Renoprotective Effects of *Punica granatum* (Pomegranate) Against Adenine-Induced Chronic Renal Failure in Male Rats

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Abstract: This study aimed to assess the nephroprotective effects of two pomegranate extracts, pomegranate juice (PJ) and pomegranate peel methanol extract (PPME) in rats with chronic renal failure (CRF) induced by adenine (AD). Thirty six male rats were allocated into six groups: Control (CO), PJ, PPME, AD, AD+PJ and AD+PPME groups. The obtained results showed a significant increase in serum levels of creatinine (Cr), blood urea nitrogen (BUN), uric acid (UA) in AD-fed rats. In addition, relative kidney weight, urine volume and urine NAG activity were significantly increased, while creatinine clearance was decreased. A significant disturbance was observed in renal antioxidant system of AD-fed rat group represented by elevations in thiobarbituric acid reactive substance (TBARS) and protein carponyl (PC) as well as depletion in the activities of SOD and CAT. Also, a significant increases in concentration of both serum tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) accompanied by decrease in nitric oxide level were observed. Administration of pomegranate extracts, either PJ or PPME significantly mitigated all the signs of AD-induced CRF. The results suggested that the renoprotective efficacy of pomegranate, in particular the methanol peel extract, can be attributed to antioxidant, anti-inflammatory and different signaling pathway mechanisms.
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1- Introduction

Many renal diseases in human are progressive in nature and eventually result in renal failure (Tong *et al.*, 2010). The incidence of chronic renal disease (CRD) appears to be on the increase, especially in some developing countries, imposing a very expensive and rising demand on health care systems already burdened by paucity of resources (Hossain*et al.*, 2009). Several factors influence the onset and progression of CRD, such as hypertension and diabetes mellitus as well as inflammation and oxidative stress (Ali *et al.*, 2013a).

Chronic renal Failure (CRF) is a progressive and irreversible loss of large number of functional nephrons caused by wide variety of disorders of the blood vessels, glomeruli, tubules and renal interstitium. CRF is characterized by the structural and functional responses of remnant nephrons, which ultimately lead to glomerulosclerosis (Guyton and Hall, 2006). The diseased kidney showed reduction in kidney size and presence of broad casts in the urine sediment, reflecting the dilated hypertrophied remaining nephrons (Brenner and Lazarus, 1991). When the kidney no longer have enough functioning nephrons to effectively ride the body of toxins, uremic poisoning results.

Adenine(AD)-induced CRF model provides valuable information about the pathomechanism of various complications associated with a persistent uremic state (Lacour*et al.*, 2005). Long-term feeding of AD to rats produced metabolic abnormalities resembling CRF complications in humans, it could increase serum uric acid, creatinine and urea nitrogen by decreasing their urinary excretion (Ali *et al.*, 2010). Exposure to a high concentration of adenine results in the production of free radicals (FR), which induces oxidative stress as shown by increased lipid peroxidation, free radical generation, and arachidonic acid release with decreased glutathione (Wang *et al.*, 2011) as well as elevation in inflammatory markers (Mahmoud *et al.*, 2012). Biological compounds with antioxidant properties and renal membrane-regenerating potential may be a benefit in alleviating adenine-induced toxicity.

Nawadays, considerable attention has been devoted to medicinal plants particularly rich in polyphenols, mainly flavonoids and phenolic acids, which exhibit antioxidant properties due to their hydrogen-donating and metal-chelating capacities as potential chemopreventive agents (Grzegorczyk *et al.*, 2007). Numerous medicinal plants and their formulations have been investigated in attempts to develop alternative therapeutic or prophylactic agents to protect against CRF with no side effects.

Punica granatum L. (*Punicaceae*), commonly called pomegranate, recently described as nature's power fruit, is a plant used in folkloric medicine for the treatment of various disease (Abdel Moneim,

2011) widely cultivated in the Mediterranean region. Pomegranate is rich in antioxidant of polyphenolic class which includes tannins, anthocynins (Nigris*et al.,* 2007) and flavonoids (Ricci, 2006). Content of soluble polyphenols in pomegranate juice varies withinthe limits of 0.2–1.0%, depending on variety and include mainly tannins, ellagic tannins, anthocyanins, catechins, gallic and ellagic acids (Gil *et al.,* 2000).

