and oxidative stress are thought to be the underlying mechanisms of toxicity[4].

Marx *et al*.,[5] were the first to describe platelet-rich plasma (PRP). It is a product containing autologous growth factors[6]. Many growth factors, like epidermal growth factor (EGF), hepatocyte growth factor (HGF), platelet derived growth factor (PDGF), and vascular endothelial growth factor (VEGF), are locally secreted by PRP over a period of three weeks following administration[7].

According to some studies exogenously administered epidermal growth factor (EGF) as well as hepatocyte growth factor (HGF) administration improves renal tubular cell regeneration, repairs, and speeds up renal function recovery[8].

Though PRP has been shown useful as a product permitting tissue regeneration by releasing growth factors that alleviate tissue destruction, its effect on nephrotoxicity caused by CsA is not tested yet upon reviewing previous literature.

The present work was designed to detect the adverse effects of cyclosporine A (CsA) on the kidney of adult male albino rat and to evaluate the therapeutic effect of platelet-rich plasma (PRP) on CsA-induced nephrotoxicity.

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MATERIAL AND METHODS

Chemicals

Cyclosporin A (CsA) is available as Sandimmune soft gelatin capsules 100 mg each, it was purchased from Novartis pharma, Egypt. Preparation of CsA was allowed to dissolve in 2.5 ml olive oil and introduced subcutaneously into rats[3].

Platelet-rich Plasma (PRP) samples were extracted from 10 healthy adult male albino rats of the same age as PRP donors. The process was held out in the Biochemistry Department of Kasr Alainy Faculty of Medicine, Cairo University. It was introduced once by being injected subcapsular in the kidney of rats[9].

Whole blood from PRP donor rats was extracted retro-orbitally. The blood was combined with 3.2 % sodium citrate in a ratio of 9/1, subjected to centrifugation at 400 g for a duration of 10 minutes, then separated and recentrifuged at 800 g for 10 minutes. The platelet which is poor in plasma (PPP) in the upper 2/3 of the sample was eliminated. The remaining 1/3 was extracted as PRP. The PRP to be used was allowed to freeze at -80°C. A sysmex XT-1600i system was applied to determine the average PRP. The count of platelet was 2410 × 10^3 platelets/µl. CaCl2 10 % (0.8 ml PRP + 0.2 ml CaCl2 10%) was utilized to allow activation of PRP prior its use[9].

Animals

The experimental study received approval of Cairo University Institutional Animal Care and Use Committee (CU-III-F-77-19). A number of forty adult male albino rats of 180-220 g were incorporated in the study. The rats were brought from the animal house, Faculty of Medicine, Cairo University. The rats were acclimatized to standard laboratory and environmental factors. They were classified into four groups, each of them contained ten animals.

Group I (control): the animals were given no medication and were sacrificed after 2 weeks.

Group II (sham control): the animals were given 1ml of olive oil (the vehicle) subcutaneously for 2 weeks. 24 hours after the last dose of olive oil, they received 1 ml of saline as a single subcapsular injection in each kidney and sacrificed after another 2 weeks[9].

Group III (CsA-treated): the animals received cyclosporin A (CsA) subcutaneously; the dose of CsA was 100 mg/kg/day dissolved in olive oil (each capsule was dissolved in 2.5 ml olive oil) over a period of 2 weeks[3] then they were sacrificed immediately after the 2 weeks.

Group IV (CsA-PRP-treated): the rats received CsA as group III, next, 24 hours later the animals received 1 ml PRP as a single subcapsular injection in each kidney, then sacrificed after another 2 weeks[9]. Rats were sacrificed by cervical dislocation.

After a 12-hour fast and acclimatization period, the animals in groups IIb (sham control) and IV (CsA-PRP-treated) were operated on. A subcostal lumbar incision was made with the animal placed in a lateral recumbent position to expose the kidney, then the procedure was repeated on the other side[8].

Activated PRP was injected subcapsular in each kidney through five punctures (anterior, posterior, lateral, upper pole, and lower pole) in the CsA-PRP-treated group (IV) to spread the whole quantity of PRP on the entire surface of each kidney[7]. 1 ml of saline was injected subcapsular on each side in the sham control group (IIb), using the same procedure as group IV.

The guidelines concerned with the care and utilization of animals on the international, national, and institutional bases were applied.

By the end of the assigned period for each experimental group, blood samples were collected from the retroorbital venous plexus for biochemical study, then rats were sacrificed by cervical dislocation and kidneys were extracted.

Histological study

Tissues obtained from kidney were fixed in 10% formalin overnight and prepared for paraffin blocks and then successive sections of 5µ thick were prepared. Sections underwent hematoxylin and eosin and Masson's trichrome staining.

Biochemical study

Samples of blood were obtained by performing retro-orbital puncture. After being centrifuged, serum was obtained and stored at -80 °C. All rats had their blood urea nitrogen and serum creatinine levels checked. Quanti Chrom TM assay kits 2 were used to estimate measurements using a traditional colorimetric approach[10].

Kidney samples were subjected to homogenization with 0.1M phosphate-buffered saline at pH7.4, to reach a concentration of 10% w/v at the end and stored at−20°C for determination of tissue levels of malondialdyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD) biochemically. The procedures were carried following the manufacturer's (Biodiagnostics) instructions.

Biochemical determination of tissue N-acetyl glucosaminidase (NAG) level by Sandwich ELISA technique (ug/ml) has been done as well. The quantitative sandwich enzyme immunoassay approach was performed using the NAG ELISA kit provided by My Biosource, USA. NAG, if present, would bind to the antibody pre-coated wells when the samples were added to the microtiter plate wells.

