Effect of Platelet Rich Plasma on an Experimental Rat Model of Adriamycin Induced Chronic Kidney Disease

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Abstract

Background: Chronic kidney disease (CKD) is a world-wide health problem with increased mortality and morbidity. Currently, there is no effective protective therapy against CKD- induced renal damage with its sequel of end stage renal disease. Platelet-rich plasma (PRP) has a progressively gained consideration in wound healing, repair/regeneration of damaged tissues and conservation of organ function.

Aim of Study: This current study was planned to evaluate the possible protective effect of PRP treatment on a rat model of Adriamycin (ADR)-induced CKD.

Material and Methods: Sixty male albino rats were included; 30 were used for PRP preparation and the other 30, were randomly categorized into three equal groups of ten rat each: Normal control group, ADR group and PRP treated ADR group. At the end of experimental regimen (6 weeks), 24h urine samples were collected for measuring 24h urinary protein excretion and creatinine clearance (Cr Cl), then rats were decapitated and blood samples were collected for measuring serum albumin, triglycerides and cholesterol along with serum creatinine and blood urea nitrogen (BUN). Renal tissue samples were harvested and used for determination of renal level and mRNA expression of nephrin along with levels of hepatocyte growth factor (HGF), malondialdehyde (MDA), super oxide dismutase (SOD) activity, tumor necrosis factor alpha (TNF-α), interleukin 1-β (IL-1β), and transforming growth factor 1β (TGF 1β) in renal homogenates.

Results: Platelet rich plasma treatment improved ADR induced proteinuria, hyperlipidemia and renal dysfunction, which was accompanied by parallel upregulation of renal nephrin and inhanced levels of HGF and SOD activity, whereas the increased levels of TNF-α, IL-1β and TGF 1β were attenuated.

Conclusion: Platelet rich plasma could potentially protect against ADR induced CKD by alleviating its associated proteinuria, hyperlipidemia and the altered oxidative stress, inflammatory and fibrogenic responses.

Key Words: *Platelet rich plasma – Adriamycin – Chronic kidney disease – Proteinuria.*

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Introduction

CHRONIC kidney disease is a worldwide health problem, that occurs as a result of exposure to wide range of deleterious agents as environmental toxins and drugs [1], with diabetes, hypertension and obesity were considered to be the main underlying risk factors [2]. Most types of CKD are characterized by a progressive loss of kidney function, reduced glomerular filtration and chronic massive proteinuria [3], with vicious cycle of nephron damage, progressive glomerulo-sclerosis, tubule-interstitial fibrosis, and finally end stage renal disease [4]. The alarming increase in chronic and end stage renal disease paid the attention to identify the possible mechanisms that perpetuate and exacerbate renal dysfunction; to develop strategies to prevent and/or modulate them [5].

Adriamycin (ADR) is an anthracycline-based anticancer drug whose therapeutic use has been limited due to the incidence of cardiac, renal, hematological and testicular toxicities [6]. Adriamycin-induced nephropathy is a classic well accepted rat model of CKD, characterized by focal glomerulo-sclerosis leading to chronic proteinuria and renal failure [7]. The exact mechanisms of renal injury induced by ADR are not yet fully elucidated [8,9]. Studies reported that ADR-induced CKD is associated with hypoalbuminemia, dyslipidemia and proteinuria [10]. Adriamycin caused a wide effacement of podocyte foot processes disrupting the integrity of slit diaphragms (SD) with subsequent proteinuria, focal segmental glomerulo-sclerosis and tubule-interstitial damage [11]. Nephrin is one of the most important podocyte-associated proteins, playing a key role in maintaining the integrity of SD, keeping the constructional and

functional properties of the glomerular filtration barrier [12]. Defects in nephrin expression had been identified as a leading cause of several inherited and acquired forms of proteinuria [13].

It had been well documented that proteinuria is not only a marker for the progression of CKD, but also acts as a pathogenic mediator that promotes tubular injury and interstitial fibrosis [14,15]. In addition, oxidative stress, inflammation, and endothelial/epithelial mesenchymal transition were also regarded as key mediators of renal injury and fibrosis with a sequel of progressive loss of renal function in CKD [16,17]. Consequently, factors having anti-proteinuric, antioxidant and anti-inflammatory effects may have a special interest in ameliorating CKD disease progression.