Apart from their antioxidant capacity, there have been numerous reports on the *in vivo* properties of pomegranates, namely on anti-atherosclerotic capacity (Kaplan and Aviram, 2001), anti-proliferative and pro-apoptotic activities of pomegranate tannin extract (Seeram*et al.,* 2005), anti-inflammatory activity (Adams *et al.,* 2006), as well as chemopreventive and chemotherapeutical potential towards prostate cancer by pomegranate juice (Malik *et al.,* 2005). Also, pomegranate has potent nephroprotective effect against ethylene glycol-induced nephrolithiasis (Tugcu*et al.,* 2008) and ferric nitrilotriacetate (Fe-TNA) induced renal damage (Ahmed and Ali, 2010).

Accordingly, the objective of this study is to examine the renoprotective effects of pomegranate (*Punica granatum*) juice and the methanol extract of pomegranate peel on the adenine-induced chronic renal failure in male rats.

2- Material and methods

2.1 Chemicals

Adenine (AD) was purchased from agents of Sigma Chemicals (St. Louis, Mo, USA). All other chemicals were purchased locally and were of analytical reagent grade.

2.2 Pomegranate juice preparation

Pomegranate Juice (PJ) was prepared as described by Abdel Moneim *et al.(*2011). The fresh ripened pomegranate fruits, free of blemishes or obvious defects were purchased from a local market at Mansoura City, Egypt. Ten kg of pomegranates (*P. granatum*) were washed and manually peeled, without separating the seeds. Juice was obtained using an electrical blender, filtrated with a buchner funnel and immediately diluted with distilled water to volume of 1:3 and stored at -20°C until used (Faria *et al.,* 2007).

2.3 Pomegranate peel methanol extract (PPME) preparation

Pomegranate peels were manually separated, sun dried and grounded to powder. The powder (25 g) was extracted by mixing using a magnetic stirrer with 100 ml methanol at 30°C for 1 hr. The extract was filtered to remove the peel particles. The residue was re-extracted with the same solvent. The extracts were pooled and concentrated under vaccum at 40°C (Singh *et al,*. 2002 and Abdel Moneim, 2012).

2.4 Experimental protocol

Thirty six adult male albino rats weighing 160 - 180 g were used in the present study. The rats were obtained from The Urology & Nephrology Center, Mansoura University, Mansoura, Egypt. Animals were kept under standard laboratory conditions of light/dark cycle (12/12h) and temperature (25±2°C). They were provided with water and normal laboratory diet *ad libitum*. Care and use of the animals were conducted under supervision of the Animal Care Committee of Mansoura University, Mansoura, Egypt.

After one week of acclimatization, the rats were randomly divided into six equal groups (six rats/ each) as follow:

1- Normal control (CO)group, fed on normal diet without treatment for 4 weeks.
2- PJ group, fed on normal diet and received oral administration of PJ by gastric tube at dose 3 ml/kg body weight for 4 weeks(Abdel Moneim *et al.,* 2011).
3- PPME group, fed on normal diet and received oral administration of 200 mg/kg b.w. of PPME (Parmar and Kar, 2008) for 4 weeks.
4- AD group, fed on normal diet containing adenine (0.75 % w/w) for 4Weeks (Yokozawa *et al.,* 1986).
5- AD and PJ group, fed on normal diet containing adenine (0.75 % w/w)and received oral administration of PJ at dose of 3 ml/kg body weight for 4 weeks.
6- AD and PPME group, fed on normal diet containing adenine (0.75 % w/w) and received oral administration of of 200 mg/kg b.w. PPME for 4 weeks. Animals body weight were recorded at the start and weekly.