In addition, mRNA expression of kidney injury molecule-1 (KIM-1) as a marker of injury of proximal tubules injury has been performed using Q-PCR. The use of fluorescent reporter molecules in this process distinguishes real-time quantitative polymerase chain reaction (qPCR) from standard PCR. Whole RNA was

isolated from homogenized tissue by the SV Total RNA Isolation method, which included a DNase treatment phase, following the manufacturer's recommendations (Thermo Scientific, USA). A high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific, USA) was utilized to convert total RNA (1 g) to cDNA. Using known gene sequences, gene-specific primers for KIM-1 were generated with the help of a digitally perogrammed Primer Express 2.0 (Applied Biosystems, Foster City, CA). The following were the primer sequences for rats: Forward primer 5′AGAGAGCAGGACACAGGCTT-3′ and reverse primer 5′ACCCGTGGTAGTCCCAAACA-3′ were used on rats. The 7900 HT Real Time PCR System (Applied Biosystems) was applied to gain multiples and to detect using SYBR Green emission (SYBR Green Master Mix, Applied Biosystems). PCR cycles involved 40 cycles at 95°C for 15 seconds and 60°C for 60 seconds. The relative standard curve approach was used to quantify gene expression relative to controls after normalizing it with the mean of β-actin mRNA content. Finally, the data was presented as 2-ΔCT (CT threshold cycle).

Immunohistochemical study

The peroxidase-labeled Streptavidine-Biotin method was used for immunohistochemical study[11]. The slices were subjected to 1.5 % normal goat serum in PBS. An anti-caspase-3 mouse monoclonal primary antibody (Dako company, Cairo, Egypt, Catalog No.IMG-144A at a dilution 1/200) was applied for determining apoptosis[12]. An anti-transforming growth factor beta-1 (TGFβ-1) rabbit monoclonal antibody (clone TB21, MCA 797, Serotec, Oxford, UK at 1:200 dilution) was used to detect tissue fibrosis[13]. Slides were subjected to rinsing well in PBS then incubated with biotinylated secondary antibody. Substrate chromagen 3,3'- diaminobenzidine (DAB) was added then cleaned well with distilled water to view the result in the form of a brown-colored product in the cytoplasm both for caspase-3 and TGFβ-1. The slides were dehydrated and mounted after being counterstained with hematoxylin. Human tonsillar tissue was used as a positive control for caspase-3, and liver tissue was used as a positive control for TGFβ-1. Negative control was acquired through omission of the primary antibody in the automated staining protocol according to the manufacturers' instructions.

Histomorphometric assessment

The information was gathered with the help of the "Leica Qwin 500 C" image analyzer computer system (Cambridge, England). In each renal cortical region, ten fields with no overlap were selected on a random base for determination of the required features at a magnification of 400. Investigations were done using the standard frame of measuring; a known area equal to 11694 μm^2. The following areas percent were measured in the renal cortex:

1. Area percent of collagen fibers stained by Masson's trichrome stain
2. Area percent of Caspase-3 immunopositive expression
3. Area percent for transforming growth factor beta-1 (TGFβ-1) immunopositive expression

Statistical analysis

Data were analyzed Statistically using statistical package for the social sciences (SPSS) version 21.0 (IBM Corporation, Somers, NY, USA) statistical software. The results were represented as means ± standard deviation (SD). Evaluation of statistics was done using one-way analysis of variance (ANOVA) followed by Bonferroni pairwise comparisons. Significance was applied when the *p value* was less than 0.05.

RESULTS

Light microscopic results

Hematoxylin and Eosin-stained sections of the control and sham control groups revealed the standard histological architecture of renal cortex with normal renal glomerulus formed of tuft of capillaries around which Bowman's capsule has a parietal layer of simple squamous epithelium and visceral podocytes with regular narrow urinary space in between. Proximal convoluted tubules exhibited well-defined brush border lined with cuboidal to low columnar cells with markedly acidophilic cytoplasm and narrow lumen, while distal convoluted tubules having lower cuboidal cells with less acidophilic cytoplasm and rounded central nucleus with less distinct brush border and wider lumen (Figures 1A,B)

In CsA treated group (III), the renal cortex revealed markedly shrunken segmented glomerulus with darkly stained nuclei of glomerular cells and widened urinary space, together with areas of interstitial hemorrhage and intraluminal casts (Figure 1C). Other sections showed hypercellular glomerulus with extremely narrow urinary space and intraglomerular hemorrhage, as well as tubular cytoplasmic vacuolation, karyolysis and intraluminal casts (Figure 1D).

In CsA-PRP treated group (IV) renal cortex showed restoration of renal glomerulus to its normal appearance with narrow urinary space, as well as restoration of proximal tubules to their control appearance with intact brush border, similarly distal tubules almost revealed a normal appearance. However, some tubules exhibited cytoplasmic vacuolation with intraluminal casts, very few tubules showed karyorrhexis and karyolysis together with interstitial hemorrhage (Figure 1E).

In Masson's trichrome stained sections, there were minimal accumulation of blue-stained collagen fibers around the glomerulus as well as the tubules in groups I and II (Figures 1F,G). However, there was too much accumulation of blue stained collagen fibers around and within the glomeruli as well as around tubules, moreover, there existed vascular dilatation together with marked accumulation of collagen fibers around blood vessels, as well as thickening of the vessel wall in group III (Figures 1H,I). Meanwhile group IV showed minimal

deposition of collagen fibers around the glomerular capillaries as well as renal tubules (Figure 1J).

Regarding the immunohistochemical expressions of control groups of caspase-3 (Figures 2A,B) and transforming growth factor beta-1 (Figures 2E,F) exhibited very weak cytoplasmic expression in cells of PCT, DCT and glomeruli. Sections from group III showed strong cytoplasmic expression in the form of dark brown granules (Figure 2C for caspase-3) and (Figure 2G for TGFβ-1). However, in group IV there was a weak cytoplasmic expression (Figure 2D for caspase-3) and (Figure 2H for TGFβ-1).