Platelet rich plasma is recently known for its powerful healing properties and an up-growing role in regenerative medicine [18]. It is an autologous derivative of whole blood rich in active growth factors (GFs). Its powerful therapeutic potentials may be attributed to its ability to deliver great variety of biologically active GFs to the site of injury with its simplicity, effectiveness, safety, and constant availability [19]. Platelets contain three types of granules containing many of GFs and bioactive molecules including ; hepatocyte growth factor (HGF); insulin-like growth factor-I (IGF-I), epidermal growth factor adenosine diphosphate (ADP) and tri-phosphate (ATP), among others [20]. These factors were shown to enhance renal tubular cell regeneration and repair kidney structure and function after damage in various experimental renal models [21]. Platelet rich plasma have underscored beneficial effects in different experimental models of acute and chronic renal injury; which could be attributed to its regenerative, antioxidant and anti-inflammatory characteristics [22,23]. Thereby, it could be anticipated that the administration of PRP as a natural cocktail of many GFs and bioactive molecules may protect against or delay the disease progression in ADR induced CKD.

Due to increased incidence of CKD and numerous side effects of chemical drugs usage, it is necessary to look for a cheap, safer and highly effective therapy. Irrespective of the role of PRP in regenerative and healing medicine, its influence on ADR-induced CKD had not been previously explored. Therefore, this study was carried out to evaluate the impact of PRP on ADR-induced CKD model in rats, with shedding the light on the possible mechanisms underlying its actions.

Material and Methods

Chemicals and reagents:

The ADR in the form of (ADR hydrochloride) vial was purchased from EIMC United Pharmaceuticals, Egypt. All other chemical reagents were of high purity and analytical grade and were obtained from (Sigma, USA), unless described otherwise.

Platelets rich plasma preparation:

Thirty male albino rats were used as blood donors, which were anesthetized with ether, sacrificed and blood was collected on sodium citrate anticoagulant solution (9 parts of blood : 1 part of sodium citrate), then centrifuged at 1000 rpm for 15min ,after which plasma was collected and centrifuged at 3000 rpm for 10min to get PRP. Then PRP concentrate was dissolved in phosphate buffer saline (PBS) (1:1) [24,25]. Platelets were counted and platelet number was ranged from 1000000/ ▗▖▃▖ to 1800000/ ▗▖▃▖

Study design and animal grouping:

This study was conducted from April 2018 to August 2018, and all procedures were performed according to the guidelines of the Research Ethical Committee of Tanta Faculty of Medicine. Sixty adult male albino rats weighing 200-250g were used in this experiment. Proper acclimatization for one week in the animal house with, 12h lighting cycle and $24\pm2°C$ temperature. The rats were housed five per cage, had free access to standard rodent chow and water. Randomly thirty male albino rats from them were used in preparing PRP as described previously [24,25], while the other thirty rats were categorized into three equal groups of 10 rats each:

- *Normal control group:* This rat group received a single intravenous injection (tail vein) of 0.5ml of 0.9% saline solution.

- *Adriamycin group (ADR):* Rats of this group received a single intravenous injection of ADR (tail vein) at a dose of (6mg/kg body weight, dissolved in 0.5ml of 0.9% saline solution), to induce chronic kidney disease [26].

- *PRP treated ADR group (PRP-ADR):* This rat group injected with a single intravenous injection of ADR as described above, then received intra peritoneal injection of PRP, at dose of 1ml/kg, twice/week for 6 weeks [23], starting from the first day of ADR injection.

Collection of urine samples:

At the end of the study period (6 weeks), rats in each group were individually housed in metabolic

cage for 24h urine collection. Total urine volume was measured; one ml was collected from the 24h urine sample, and used for measurement of 24h urinary protein excretion and Cr Cl.