At the end of the 4th week, the rats were placed individually in metabolic cages for 24 h to collect urine, then the animals were sacrificed under ether anesthesia. Blood samples were collected to obtain sera. The blood and urine samples were centrifuged at 850g for 15 min at 4 °C. The obtained serum and urine samples were stored frozen at -80 °C until analysis. The rats were dissected, the kidneys were removed, cleared and weighed. A known weight of each kidney was homogenized in potassium phosphate buffer (pH 7.2) using Tephlon homogenizer at 4°C. The homogenate was centrifuged and the supernatant was used for biochemical analysis.

2.5 Biochemical analysis

Serum and urine creatinine (Cr), serum blood urea nitrogen (BUN) and serum uric acid (UA) were assayed using kits provided from Biodiagnostic Company, Dokki, Giza, Egypt according to the methods described by Szasz *et al.* (1979), Fawcett and Soctt (1960) and Fossati *et al.* (1980) respectively. N-acetyl-beta-D-glucosaminidase (NAG) activity was assayed in urine according to the method of Yuen *et*

al. (1982) using assay kit obtained from the Egyptian American Company for Laboratory Services, Egypt. Creatinine clearance (Crcl) measurements were calculated using the standard formula:

Crcl (ml/min) = (Ucr x Vu)/Scr, where Ucr = Urine creatinine concentration ; Vu = Urine volume (ml/24h) and Scr= Serum creatinine concentration.

Renal lipid peroxidation product, thiobarbituric acid reactive substance (TBARS) and protein carbonyl (PC) as oxidative stress indices were assayed according to the methods of Ohkawa *et al.* (1982) and Smith *et al.* (1991) respectively. Superoxide dismutase (SOD) and catalase (CAT) activities were determined by the methods of Nishikimi *et al.* (1972) and Bock *et al.* (1980) respectively. Additionally, reduced glutathione (GSH) content was estimated according to the method of Prins and Loose (1969). C-reactive protein (CRP) concentration and nitric oxide (NO) level were determined in serum using kits provided from Biodiagnostic Company, Dokki, Giza, Egypt, according to the methods of Peltola *et al.* (1983) and Montgomery and Dymock (1961) respectively. Serum tumer necrosis factor-α (TNF-α) was measured using ELIZA technique according to Aggarwal *et al.* (1985).

2.6 Statistical analysis

All data were analyzed by one way analysis of variance (One-way ANOVA) followed by Least Significant Difference (LSD) test, using SPSS statistical package, version 17.00 software. The results were expressed as means ± S.E and values were considered to be statistically significant at $p< 0.05$ (Snedecor and Cochran, 1980).

3- Results

Table 1 shows body weight, relative kidney weight and urine volume in different rat groups. The data revealed that feeding of rats with 0.75% w/w AD for 4 weeks significantly decreased the final body weight compared to weight at the start and compared to control rats at the end of the experiment. On the other hand, co-treatment of AD fed rats with either PJ or PPME increased body weight in comparison to AD treated rats. Also, the results show that, at the end of the experiment, the relative kidney weights and the urine volumes were significantly increased while Crcl was significantly decreased in AD administered rats compared to control. However, co-treatment of AD fed rats with PJ or PPME ameliorated these effects comparing to AD-fed rats.

In Table 2, serum Cr, BUN, UA levels as well as the activity of NAG in urine were significantly increased in AD-administered group compared to control. Meanwhile, concomitant treatment with AD and PJ or PPME caused significant improvement of these parameters towards the normal levels.

The levels of oxidative stress markers (TBARS) and (PC) as well as the activities of antioxidant enzymes (SOD) and (CAT) and reduced GSH levels in renal tissue of various rat groups was shown in Table 3. The results indicate that both TBARS and PC were significantly increased while SOD and CAT activities as well as GSH levels were significantly decreased in renal tissue homogenate of AD fed rats compared to control. However, administration of AD fed rats with PJ or PPME significantly reduced the elevations in renal TBARS and PC associated with a significant increase in the activities of SOD and CAT as well as GSH level compared to AD administered rats.