The area percent of Masson's trichrome, caspase-3 and TGFβ-1 in the renal cortex of group III has been significantly higher (P = 0.000) than the control, sham control groups as well as group IV. However, comparing the results of group IV with that of group II there was a significantly higher value (P = 0.000) (Figures 3A,B,C respectively).

Biochemical and Molecular Results

Serum urea was significantly increased (P = 0.000) in group III whose mean value was 122.6 ± 0.83 mg/dl compared to group I, IV whose mean values were 34.24 ± 0.55 mg/dl and 74.49 ± 4.5 mg/dl respectively. Meanwhile serum urea level was a significantly higher (P = 0.000) in group IV in comparison to sham control group II whose mean value was 34.72 ± 1.22 mg/dl (Figure 3D).

Serum creatinine was significantly higher (P = 0.000) in group III with mean value 1.42 ± 0.09 mg/dl as compared to group I, IV whose mean values were 0.28 ± 0.15 mg/dl and 0.46 ± 0.12 mg/dl consecutively. At the same time group IV had a significantly higher (P = 0.037) value when compared to group II whose mean value was 0.28 ± 0.12 mg/dl (Figure 3E).

Oxidative markers

MDA level in nmol/gm tissue

Kidney sections of group III showed marked increase in renal MDA where the mean value was 118.04 ± 1.66 nmol/mg protein. On comparing its results with those of group I and IV whose mean values were 41.62 ± 0.63 nmol/mg and 60.31 ± 4.07 nmol/mg protein respectively there was a significant increase (P = 0.000) in mean MDA level. Similarly on comparing the results of group IV with those of group II whose mean value was 42.19 ± 0.57 nmol/mg protein, there existed a significantly higher difference (P = 0.000) (Figure 3F).

SOD level in µ/mg tissue

Renal sections of group III showed marked decrease in renal SOD level with a mean value of 9.90 ± 0.53 µ/mg protein with a significantly lower value (P = 0.000) on comparing its results with those of group I and IV whose mean values were 21.98 ± 0.70 µ/mg protein and 18.46 ± 1.36 µ/mg protein respectively. Comparing the results of group IV with those of group II there was also a significantly lower value (P = 0.000) (Figure 3G).

Glutathione reductase (GSH) level in mmol/g tissue

Kidney sections of group III showed marked decrease in renal GSH levels with a mean value of 38.36 ± 3.94 mmol/g protein and recovery group (IIIb) had a mean value of 40.02 ± 1.81mmol/g protein with a significant decrease (P = 0.000) has been encountered on comparing its results with those of group I and IV whose mean values were 91 ± 2.08 and 69.88 ± 8.01 mmol/g protein respectively. Group IV also revealed a significant decrease (P = 0.000) in the renal GSH level in contrast to group II whose mean value was 91.98 ± 1.86 mmol/g protein (Figure 3H).

Gene expression result

Kidney injury molecule-1 (KIM-1)

Statistical study in group III whose mean value was 5.24 ± 0.52 revealed a significant increase (P = 0.000) in KIM-1 expression (fold change) in contrast to group I and IV whose mean values were 1.10 ± 0.04 and 2.14 ± 0.12 respectively. Meanwhile, Group IV displayed a significant higher (P = 0.000) KIM-1 expression when compared to group II whose mean value was 1.11 ± 0.06 (Figure 3I).

N-acetyl glucosaminidase (NAG) expression in µg/ml

The mean level of NAG expression in group III was 9.18 ± 0.81 µg/ml, with a significant increase (P = 0.000) in contrast to mean values of group I, IV whose mean values were 2.40 ± 0.15 and 4.62 ± 0.99 µg/ml respectively. In addition, the mean value of group IV was significantly higher (P = 0.000) in contrast to group II whose mean value was 2.48 ± 0.18 µg/ml (Figure 3J).

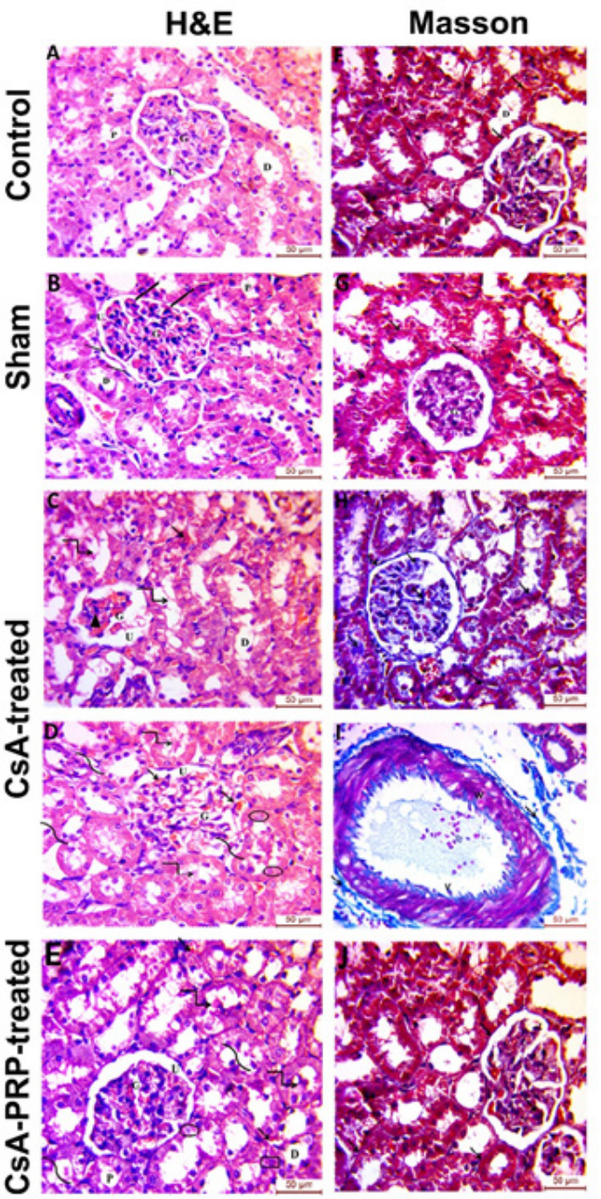

Fig. 1 × 400 :

Haematoxylin and eosin stained section of control and sham control groups (A,B) showing a normal renal glomerulus (G) with regular narrow urinary space (U) formed of tuft of capillaries surrounded with bowman's capsule formed of a parietal layer of simple squamous epithelium (thin line) and visceral podocytes (thick line) with regular narrow urinary space (U) in between , proximal convoluted tubules (P) with well-defined brush border lined by cuboidal to low columnar cells with strongly acidophilic cytoplasm and distal convoluted tubules (D) with less distinct brush border lined by lower cuboidal epithelium, with less acidophilic cytoplasm and rounded central nucleus .