Blood and tissue sampling:

All rats were anesthetized with ether, then sacrificed and blood samples were collected and centrifuged at 3000xg for 15min, and stored at –20°C till usage for determination of serum biochemical parameters. Then kidneys of each rat were gently dissected, weighed, washed with ice cold saline, then they were homogenized as 10% (w/v) in 50mM phosphate buffer (pH 7.4) and centrifuged at 5000xg for 20min at 4°C. The resultant supernatant was then divided into aliquots and kept frozen at –80°C until used for biochemical analysis. Total protein content was measured in renal tissue homogenates using the Lowry method as described previously [27].

Determination of 24 h urinary protein excretion and serum albumin, triglyceride and cholesterol:

Measurement of total urinary protein concentration was assayed using the method of Bradford [28]. Serum albumin was determined according to the method described by Doumas and Watson [29], while serum triglycerides and cholesterol were assayed using colorimetric kits supplied by (Biomed Diagnostics. EGY-Chem., Egypt).

Biochemical assessment of renal function:

Serum and urinary creatinine were measured using colorimetric kits supplied by (Diamond Diagnostics, Egypt). Blood urea nitrogen was determined based on urease-colorimetric method using spectrum diagnostics kits. Creatinine clearance, as index for glomerular filtration rate was measured according to the standard equation:

Cr Cl (ml/min) = Urine creatinine (mg/dl) x Urine output (ml/24 hrs) / Serum creatinine) mg/dl) x 1440 (min) [30].

Assessment of renal nephrin and HGF levels:

Levels of renal nephrin contents were quantified in kidney homogenate with ELISA kits (Cusabio biotech CO., Ltd, Wuhan, China), while renal HGF was determined using ELISA supplied by (Sunred Biological Technology, China),following the manufacturer's protocol.

Assesment of renal redox status, TNF-α, IL 1β and TGFβ:

Malondialdehyde and SOD activity levels were determined according to the methods described by Ohkawa et al. [31] and Misra and Fridovich [32],

respectively. Meanwhile, renal assay for TNF-α, IL 1β and TGF-β were performed using their respective ELISA kits supplied by (RayBiotech, Inc., USA), (abcam, MA, USA) and (MyBiosource USA), respectively. All procedures were performed according to the manufacturers' instructions.

Assessment of nephrin gene expression using quantitative real Time PCR:

Isolation of total RNA from kidney samples was performed using Gene JET RNA Purification Kit (Thermo Scientific, # K0731, USA). Total RNA (5) was then reverse transcribed producing cDNA using Revert Aid H Minus Reverse Transcriptase (Thermo Scientific, #EP0451, USA). The cDNA was used as a template to determine the relative expression of nephrin genes using Step One Plus real time PCR system (Applied Biosystem, USA). The PCR primer sequence was as follow: (Forward: 5'-TGTGAGGATCCGAGGGG AAT-3', Reverse: 5'-GGAAACGGGTGTTG TGAAGC-3') the housekeeping gene β-actin (Forward: 5'-TATCCTGGCCTCACTGTCCA-3', Reverse: 5'-AACGCAGCTCAGTAACAGTC-3'). The relative gene expression was calculated using $2^{-\Delta\Delta Ct}$ method [33].

Statistical analysis:

Statistical analysis was carried out using the mean and standard deviation (SD) using SPSS for windows (Version 21.0). One-way analysis of variance (ANOVA) was used for multiple comparison to evaluate the statistical significance between experimental groups with Tukey post hoc test analysis, p-value <0.05 was considered significant. Correlation between variables was evaluated using Pearson's correlation coefficient with level of significance <0.05.

Results

Effect of PRP treatment on urinary 24h urinary protein excretion and serum albumin, triglycerides and cholesterol levels:

As shown in Table (1), rats in the ADR group exhibited significantly higher levels of 24h urinary protein excretion, serum triglycerides and cholesterol, which was associated with significantly lower serum albumin levels, when compared with the normal control group (p<0.05). Concomitant administration of PRP with ADR significantly reduced the levels of 24h urinary protein excretion, serum triglycerides and cholesterol, whereas it significantly increased serum albumin levels in rats of the PRP-ADR group versus the ADR group (p<0.05).