The results in Table 4 revealed that a significant elevations were observed in the concentrations of TNF- α and CRP in serum of AD-treated rats compared to normal control. On the contrary, the serum level of NO in AD fed rats showed significant decrease in comparison to control group. Moreover, the results indicate that, serum TNF- α and CRP concentrations were significantly reduced accompanied with significant elevation of NO in AD treated rats co-administered with either PJ or PPME compared to AD treated rats.

Table (1): Body weight, relative kidney weight, urine volume and Crcl in control and different treated groups.

	Initial weight (g)	Final weight (g)	Relative kidney weight (g%)	Urine volume (ml/24 h)	Crcl (ml/min)
CO	174.15±5.14 ^{a}	253.70±10.31 ^{a}	0.71±0.032 ^{a}	12.09±1.69 ^{a}	2.32±0.32 ^{a}
PJ	168.90±4.82 ^{a}	260.38±8.52 ^{a}	0.69±0.028 ^{a}	11.73±2.01 ^{a}	2.40±0.50 ^{a}
PPME	177.20±6.31 ^{a}	270.60±10.62 ^{a}	0.72±0.030 ^{a}	12.48±2.17 ^{a}	2.29±0.30 ^{a}
AD	172.41±6.24 ^{a}	141.25±7.48 ^{b}	2.48±0.036 ^{b}	34.09±3.62 ^{b}	0.76±0.18 ^{b}
AD+PJ	171.20±5.24 ^{a}	198.15±8.77 ^{ac}	1.25±0.030 ^{a}	21.70±2.66 ^{c}	1.77±0.20 ^{a}
AD+PPME	175.40±7.30 ^{a}	202.40±5.17 ^{ac}	1.11±0.042 ^{a}	18.36±2.13 ^{c}	1.54±0.26 ^{ac}

Values are means±SE of six animals for each groups. Values superscripts with different letters (a-c) were significantly different ($P\leq 0.05$). CO = control, PJ = Pomegranate Juice, PPME= Pomegranate Peel methanol extract, AD = Adeninie.

Table (2): Serum Cr, BUN, UA and urine NAG activity in control and different treated groups.

	Cr (mg/dl)	BUN (mg/dl)	UA (mg/dl)	Urine NAG (IU/L)
CO	0.64±0.02 [a]	15.93±0.61 [a]	2.73±0.08 [a]	7.86±1.22 [a]
PJ	0.62±0.02 [a]	14.86±0.83 [a]	2.51±0.07 [a]	7.38±0.70 [a]
PPME	0.59±0.03 [a]	13.65±0.88 [a]	2.48±0.05 [a]	7.10±0.84 [a]
AD	2.79±0.18 [b]	62.42±1.92 [b]	4.41±0.06 [b]	54.22±2.10 [b]
AD+PJ	1.93±0.08 [bc]	25.13±1.06 [bc]	3.24±0.07 [bc]	30.13±2.45 [bc]
AD+PPME	1.61±0.04 [bc]	21.70±1.20 [bc]	2.95±0.04 [c]	27.90±2.80 [c]

Values are means±SE of six animals for each groups. Values superscripts with different letters (a-c) were significantly different ($P \leq 0.05$). CO = control, PJ = Pomegranate Juice, PPME= Pomegranate Peel methanol extract, AD = Adeninie.

Table (3): Renal TBARS, PC, SOD, CAT and GSH in control and different treated groups.

	TBARS (n mol/mg)	PC (µ mol/g)	SOD (U/min/g)	CAT (µmol/sec/g)	GSH (mg/g)
CO	96.08±0.91 [a]	0.43±0.02 [a]	1.79±0.02 [a]	2.68±0.04 [a]	3.80±0.30 [a]
PJ	95.90±0.80 [a]	0.41±0.04 [a]	1.80±0.03 [a]	2.76±0.04 [a]	4.00±0.30 [a]
PPME	95.61±1.40 [a]	0.42±0.03 [a]	1.83±0.02 [a]	2.80±0.03 [a]	3.80±0.22 [a]
AD	203.60±1.74 [b]	0.95±0.11 [b]	0.84±0.01 [b]	1.45±0.01 [b]	1.10±0.14 [b]
AD+PJ	137.22±1.56 [c]	0.79±0.13 [c]	1.60±0.02 [c]	2.15±0.02 [c]	3.20±0.32 [c]
AD+PPME	124.50±1.08 [c]	0.74±0.07 [c]	1.65±0.02 [c]	2.36±0.03 [c]	3.51±0.27 [c]

Values are means±SE of six animals for each groups. Values superscripts with different letters (a-c) were significantly different ($P \leq 0.05$). CO = control, PJ = Pomegranate Juice, PPME= Pomegranate Peel methanol extract, AD = Adeninie.