Haematoxylin and eosin stained section of CsA treated group (C, D) showing markedly shrunken segmented glomerulus (G) with darkly stained nuclei of glomerular cells (black arrowhead) and widened urinary space (U). There are areas of interstitial hemorrhage (thin arrow) together with intraluminal casts in distal tubule (elbow arrow). Other sections showed hypercellular glomerulus (G) with extremely narrow urinary space (U) and intraglomerular hemorrhage (thin arrow), as well as tubular cytoplasmic vacuolation (curved connector), karyolysis (oval), intraluminal casts (elbow arrow).

Haematoxylin and eosin stained section of CsA-PRP treated group (E) showing restoration of glomerulus to its normal appearance (G) with beginning of narrowing of the urinary space (U), as well as restoration of proximal tubules (P) to their normal appearance with intact brush border, distal tubules (D) almost have a normal appearance. However, some tubules exhibited cytoplasmic vacuolation (curved connector) with intraluminal casts (elbow arrow) and very few tubules showed karyorrhexis (rectangle) and karyolysis (oval) together with minimal interstitial hemorrhage (thin arrow)

Masson's trichrome stained section of control and sham control groups (F, G)are showing minimal blue stained collagen fibers around glomerulus (G) and around tubules (P, D) (thin arrow).

Masson's trichrome stained section of CsA-treated group (H, I) showing excessive deposition of collagen fibers around and within the glomerulus (G), as well as around and within tubules (thin arrow). Other sections are showing showing vascular dilatation (V), together with excess deposition of collagen fibers (thin arrow) around blood vessel, as well as thickening of the vessel wall (W).

Masson's trichrome stained section of CsA-PRP treated group (J) showing minimal blue stained collagen fibers around and within the glomerulus, as well as around tubules (thin arrow).

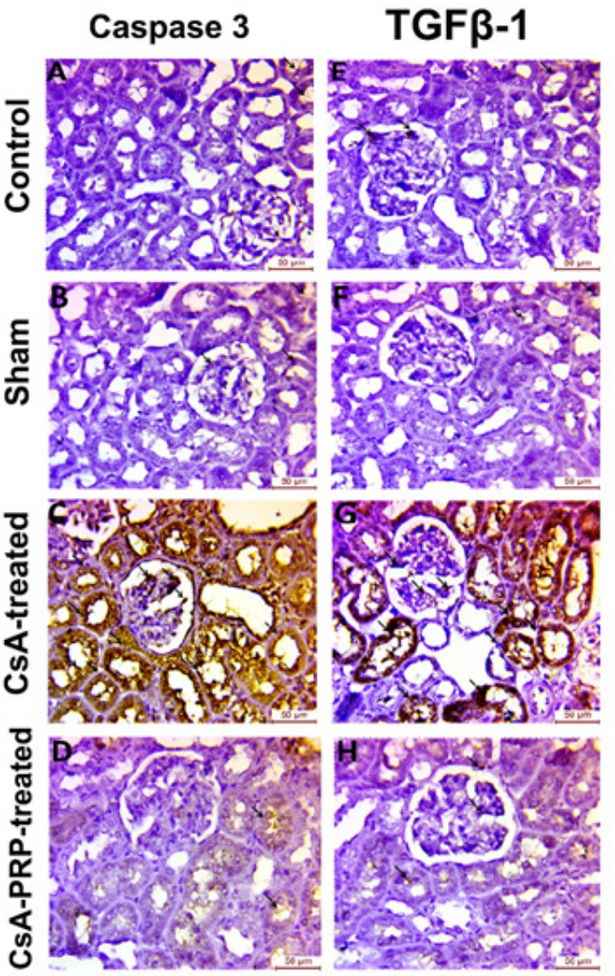

Fig. 2 × 400:
Caspase-3 immuno-expression in control and sham control groups (A, B) is very weak within the glomeruli and tubules (thin arrow).
Caspase-3 immuno-expression in CsA treated group(C) is strong within the glomeruli and tubules appearing as dark brown granules (thin arrow).
Caspase-3 immuno-expression in CsA-PRP treated group (D) is weak within the glomeruli and tubules (thin arrow).
TGFβ-1 immunoexpression in control and sham control groups (E, F)is very weak within the glomeruli and tubules (thin arrow).
TGFβ-1 immunoexpression in CsA treated group (G) is strong within the glomeruli and tubules appearing as dark brown granules (thin arrow).
TGFβ-1 immunoexpression in CsA-PRP treated group (H) is weak within the glomeruli and tubules (thin arrow).