Effects of PRP treatment on renal function related parameters:

As compiled in Table (2), serum creatinine and BUN were significantly higher, whereas Cr Cl was significantly lower in rats of ADR group compared to normal control group ($p<0.05$). PRP administration significantly decreased both serum creatinine and BUN, while significantly increased Cr Cl, in the PRP-ADR group compared to the ADR group. ($p \leq 0.05$).

Effect of PRP on renal oxidative stress markers, inflammatory cytokines and TGF β-1:

Compared with the normal control group, renal MDA levels were significantly higher, whereas renal SOD activity levels were significantly lower in the ADR group ($p \leq 0.05$). On the other hand, PRP administration resulted in a significant decrease in MDA levels along with a significant increase in SOD activity levels in the rats of PRP-ADR group versus the ADR group ($p \leq 0.05$). (Table 3).

In addition, results of Table (3) also elucidated significantly increased levels of TNF-α, IL 1β and TGF β-1 in renal homogenates obtained from ADR group compared to normal control one, whereas these levels were significantly decreased in the rats of PRP-ADR group compared to ADR group ($p \leq 0.05$).

Effect of PRP on levels of nephrin and HGF in renal tissues of experimental rats:

As depicted in Figs. (1,2), ADR administration significantly decreased renal nephrin level and mRNA expression which was associated with lower levels of renal HGF in rats of ADR group compared with the normal control group ($p \leq 0.05$). Meanwhile, these values were significantly increased upon PRP administration in rats of PRP-ADR group compared with the ADR group ($p \leq 0.05$).

Pearson correlation analysis of HGF with 24h urinary protein excretion and renal biochemical markers in different studied groups:

Results of Table (4) revealed that HGF presented a significant positive correlation ($p<0.05$) with renal nephrin level whereas, a statistically significant negative correlation ($p<0.05$) was found between renal HGF level in one hand and MDA, TNFα and TGF-β 1 levels on the other hand ($p<0.05$).

Table (1): Effect of PRP treatment on 24h urinary protein excretion and serum albumin, triglyceride and cholesterol in experimental rats.

Pararmeters / groups	Normal control group	ADR group	PRP-ADR group
Urinary protein excretion (mg/24-h urine)	3.6±1.25	89±21.43 [a]	21±8.78 [ab]
Serum albumin (g/dl)	2.44±0.38	1.36±0.47 [a]	2.28±0.51 [b]
Serum triglycerides (mg/dl)	64±9.25	421±101.67 [a]	190±76.53 [ab]
Serum cholesterol (mg/dl)	75±15.39	435±96.74 [a]	210±83 [ab]

- Data are mean ± standard deviation of a group of 10 rats. Statistical analysis is carried out using one-way ANOVA with Tukey's post hoc test using SPSS program.
a Significant difference vs. normal control group ($p<0.05$).
b Significant difference vs. ADR group ($p<0.05$).

Table (2): Effect of PRP treatment on renal function related parameters in experimental rats.

Pararmeters / groups	Normal control group	ADR group	PRP+ADR group
serum creatinine (mg/dl)	0.4833±0.12	1.17±0.35 [a]	0.633±0.17 [b]
Blood urea nitrogen (mg/dl)	24±9.6	83±17.93 [a]	31±11.75 [b]
Creatinine clearance (ml/min)	1.194±0.47	0.631±0.13 [a]	0.975±0.29 [ab]

- Data are mean ± standard deviation of a group of 10 rats. Statistical analysis is carried out using one-way ANOVA with Tukey's post hoc test using SPSS program.
a Significant difference vs. normal control group ($p<0.05$).
b Significant difference vs. ADR group ($p<0.05$).

Table (3): Effect of PRP treatment on renal oxidative stress markers, inflammatory cytokines and TGF β in experimental rats.

Pararmeters / groups	Normal control group	ADR group	PRP+ADR group
Renal MDA level (nmol/mg protein)	0.24±0.06	0.78±0.15 [a]	0.33±0.08 [a]
Renal SOD activity (units/mg protein)	7.38±1.12	3.47±0.76 [a]	6.95±0.93 [b]
Renal TNF-α level (pg/mg protein)	13±4.63	48±9.57 [a]	17.83±6.39 [b]
IL 1 B (pg/mg protein)	25±6.89	87±20.33 [a]	41±9.45 [ab]
TGF β 1 (pg/mg protein)	23±7.97	62±17.58 [a]	31±8.97 [a]

- Data are mean ± standard deviation of a group of 10 rats.
MDA : Malondialdehyde.
SOD : Superoxide dismutase.
TNF-α : Tumor necrosis factor-α.
TGF β 1: transforming factor β 1.
- Statistical analysis is carried out using one-way ANOVA with Tukey's post hoc test using SPSS program.
a Significant difference vs. normal control group ($p<0.05$).
b Significant difference vs. ADR group ($p<0.05$).