Table (4): Serum TNF-α, CRP, NO in control and different treated rat groups.

	TNF-α (pg/L)	CRP (mg/L)	NO (µ mol/L)
CO	4.68±0.14 [a]	12.25±0.68 [a]	56.29±1.77 [a]
PJ	4.05±0.32 [a]	12.16±0.72 [a]	56.82±2.14 [a]
PPME	4.28±0.26 [a]	12.08±0.74 [a]	55.19±2.05 [a]
AD	9.36±0.34 [b]	26.30±0.94 [b]	38.27±2.10 [b]
AD+PJ	6.84±0.39 [bc]	19.65±0.80 [bc]	43.60±3.00 [c]
AD+PPME	5.90±0.25 [ac]	16.34±0.73 [c]	46.02±2.65 [ac]

Values are means±SE of six animals for each groups. Values superscripts with different letters (a-c) were significantly different ($P \leq 0.05$). CO = control, PJ = Pomegranate Juice, PPME= Pomegranate Peel methanol extract, AD = Adeninie.

4- Discussion

The worldwide incidence of CRD is increasing (Locatelli *et al.,* 2006), but access to renal replacement therapy, either transplantation or dialysis is limited in several regions of the world due to a lack of financial and clinical resources (Jian*et al.,* 2012). Strategies to delay the onset of dialysis or to attenuate uremia often rely on dietary supplements.

Adenine-induced renal failure rats were used as the disease- model for evaluation of drug efficacy (Lacour *et al.,* 2005). Orally administered adenine is immediately metabolized to 2,8-dihydroxyadenine (DHA), which formed crystals in the apical region of the proximal tubular epithelia. Increased crystals induced damages in the cells of these tissues and caused renal dysfunction (Yokozawa *et at.,* 1986 and Koeda *et al.,* 1998). Therefore, the rats orally administered 0.75% adenine for more than four weeks are considered to be a model of rapidly progressive type of CRF, which are more compatible with the clinical findings. So, this model is suitable for testing new therapy (Tong *et al.,* 2010).

In the present study, the obtained data reveal that treatment of rats with AD significantly decreased body weight associated with significant increase in relative kidney weight and urine volume (Table-1). These results confirmed with previous findings of Ali *et al.* (2010); Wang *et al.,* (2011) and Kim *et al.*

(2013) who reported that body weight was significantly lower, and the ratio of kidney weight/body weight was significantly higher in AD fed rats compared to control. These changes could be attributed to malnutrition associated with AD administration, mainly due to a reduction of food intake and increase excretion of protein as albumin in urine (Tong *et al.*, 2010). Meanwhile, Co-treatment with PJ or PPME attenuate the decrease in body weight as well as the increase in urine volume and relative kidney weight.

It has been reported by Choi *et al.* (2012) that long-term feeding of AD in rats suppressed the excretion of nitrogenous compounds by means of renal tubular occlusion, and produced metabolic abnormalities resembling CRF in humans. The intake of AD produced extraordinary increase of Cr, BUN and urea in the serum of rats as well as a reduction in their urinary excretion.

The results in table-2 indicate that feeding of rats with AD (0.75% w/w for four weeks) significantly increased the markers of kidney function, serum Cr, BUN, and UA while CrCL was significantly decreased compared to normal control. These data are in consistent with previous studies confirming that AD feeding caused significant increase in concentrations of urea and Cr in plasma (Ali *et al.*, 2010 and Ali *et al.*, 2011) and significant decrease in CrCL (Ali *et al.*, 2013b and Kim *et al.*, 2013). These findings indicate that the obtained rises in serum Cr, BUN and UA reflects a disease or damage in the kidneys (Wang *et al.*, 2011). In addition, in the present study, NAG activity in urine was elevated in AD treated rats confirming kidney damage as NAG is biomarker of proximal tubular damage (Bosomworth *et al.*, 1999) and may be a biomarker of injury to other parts of the nephron.