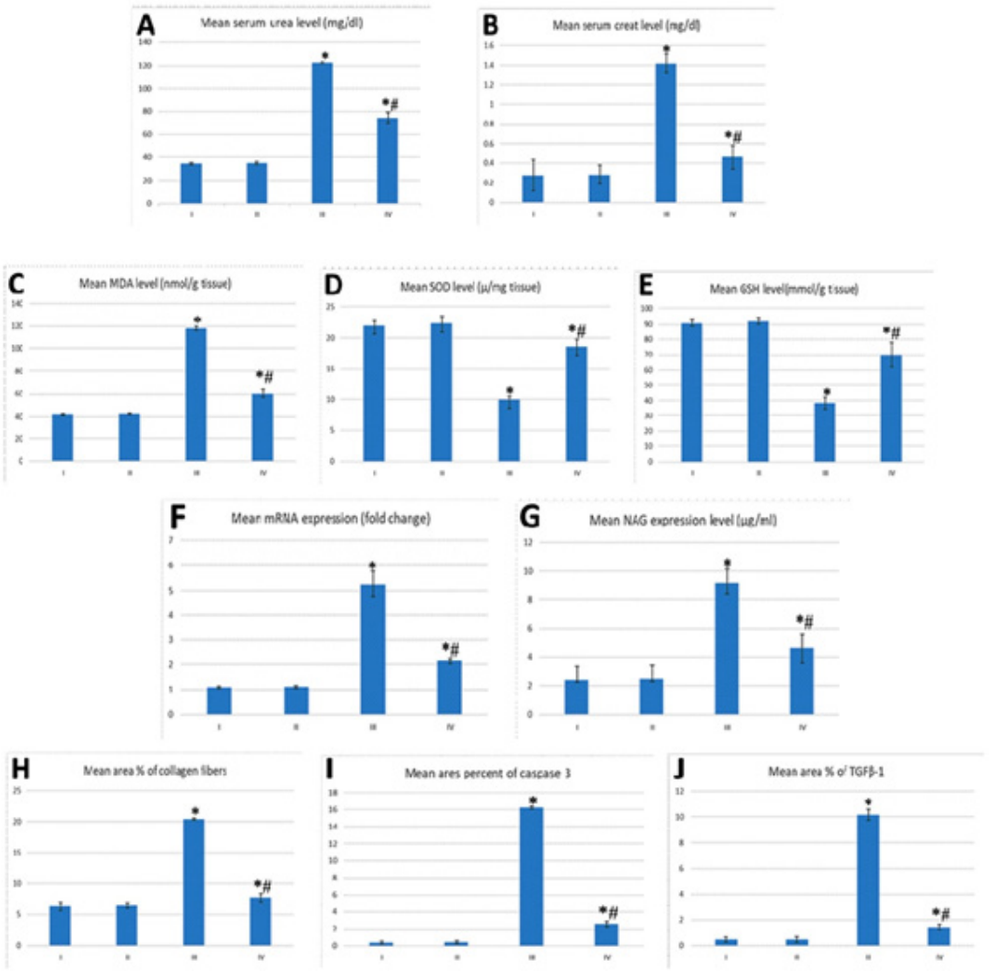

Fig. 3: Different levels of biochemical, molecular markers and mean area percent of collagen fibers, caspase-3 and TGFβ-1 expression.
Data were expressed as Mean ± SD, P value was significant if ≤ 0.05.
* significant versus control
# significant versus CsA treated group

DISCUSSION

CsA has improved transplant outcomes dramatically in various organs from the beginning of its utilization in the 1980s for decreasing transplant rejection[14]. The incidence of longstanding CsA toxic effect on kidney following its long use, as in patients with organ transplantation or nephrotic syndrome resistant to steroids, is around 30–40%[15]. The aim of this work was to assess the potential therapeutic effect of PRP in chronic CsA related nephrotoxicity in rats. This was applied in relation to histopathological, immunohistochemical, biochemical and molecular changes.

The present study revealed histopathological changes in renal cortex as evidenced by light microscopic examination of hematoxylin and eosin-stained sections of CsA-treated group (III). The renal cortex showed markedly shrunken segmented glomeruli with widened urinary space, together with areas of interstitial hemorrhage and intraluminal casts. These findings are in concordance with those of Salem *et al*.2018[9] and Baher *et al*.2019[16].

The increased endothelial damage could explain the smaller segmented glomeruli[17]. Bowman's space dilation could be caused by vascular alterations (afferent arteriolopathy combined with hyaline thickening of the arteriolar wall) that cause glomerular capillaries to collapse[3]. Interstitial hemorrhage is thought to be caused by either an active or passive vasoactive action on the endothelium of blood vessels through the formation of reactive oxygen species, according to Prozialeck *et al*. 2008[18]. Finally, protein leaking from injured glomerular cells could be the cause of intraluminal casts[19].

In addition, other sections of group III revealed hypercellular glomeruli with extremely narrow urinary spaces and intraglomerular hemorrhage. This agrees with the findings of Elwan *et al*. 2018[20] and Sewelam and Mokhtar 2019[21]. Hypercellular glomeruli with a very

little urinary space may be linked to significant vascular congestion[22].

In the current study, group III showed involvement of many tubular cells which showed cytoplasmic vacuolations as well as cast formation. Ghoniem *et al*. 2012[23] discovered similar results. According to Therien and Blostein 2000[24], tubular vacuolations are caused by a malfunctioning tubular sodium pump, which leads to hydropic tubular cell degeneration. Alwin and Arthur 2009[25] added that a rise in the osmotic gradient across the plasma membranes of cells caused tubular cells to withdraw water, and consequently swell and vacuolate.

Sakr *et al*. 2017[26] linked the formation of intraluminal casts to a mixture of sloughed tubular cells and Tamm-Horsfall protein is a glycoprotein generated exclusively via renal tubular epithelial cells inside the tubular lumen. Furthermore, the author claimed that an increase in sodium concentration in the lumen caused by impaired sodium reabsorption by injured tubular cells resulted in Tamm-Horsfall protein polymerization, and thus the production of a gel-like substance, which contributed to cast formation.

In the current study, nuclear alterations such as karyorrhexis and karyolysis were also found in the CsA-treated group. Navarro-Moreno *et al*. 2009[27] discovered similar results. Silva 2004[28] interpreted these nuclear alterations as a result of protein synthesis inhibition and interference with nucleic acid production.