Table (4): Correlation analysis of renal HGF with 24h urinary protein excretion and renal biochemical parameters in different studied groups.

	Renal HGF level
24 h urinary protein excretion	$r = -0.714*$
Nephrin	$r = 0.762*$
MDA	$r = -0.611*$
SOD activity	$r = 0.218$
TNF a	$r = -0.731*$
IL-1 p	$r = -0.318$
TGF-β 1	$r = -0.647*$

Correlation study was performed using Pearson correlation.
*Significance was considered at level of $p<0.05$.

Fig. (2): Effect of PRP on renal HGF level in experimental rats.

NC : Normal control group.
ADR: ADR treated group.
ADR+PRP: PRP treated ADR group.
- Statistical analysis is carried out using one-way ANOVA with Tukey's post hoc test using SPSS program.
a Significant difference vs. control group ($p<0.05$).
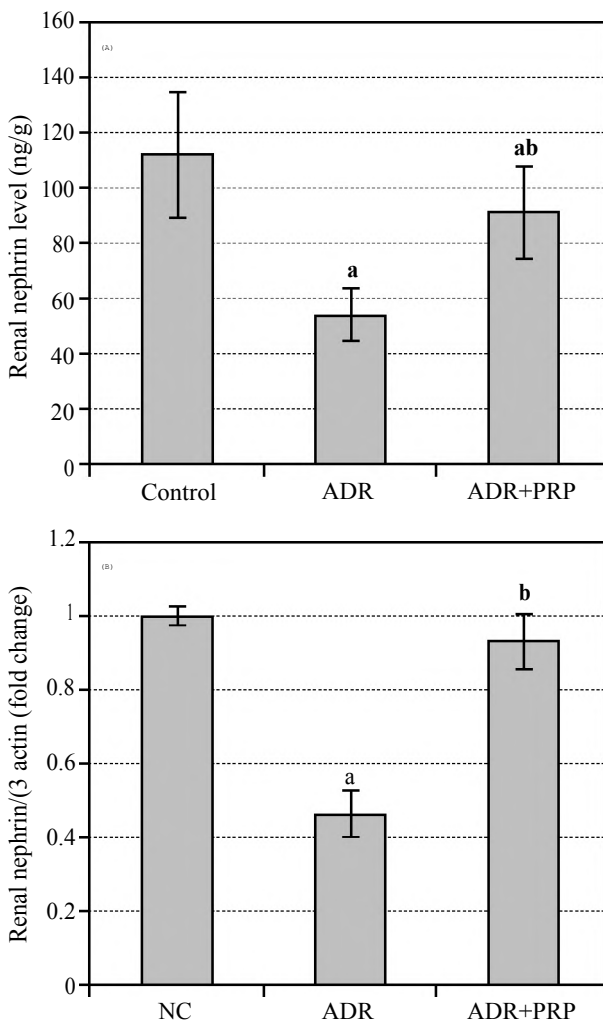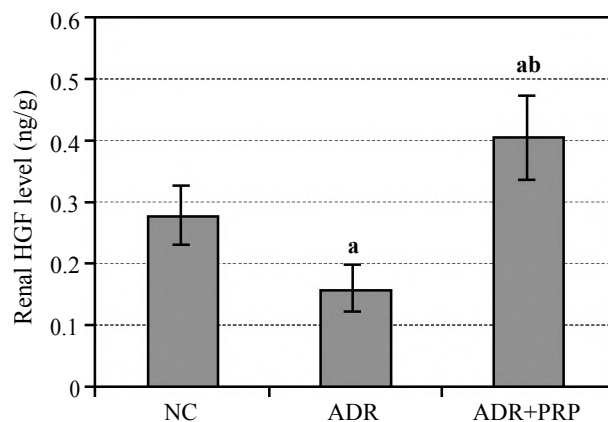**b** Significant difference vs. ADR group ($p<0.05$).