Moreover, the co-administration of PJ or PPME to AD feeding rats caused significant reduction in serum Cr, BUN and UA as well as significant CrCL increase accompanied with decreased NAG activity in urine compared to AD fed rats, indicating improvement in renal function. These effects are assumed to be related to the antioxidant property of pomegranate, as shown in Table-3, through scavenger of FR released as a consequence of oxidative damage (Singh *et al.*, 2011).

In mammalian metabolism, when AD is present in excess, it become a significant substrate for xanthene dehydrogenase. This enzyme can oxidize AD to 2,8-dihydroxyadenine (DHA) forming crystals in renal tubules (Ali *et al.*, 2010). The DHA precipitated crystals could enhance the production of reactive oxygen species (ROS) as peroxides and superoxide anion radicals (Veena *et al.*, 2006) causing oxidative stress. Ali *et al.* (2013a) reported that superoxide formation was significantly higher in the kidneys of AD-treated rats compared to the kidneys of control. These produced FR interact with renal epithelia damaging the renal membranes and lead to the tubules dysfunction and damage (Grases *et al.*, 1998).

When ROS are generated as a consequence to tissue injury induced by AD and is not eliminated, it well attack different cell components as DNA, RNA, proteins, lipids and enzymes leading to many degenerative processes in the renal cells manifested as glomerular disease, renal ischemia, perfusion injury and evantually renal failure. (Abdel-Raheem *et al.*, 2010).

Altered antioxidant enzymatic and non-enzymatic system function was observed in CRF rats where, oxidative stress results from the excessive generation of oxidants, which overwhelms antioxidant defense mechanisms. Wang *et al.* (2011) found a negative correlation in BUN, Cr and antioxidant enzymes in AD-treated rats demonstrated that FR production activation is highly influenced by the damage of kidney. Also, oxidative stress is already found in early stages of renal disease and increase with declining kidney function (Dounousi *et al.*, 2006).

The obtained results in Table 3 demonstrated that feeding of rats with AD (0.75% w/w for 4 weeks), induced biochemical signs of kidney tissue injury, evidenced by increased TBARS and PC levels and decreased the antioxidant enzyme activities of SOD and CAT as well as decreased GSH content in renal tissue homogenate. Evidence of these results are means of associated oxidative stress which identify FR-induced injury (Rosenblat *et al.*, 2006) with an overall decrease in cellular function. With regard to antioxidant defense system in this study, AD-treatment significantly reduced the total content of GSH and inhibited the activities of SOD and CAT confirming that AD feeding caused oxidative stress in renal tissue. These results are in accordance with recent findings by Kim *et al.* (2013) and Ali *et al.* (2013a&b) who confirmed that feeding of rats with AD for 4 weeks significantly increased oxidative stress markers and decreased the activities of antioxidant enzymes as well as GSH level in renal tissue.

In the present study, the resulted decrease in SOD activity can be attributed to the increased superoxide radical by AD. This finding confirmed by Vaziri (2004) who showed that oxidative stress in CRF animals is associated with and, in part, owing to upregulation of superoxide producing enzyme, nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, and dwon regulation of SOD. The decrease in the activity of CAT in the kidney of AD-

treated rats can be attributed to direct inhibition of CAT by the DHA crystals and decreased regeneration of CAT from its inactive form, due to a lesser availability of NADH (Kirkman and Gaetani, 1984).