Based on all the preceding data in this study, it was observed that tubular affection was greater than glomerular affection. This is supported by most of the published research. Though glomeruli are undoubtedly important, tubular damage and interstitial fibrosis in chronic kidney disease correlate more strongly with renal tubular dysfunction than glomerular alterations. Tubular cells are particularly vulnerable to diseases due to their reliance on aerobic metabolism and high energy consumption[29].

Although PRP has been shown as useful product allowing tissue regeneration by releasing growth factors that alleviate tissue destruction[9], its effect on CsA-induced nephrotoxicity has not been studied yet in the available literature. Many studies, however, have expressed concern on the therapeutic and preventive effects of PRP in chronic nephrotoxicity[9,16].

In this study, the microscopic findings of the CsA-PRP-treated group (group IV) revealed considerable improvement. There was reduction of the degenerative alterations seen in group III and restoration of majority of the glomeruli and tubules of the kidney to their standard control look. The glomeruli had a rounded appearance with maintained architecture and narrow urinary spaces. The appearance of the proximal convoluted tubules was restored. However, only few tubules had cytoplasmic vacuolation in conjunction with intraluminal casts, and only few tubules had karyorrhexis and karyolysis in conjunction with interstitial hemorrhage.

The results of this work were like those of Salem *et al*. 2018[9], Ahmed and Fouad 2019[30], Keshk and Zahran 2019[31] and El Agwany *et al*. 2022[32].

PRP enhances regeneration of tissues by improving cellular recruitment, development, and differentiation. It has been proved that PRP also show anti-inflammatory and immunomodulation properties[6]. PRP promotes healing by releasing cytokines and growth factors (GFs) from platel *et al* pha granules[9]. Vascular endothelial growth factor (VEGF) increases endothelial cell chemotaxis and proliferation, as well as formation of new vessels, vascular hyperpermeability, and renal stem cell differentiation[33]. In addition, it reduces tubular damage and preserves renal parenchymal integrity, filtration capacity of kidney, blood flow to renal vessels, and excretory function of the kidney as proved by Sanchez-Gonzalez *et al*. 2011[33].

In the current study Masson's trichrome stained sections of group III revealed excessive accumulation of blue stained collagen fibers around as well as within the glomeruli, as well as around tubules. Consequently there was a significantly larger mean area percentage of collagen fibers expression in Masson's trichrome stained sections group III than group I, II and IV. These results are similar to those of Korolczuk *et al*. 2010[3] and Xiao *et al*. 2013[34]. In addition to vascular dilatation, excessive collagen fibers deposition around blood vessels, as well as thickening of blood vessel walls. These findings agree with those of Uz *et al*. 2008[35], where they found vessel walls' thickening in a segmental pattern in the form of a 'necklace' along the exterior adventitial side of arterioles, as well as interstitial fibrosis.

CsA enhances the epithelial-mesenchymal transition (EMT) in renal cells, according to Liu *et al*. 2017[36]. The inflammatory-induced fibrosis and eventual kidney failure are hypothesized to be aided by EMT[37]. Those suffering from systemic lupus erythmatosus, an autoimmune illness associated with renal fibrosis, exhibits a high value of osteopontin circulating in blood, according to Wirestam *et al*. 2017[38]. Osteopontin may contribute to kidney inflammation, which leads to interstitial fibrosis; these data imply that it could be involved in interstitial fibrosis of kidney caused by CsA[39].

Unlike the current findings, Uludağ *et al*. 2018[2] declared that interstitial fibrosis was not detected in any sample. There was no wall thickening of arteries and arterioles. This controverse could be attributed to the application of different dose of CsA. Elzinga *et al*. 1993[40] mentioned that an amount of 15 mg/kg/day for 15 days could cause renal toxic effect if it was combined with low sodium diet. Uludağ *et al*.[2] gave the amount of 15 mg kg/day for 15 days without diet low in sodium, which could explain the absence of fibrosis.

Fortunately, group IV showed minimal accumulation of collagen fibers around glomerular capillaries and tubules of kidney, indicating that the substantial collagen deposition reported in group III had resolved. This agrees with Salem *et al*. 2018[9].

This improvement may be attributed to the existence of hepatocyte growth factor (HGF) in PRP, which inhibits the activation of fibroblasts in interstitial tissue and epithelial-to-mesenchymal transition of tubules[8]. Many studies, including those of Anitua *et al*. 2012[41], Chellini *et al*.2019[42], Van der Bijl *et al*. 2019[43], and Squecco *et al*. 2020[44], support the power of PRP to reverse the basic fibrotic response of cells, particularly the transformation of fibroblasts into myofibroblasts caused by profibrotic member; transforming growth factor beta-1 (TGFβ-1).

Two significant markers have been studied immunohistochemically in this study: caspase 3 and transforming growth factor beta-1 (TGFβ-1). Caspases are a class of aspartate-specific cysteine proteases that are key mediators in apoptotic and inflammatory pathway. Apoptosis (programmed cell death) is a compound vital mechanism that regulates cell survival by eliminating damaged or diseased cells[45].

Sections from CsA-treated group III showed strong cytoplasmic caspase-3 immunoreactivity in PCT, DCT and glomeruli. Consequently, the histomorphometric analysis of this study has reflected this result. In contrast to control and PRP treated groups, group III had a considerably higher mean area percent of caspase-3 immunoexpression in the kidney sections. These data are like those of Topcu *et al*. 2016[46] and Yang *et al*. 2001[47].

Chronic ischemic processes caused by chronic CsA consumption produce more reactive oxygen species than the compensatory capacity of biological antioxidant molecules. Death of kidney cells by apoptosis, interstitial fibrosis, and atrophy of tubules is caused by increased oxidative stress[48].

CsA-PRP-treated group (IV) showed little caspase-3 expression similar to the findings of Salem *et al*. 2018[9]. Hepatocyte growth factor (HGF) considered a prominent factor in PRP has been discovered to prevent renal cell death[49]. Additionally, epidermal growth factor (EGF) secreted in response to damage has been shown to hasten the renal healing process[50].