Fig. (1): Effect of PRP on renal nephrin level (1A) and nephrin expression (1B) in experimental rats.

NC : Normal control group.
ADR: ADR treated group.
ADR+PRP: PRP treated ADR group.
- Statistical analysis is carried out using one-way ANOVA with Tukey's post hoc test using SPSS program.
a Significant difference vs. control group ($p<0.05$).
b Significant difference vs. ADR group ($p<0.05$).

Discussion

The major findings of this current study can be summarized as follows: (i) Treatment with ADR caused deterioration of the renal function and serum biomarkers which was accompanied by exacerbated renal oxidative stress, inflammatory and fibrogenic responses. (b) PRP could protect against ADR induced renal pathology by several mechanisms as proposed in this current study.

The results obtained herein showed that injection of ADR was associated with proteinuria, hypoalbuminemia and hyperlipidemia which were accompanied by significant reduction in renal nephrin levels and mRNA expression in ADR group compared to the control one. Similar results were recorded by earlier studies [10,34].

The ADR induced proteinuria could be as a result of its direct toxicity through releasing free radicals that damage the glomerular structure with loss of nephrin [35]; or may be indirectly through the released inflammatory cytokines which inhibit the activity of the nephrin gene promoter [36], this in turn disturb the integrity of SD with sustained proteinuria [37]. The resultant proteinuria and its sequel of hypoalbuminemia and decreased serum oncotic pressure leads to stimulation of the hepatic synthesis of triglycerides and cholesterol with subsequent hyperlipidemia [38]. Moreover, Hong et al. [39] emphasized that ADR induced hyperlipidemia could be attributed to the inhibition of carnitine palmitoyl transferase system and the lowered level of cytochrome P450 which depress

cholesterol 7α-hydroxylase activities, the key enzyme in conversion of cholesterol to bile acids.

In addition, our investigations showed that proteinuria was associated with renal function impairment in ADR-treated rats, as indicated by significantly increased levels of serum creatinine, BUN with decreased Cr Cl in the ADR group compared with the normal control group, which was in harmony with previous studies [25,40-42]. This could be attributed to either; direct ADR toxicity mediated via tubular cell chemokine release and oxidant injury [11]; or indirectly through ADR induced proteinuria which triggers tubular cell activation and injury with attraction of interstitial inflammatory cytokines resulting in tubular interstitial damage with gradual loss of renal function [43,44].

From the over mentioned data, the role of oxidative stress and inflammation in ADR pathophysiology could not be ruled out. In accordance, our results showed an imbalance of redox status as revealed by significantly increased levels of MDA and decreased SOD levels, which was associated with increased inflammatory cytokines; TNF α and IL-1 β in kidney tissues of ADR-treated rats compared to control ones. These findings came in line with previous studies [45,46].

Oxidative stress may cause direct toxicity to the cells, causing damage to DNA, protein, and lipids or may indirectly trigger the release of chemokine from injured renal cells with subsequent renal infiltration or activation of macrophages and T cells [47,48]. Activated or recruited macrophages and T cells secrete inflammatory cytokines as TNFα and IL-1 β which could induce mesangial expansion, podocyte injury, and endothelial damage, instigating glomerulo-sclerosis and interstitial fibrosis which mostly lead to renal failure [11,46].

Several lines of evidence emphasized the pivotal role of TGF-β 1 in the progression of fibrosis in CKD [49] (Chen et al., 2018). In accordance, this current study elucidated a significantly increased renal level of TGF-β 1 in ADR group compared to control one, which agree previous studies [50,51]. The pro fibrotic TGF-β 1 had been involved in the synthesis and deposition of extracellular matrix (ECM) together with stimulation of the pro inflammatory and pro fibrotic mediators promoting the ADR induced tubule-interstitial fibrosis [52]. Moreover, it could down regulate the expression of nephrin, exacerbating the proteinuric injury and its associated pathology [53].