The observed decrease of GSH content in AD-treated rats might be due its increased conversion to GSSG. GSH acts as a radical scavenger by itself and as a detoxicant in eliminating different electrophilic toxic compounds (CeballosPicot *et al.,* 1996). Hanly *et al.* (2009) reported that GSH depletion induces LPO and ultimately cell lyses. Replenishing the GSH level is, therefore, necessary for the maintenance of the overall thiol status in the cell. Indeed, GSH concentration closely correlated with the degree of renal failure (Wang *et al.*,2011). Depletion in GSH by itself could contribute to the progression of uremia because it has been demonstrated that GSH depletion in rats leads to an acute renal failure (Abulezz *et al.,* 1991).

The obtained results in Table-3 revealed that treatment of rats with P. extracts ameliorated the oxidative stress induced in renal tissue homogenate by AD. This amelioration represented by decreasing TBARS and PC levels in renal tissue associated with elevation in the activities of the antioxidant enzymes, SOD and CAT. GSH content also significantly elevated in the kidney of rats co-administered with AD and P. extracts. These effects were evident from the significant decrease in serum levels of urea, Cr and UA, as marker parameters of kidney toxicity, compared to AD- treated rats and they were close to those in the control group (Ali and saeed, 2012). Meanwhile, the observed improvement in the measured parameters was more obvious with PPME more than PJ and this can be attributed to its high content of polyphenols in peel, such as condensed tannins and anthocyanins (Wang *et al.*, 2011). It has been reported by Ahn *et al.* (2004) and QU *et al.* (2010) that the peel possesses relatively higher antioxidant activity than seed and pulp and therefore might be a rich sources of natural antioxidants. Also, methanol extracts of peels had higher total phenolics than water extract or ether extract (Shiban *et al.,* 2012).

Previous studies demonstrated the effect of different pomegranate extracts on rats with induced renal failure. Ahmed and Ali, (2010) reported that pomegranate peel ethanol extract ameliorated the ferric nitrilotriacetate (Fe-NTA)-induced inhibition of the activity of antioxidant enzymes (CAT, GR and GPx) and GSH concentration. Also, Ali and Saeed (2012) found that co-treatment of aqueous extract of pomegranate (*Punica granatum*), attenuated gentamicin-induced renal oxidative damage in rats. In addition, hydroalcoholic extract of flowers (Singh *et al.,* 2011) and seed oil (Jurenka, 2008) of *Punica granatum* has ameliorative potential in attenuating glycerol-induced acute renal failure and paracetamol nephrotoxicity respectively in rats.

The nephroprotective effect of P. extracts may be related to different mechanisms. One of these mechanisms is the antioxidant property of P. through scavenger of free radicals released as a consequence of oxidative damage as reported in numerous studies and (Ahmed and Ali, 2010; Singh *et al.,* 2011).

Aviram *et al.* (2002) and Yasoubi *et al.* (2007) confirmed that the antioxidants, polyphenols are rich in *P.* and they are more potent, on a molar basis, than many other antioxidants, like vitamins C and E and coenzyme Q10. Pomegranate is an important source of anthocyanins, hydrolysable tannins punicalagin and punicalin (Afaq *et al.,* 2005), ellagic and gallic acids (Lansky and Newman, 2007) and also contains vitamin C (Turk *et al.,* 2008).

In the present study, the levels of inflammatory markers, Tumor necrosis factor-α (TNF-α) and C-Reactive protein (CRP) were significantly elevated associated with significant decrease of NO level in rats treated with AD compared to control. These results are in agreement with previous findings (Ali *et al.*, 2013a&b and Mahmoud *et al*., 2012) who reported that AD feeding to rats induced significant increase in the concentrations of the inflammatory mediators, TNF-α and CRP, as a signs of inflammation.

CRP has long been used as biomarker is increased in inflammation and infection (Standage and Wong, 2011). It has been shown to be increased in plasma of rats with kidney damage (Korish, 2009) and patients with advanced kidney failure (Shlipak *et al.*, 2003). Elevated CRP is associated with endothelial injury and impaired vasodilatation, both of which may lead to glomerular damage and progressive loss of kidney function (Arici and Walls, 2001). In addition, CRP is known as a mediator stimulating the release of other pro-inflammatory cytokines such as interleukin-6 (IL-6) and TNF-α (Elenkov*et al.,* 2005).