Transforming growth factor beta-1 (TGFβ-1) is a mediator that aids in the progression of glomerulosclerosis and tubulointerstitial fibrosis[51]. TGF-1 overproduction, as in the case of chronic inflammation, results in excess accumulation of extracellular matrix at the site of damage, resulting in fibrosis[52].

In contrast to group I and IV, group III showed highly positive TGFβ-1 immunoreactivity. With a consequent, persistent increase rise in mean area percent of TGFβ-1 immunoexpression. These results are similar to those of Kim *et al*. 2016[14] and Kilari *et al*. 2018[53]. The reduction in TGFβ-1 immunoexpression in group IV agrees with the findings of Salem *et al*. 2018[9]. According to the research of Sampson *et al*. 2008[54] PRP can promote fibrosis due to high levels of TGF-1 contained in platel*et al*pha granules, which encourage type I collagen formation. It is also an antiapoptotic mediator for myfibroblasts, and it has the potential to promote fibroblast differentiation into active myofibroblast. However, Sugaria *et al*. 2010[55] suggested that the effects of PRP are opposite to each other regarding the progression of fibrosis; as PRP contains anti-fibrotic molecules, hepatocyte growth factors (HGF), and serum amyloid protein, which has the ability to prevent fibrosis and modulate the role of macrophage function in different models. HGF inhibits TGF-beta receptor-related expression as well as other mediators that favors fibrosis such collagen type I and fibronectin, making it an effective antifibrotic agent in the kidney[56].

The considerable increase in the levels of urea and creatinine in serum in group III in contrast to group I was one of the notable findings in such work. This goes with the studies of Korolczuk *et al*. 2010[3], El Bassosy and Eid 2018[22], and Uludağ *et al*. 2018[2]. Persistent CsA use causes glomerular arteriolopathy, atrophy of tubules, and fibrosis of interstitial tissue, and all these effects could lead to chronic renal failure[57,58]. Furthermore, CsA causes oxidative stress, which leads to tissue harm[59].

However, these high values were notably decreased secondly to receiving PRP with a significant decrease on comparing the results of group IV with those of group III group. Same findings have been recorded by Salem *et al*. 2018[9] who attributed the potential of PRP to reduce malfunction of the kidney and leakage of enzymes from tubules to the activation of antioxidant enzymes inside renal cells, particularly glutathione peroxidase enzyme (GPx). HGF, adenosine diphosphate (ADP), adenosine triphosphate (ATP), insulin growth factor-1 I(IGF-1) and epidermal growth factor (EGF) are only few of the growth factors released by PRP[60] that promote regeneration of tubular cells and restoration of normal kidney functions[61]. Furthermore, IGF-I proved to preserve glomerular filtration, blood flow in renal vessels, and excretory function of the kidney in persons suffering from longstanding kidney disease, possibly by lowering the resistance of renal arterioles and increasing the glomerular ultrafiltration coefficient, which could be mediated by increasing nitric oxide production[62].

Kidney injury molecule-1 (KIM-1) was described for the first time by Ichimura *et al*. 1998 as a kind of transmembrane protein. KIM-1 is an early indicator for renal proximal tubular injury. In various kinds of injuries, it appeared as a highly selective and elevated marker in cells of renal tubules[64]. This study revealed that the gene expression of kidney injury molecule-1 (KIM-1) was considerably higher in group III compared to group I and IV which goes with the observation of Zhou *et al*. 2008[65] and Prozialeck *et al*. 2009[66]. KIM-1 is known to rise in response to ischemia / reperfusion injury. This idea is also supported by position of KIM-1 expression, as proximal convoluted tubular cells are characterized by being more vulnerable to ischemia injury[63]. Losing the polarity of tubular cells, increased permeability of transepithelial

membrane, and destruction of actin cytoskeletal architecture in microvascular cells of the kidney because of oxidative stress may also explain the upregulation of KIM-1[67].

The strength point of the current study was the reduction in KIM-1 expression following PRP therapy compared to group III which agrees with the results of Salem *et al*. 2018[9]. According to Lee *et al*. 2006[68], PRP suppresses renal KIM-1 by strengthening a special pathway, which inhibits the release of reactive oxygen species (ROS) and increases the resistance to oxidation. PRP has also been shown to boost the expression of anti-inflammatory mediators intercellularly (IL-4, IL-10, and IL-13), which are capable of lowering IL-1-mediated catabolic effects and suppressing inflammation[69].

N-acetyl-beta-glucosaminidase (NAG) is an intralysosomal membrane-bound enzyme generated by the epithelium of proximal tubule when lysosomal membranes are ruptured. The most commonly utilised urinary enzyme for assessing renal illness and detecting nephrotoxicity is NAG[70]. In the current work receiving CsA was linked to a marked rise in tissue N-acetyl beta glucosaminidase (NAG) expression when compared to group I and IV. Burdmann *et al*. 1994[71], Saad and Al-Rikabi 2002[72] and Salem *et al*. 2018[9] also recorded a rise in NAG values in serum in association to tubular injury. A rise of NAG level could be explained by the fact that the upper border of the epithelial cells of proximal tubules involves many microvilli that constitute the brush border and contain proteins posing enzymatic functions to perform the assigned functions of proximal tubules[73]. Intracellular enzymes are excreted in urine secondary to injury whether by exocytosis or leakage. Thus, elevated level of NAG could be attributed to malfunction of epithelial cells of proximal tubules caused by accumulation of proteins in the lumen of tubules[74]. PRP treatment reduced tissue NAG expression, which goes with the results of Salem *et al*.2018[9], who related the power of PRP to reduce malfunction of the kidney and leakage of enzymes from tubules to having many growth factors that help to regenerate renal tubular cells, restore kidney function, and regenerate the renal structure and physiology secondary to malefices of toxicity.