The second major objective in this current work was to investigate the impact of PRP on ADR induced renal pathology. The present study demonstrated a protective role for PRP against ADR-induced CKD as evidenced by significantly improved 24h urinary protein excretion ,serum albumin and lipids along with decreased levels of serum creatinine and BUN, whereas Cr Cl was significantly increased in the PRP-ADR treated group compared to the ADR-treated group. These protective potentials of PRP were associated with parallel accentuation of renal nephrin levels and expression with reduction in renal oxidative and inflammatory markers with fibrogenic cytokine; TGF-β 1.

The effect of PRP on proteinuria may be mediated mostly, via its released HGF which is a multifunctional pleiotropic growth factor that was shown to abrogate CKD and many podocyte injury-based proteinuric renal diseases [54]. Moreover, stimulation of renal HGF was recorded to ameliorate proteinuria, podocytopenia and glomerulo-sclerosis in nephrotic models [55-57]. Thus, renal level of HGF was estimated in this current study; to evaluate its role in mediating the anti- proteinuric effect of PRP in this rat model. Noteworthy, our results showed a significant decreased level of renal HGF upon ADR injection ,which was significantly increased upon PRP injection. Similar results were recorded previously [23,57]. The increased renal levels of HGF by PRP could be attributed to its own content of this growth factor, beside its stimulation to HGF production; possibly via its ability to increase the intracellular cyclic adenosine monophosphate (cAMP) in the kidney [20,23]. HGF production in cultured cells was shown to be induced by cAMP activating agents and growth factors whereas, it is inhibited by TGF-β [58].

Hepatocyte growth factor could ameliorate proteinuria through several proposed mechanisms including; first: Its ability to directly target the podocytes ,preventing their foot process effacement, stabilizing nephrin and protecting the integrity of the SD [59]; second: Its suppression to TGF-β 1 production in mesangial cells [60] which may reverse its down regulation to nephrin expression [52]; third: Its effect on inflammatory cytokines [61] which may counteract their inhibition to the transcriptional activity of nephrin promoters [36]; lastly: Its anti-apoptotic effect which could protect against ADR induced podocyte apoptosis [56]. In accordance to these findings, our results revealed that the renal HGF level was positively correlated with renal nephrin levels, whereas, negatively correlated with 24h urinary excretion along with

MDA, TNFα and TGF-β 1 levels, the main promoters of proteinuria in this rat model, supporting our hypothesis.

As a consequence of reduction of proteinuria upon PRP treatment, there was also a significant improvement in serum albumin and marked reduction in serum triglycerides and cholesterol. Consistently, agents targeting proteinuria was shown to be associated with improvement of hyperlipidemia with its sequel of cardiovascular risk [62,63], the main leading cause of mortality in CKD [64]. Moreover, this attenuation of proteinuria could protect against its associated tubular injury and interstitial fibrosis which may have a role in mitigating ADR induced renal dysfunction. In accordance, a variety of approaches targeting proteinuria were reported to improve the renal function in CKD models [40].

A part from the anti-proteinuric effect of PRP mediated via HGF, its other released GFs and bioactive molecules may contribute in mitigating the ADR induced renal pathology and dysfunction in this rat model. For example; IGF-I was recorded to preserve glomerular filtration rate, renal blood flow, and renal excretory function in CKD, possibly via reducing renal arteriolar resistance and increasing the glomerular ultrafiltration coefficient, which could be mediated via increasing the nitric oxide production [65,66]. Epidermal growth factor could promote epithelization, accelerate the healing process and boost DNA synthesis in regenerating renal tubules [67]. Hepatocyte growth factor was shown to ameliorate CKD; also via suppressing tubular epithelial to mesenchymal transition, apoptosis and inflammation [68]. Moreover, the adenosine produced by adenine nucleotides degradation could mediate tissue protection and repair by increasing oxygen supply, suppressing inflammation, and promoting angiogenesis [69].

In addition, the antioxidant potentials of PRP as recorded herein, may also explain its renoprotective effect in this rat model, which came in parallel with previous studies [20,23]. Its antioxidant capacity could be attributed to its ability to induce the expression of SOD enzyme and the nuclear factor-erythroid-2-related factor 2, the master regulator of anti-oxidant enzymes expression [70,71], which in turn could dampen the ADR induced oxidative stress and its associated pathology.

Moreover, the present work demonstrated the ability of PRP to suppress the released inflammatory cytokines, which could play a role in ameliorating ADR induced nephropathy. Consistently, the anti-inflammatory agents were recorded to reduce proteinuria via restoring the nephrin expression [72] and could ameliorate the tubule-interstitial damage, decreasing the disease progression in ADR induced nephropathy models [73]. The anti-inflammatory effect of PRP could be attributed to its ability to suppress the translocation of nuclear factor κ B and its downstream molecules, TNF α [74,75], or may be due to its capability to increase the intracellular expression of the anti-inflammatory mediators (IL-4, IL-10, and IL-13) and to decrease the IL-1 β -mediated catabolic effects [76].

Our investigations also declared a significantly reduced levels of TGF-β 1 upon PRP treatment, signifying its anti-fibrotic potentials which was supported previously [23]. This effect may be due to its content of HGF and its ability to increase cAMP and HGF levels [20,23], which were documented for their ability to down regulate the TGF-β and the other fibrosis-related genes as α-SMA, collagens and fibronectin [77,78]. Moreover, the anti-inflammatory and anti-apoptotic effects of PRP together with its suppression to ECM accumulation may contribute to its anti-fibrotic potentials as recorded previously [21,79].

Conclusion:

From the results of this current study, PRP exhibited a protective effect against ADR-induced CKD, which could be attributed to its anti-proteinuric, anti-hyperlipidemic, antioxidant, anti-inflammatory and anti-fibrotic potentials as recorded herein in this current study. Thus, PRP may play a role in clinical innovations for protection against CKD. The efficacy and safety of PRP for treating CKD and its underlying mechanisms need further studies.

Conflict of interest:

The authors declare that they have no conflict of interest.

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تأثيو البلازما الغنية بالصفائح الدموية على نموذج تجريبى
لأمراض الكلى المزمنة المستحثة بالادرياميسين فى الجرذان

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الهدف من الدراسة: تم تصميم هذه الدراسة الحالية لفحص تأثير العلاج بالبلازما الغنية بالصفائح الدموية على نموذج تجريبى لأمراض الكلى المزمنة المستحثة بالادرياميسين فى الجرذان.

المواد والطرق: تم تقسيم الجرذان إلى مجموعتين، كل واحدة مكونة من ثلاثين فأراً، تم استخدام المجموعة الأولى لإعداد البلازما الغنية بعوامل النمو، بينما تم تصنيف المجموعة الثانية إلى ثلاث مجموعات كل واحدة مكونة من عشرة جرذان المجموعة الأولى وتمثل المجموعة الضابطة، المجموعة الثانية والتى تم أعطاؤها الادرياميسين، بينما تم أعطاء المجموعة الثالثة الادرياميسين ثم تبعه العلاج بالبلازما الغنية بعومل النمو. وفى نهاية التجارب تم تجميع عينات البول لقياس نسبة افراز البروتين فى البول. ثم تم ذبح الفئران وتجميع عينات الدم لقياس وظائف الكلى ونسبة الزلال والدهون فى البلازما ثم تم تحضير عينات أنسجة الكلى لقياس الدلالات الكيميائية بها.

النتائج: كشف هذه الدراسة أن العلاج بالبلازما الغنية بالصفائح الدموية لمدة ستة أسابيع بعد الحقن بالادرياميسين قد أدى إلى تحسين ملحوظ فى وظائف الكلى وتقليل افراز البروتين فى البول، والذى ارتبط بزيادة ملحوظة فى مستوى النيفرين وعامل النمو الكبدى فى أنسجة الكلى، والذى كان موازياً لإنخفاض ذى دلالة إحصائية فى مستوى الاجهاد التأكسدى والالتهابى الكلوى.

الخلاصة: هذه النتائج تشير إلى الأثار المفيدة للعلاج بالبلازما الغنية بالصفائح الدموية على مرض الكلى المزمن المستحث بالادرياميسين فى الفئران.