Although the clear pathogenesis of CsA-caused nephrotoxicity is obscure, many studies attributed it to the oxidative stress. CsA produces endoplasmic reticulum stress and a rise in mitochondrial reactive oxygen species generation, altering redox balance and causing lipid peroxidation, which leads to nephrotoxicity[75]. Increased oxidative stress and free radical-induced damage may result from a reduction in physiological antioxidant defense mechanisms[76]. In the current study when compared to group I and IV, tissue MDA levels increased significantly in group III. This agrees with the data of UZ *et al*. 2008[35] and Uludağ *et al*. 2018[2]. Increased MDA could be linked to increased antioxidant consumption in an attempt to detoxify free radicals, which likely resulted in a decrease in SOD levels[77]. According to Nada *et al*. 2103[78], increasing ROS activity leads to an increase in MDA generation.

Tissue SOD levels in group III was significantly lower than in group I and IV. This is similar to the findings of Ismail and Aboulkhair 2019[79], who found that ischemia reperfusion damage was linked to a lower level of SOD. SOD depletion is linked to chronic ischemic process caused by chronic CsA use, which causes reactive oxygen species (ROS) more than the biological antioxidant molecule's compensating capacity[48]. Furthermore, Damiano *et al*. 2013[80] speculated that oxidative stress may be involved in CsA renal toxic effect, citing the fact that prolonged CsA treatment dramatically increases ROS production in the kidney.

Tissue GSH was shown to be considerably decreased in group III in contrast to group I and IV. In a similar study, Saad and Al Rakibi 2002[75] found that nephrotoxicity was associated with a reduction in kidney GSH. This is due to the fact that lower GSH levels and antioxidant system activity, are linked to a rise in the pro-oxidant system secondary to oxidative stress[81].

It is clear that PRP treatment aided in the restoration of antioxidant defense system to its normal function in this study, which agrees with El-Sharouny *et al*. 2019[82] and Gazia 2020[83] where both of them recorded recovery of antioxidant defense system up on administration of PRP. Hesami *et al*. 2014[84] attributed the effect of PRP treatment to prevention of lipid peroxidation and restoration of the antioxidant defense system to its normal function plus its remarkable effect on signal transduction.

CONCLUSION

Cyclosporin A at a dose of 100 mg/kg/day could induce chronic nephrotoxicity. Tubular damage and fibrosis of interstitial tissue in chronic renal conditions are related more strongly with malfunction of the kidney than alterations in the glomeruli. The procedure of subcapsular injection of PRP appeared to be safe with no side effects as evidenced from sham control group. PRP could ameliorate chronic nephrotoxic effects induced by CsA with persistence of minimal histological and immunohistochemical alterations of chronic nephrotoxicity. PRP produces marked improvement in the biochemical markers and gene expression values but with no return to control values.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تحسن KIN_1 and NAG في الكلية بعد اعطاء البلازما الغنية بالصفائح لعلاج سمية الكلية الناتجه عن السيكلوسبورين في ذكر الفأر الأبيض البالغ

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المقدمة: السيكلوسبورين هو عقار يستخدم لقمع الجهاز المناعي، مما يعالج رفض الزرع وأمراض المناعة الذاتية. غير أن الآثار الضارة تحد من استخدامها في الممارسة السريرية. والبلازما الغنية بالصفائح هي منتج زاخر بنمو تلقائي ومفيدا كمنتج يسمح بتجديد الأنسجة بإطلاق عوامل نمو تخفف من تدمير الأنسجة.

المواد والأساليب: تم استخدام اربعون فأرا ذكرا أبيضا في هذه الدراسة. وقد تم تقسيمهم الى أربع مجموعات متساوية ؛ مجموعة ضابطة ، مجموعة ضابطة كاذبة ، مجموعة معالجة بالسيكلوسيورين ، مجموعة معالجة بالسيكلوسيورين والبلازما الغنية بالصفائح. أُجري تقييم نسيجى

و كيميائى حيوى (Caspase 3 and TGFβ) ومناعى هيستوكيميائى (Hx & E and Masson Trichrome) (serum urea, create, tissue level of Glutathione reductase, SOD and MDA, and tissue expression of K I M -1 and NAG)

النتائج: وقد اظهرت القشرة الكلوية الكظرية في المجموعة المعالجة بالسيكلوسبورين عن تغيرات فى شكل كبيبي مجزأ منكمش مع نوي ملطخة داكنة و اتساع في التجويف البولي مع مناطق من النزيف الخلالي و تكون قوالب نسيجية داخل تجويفها، كما أظهرت أجزاء أخري زيادة في الخلايا الكبيبية مع ضيق شديد في التجويف البولي و نزيف كبيبي داخلي مع تكوين قوالب نسيجية داخل تجويفها و فراغات بالسيتوبلازم النبيبي و انحلال للنواة و قوالب نسيجية داخل التجويف النبيبي.اظهرت دراسات الفحص النسيجي تراكم الكثير من ألياف الكولاجين حول و داخل الكبيبات وحول الأنابيب

في المجموعة الثالثة. اما الفحص المناعى الهسنوكميائى أظهر رد فعل إيجابي قوي للكاسبيز-٣ وTGFβ-١. كيميائيا هناك ارتفاع في المصل مستوى اليوريا والكرياتينين والأنسجة MDA مع انخفاض مستوى الأنسجة SOD واختزال الجلوتاثيون في المجموعة الثالثة. يمكن للبلازما الغنية بالصفائح أن تخفف من التأثيرات السمية الكلوية النسيجية الناجمة عن السيكلوسبورين. إلى جانب تحسين العلامات البيوكيميائية وقيم التعبير الجيني.

الاستنتاج: البلازما الغنية بالصفائح منتج آمن ويمكن استخدامه لعكس الآثار الضارة الناجمة عن السيكلوسبورين.