TNF-α is a central proinflammatory agonist mediator that is generated in a wide variety of innate and adaptive immune responses, including some forms of chronic kidney disease. TNF-α binds to cell surface receptors on target cells and induces expression of adhesion molecules, chemokines for leukocytes, and apoptosis in susceptible cells (Laster *et al.,* 1988). Soluble TNF receptors are elevated in the setting of inflammation and chronic kidney disease (Knight *et al.,* 2004). Thus, TNF-α also appears to have multiple roles that could mediate progressive renal injury, and both soluble TNF receptor II (sTNFrii) and CRP may be used as markers of inflammation (Tonelli *et al.,* 2005).

Numerous studies have reported an association between renal impairment and different mediators and

markers of inflammation including CRP, IL-6, TNF-α and fibrinogen even among patients with moderate renal impairment, suggesting that CKD is a low – grade inflammatory process (Stenvinkel, 2006) with polymorphonuclear leukocyte and CD14+/CD16+ cells being key mediators in this process (Merino *et al.*, 2008).

Persistent inflammation may also be a risk factor *per se* for progression of CKD, as inflammatory markers are predictors of kidney function deterioration (Fried *et al.,* 2004) This could be a consequence of inflammatory mediators as TNF-α or IL-6 being able to act as toxins participating in uremia complications (Vanholder *et al.,* 2003). Moreover, CRP formed locally in the renal inflammatory process reduces NO production (Jabs *et al.,* 2003).

In the present study, co-administration of AD fed rats with PJ or PPME significantly attenuated the changes in inflammatory markers, TNF-α and CRP concentrations accompanied with increase of NO level compared to AD fed rats.

The results in the current study confirm the hypothesis supported by observation that, markers of oxidative stress (MDA and PC) associated with decrease in antioxidants (SOD, CAT, and GSH), are correlated with markers of inflammation (TNF-α and CRP) in CRD as reported by Himmelfrab *et al.* (2002).

According to the obtained results, it appears that, besides the antioxidant effect mechanism, an anti-inflammatory has been proposed as another mechanism for Pomegranate to which part of the effects of pomegranate could be attributed. A view which supported by the findings of (Lee *et al.,* 2010) who found that pomegranate seed oil is shown to limit LPO concequences that triggers the activation and the inflammatory responses of the immune system within the cells and induces release of the inflammatory mediators such as cytokines, chemokines, and reactive oxygen and nitrogen species that contribute to the progression of kidney injury.

In the present study, the obtained improvement in renal physiology of AD-treated rats co-administered with PJ or PPME can be attributed to the activation of PPAR-γ receptors induced by pomegranate (Huang *et al.,* 2005) and increased NO production.

A recent study by Singh *et al.* (2011) reported that the renoprotective effects of pomegranate involve the activation of nitric oxide-dependent and peroxisome proliferator-activated receptor (PPAR-γ) signaling pathway. There have been reports suggesting that ethanolic extract of flowers of *P. granatum* modulate different functions through NO signaling pathway (Kaur *et al.,* 2006). The protective role of NO in different models of renal failure has been documented (Valdivielso *et al.,* 2000), including glycerol-induced renal failure (Aydogdu *et al.,* 2006) and nephrolithiasis induced by ethylene glycol (Tugcu *et al.,* 2008). These studies have demonstrated that levels of NO are decreased in glycerol-induced renal failure and different agents have shown to produce renoprotection by increasing the NO production.

The present data demonstrate that PPME was more potent than PJ in alleviating the renal damage induced by AD. These findings confirm previous reports of Singh *et al.,* (2002), who investigated the antioxidative activity of methanol, water and acetone extracts of pomegranate peel using β-carotene-linoleate model system and found a positive correlation between the phenolic content and the antioxidant activity of the three extracts. Also, Kulkarni*et al.* (2004) compared the antioxidative activity of pomegranate peel extracts with punicalagin, a major pomegranate polyphenol. They found that the extract had higher antioxidative activity than punicalagin, which showed a synergetic effect between different phenolic compounds present in the peel extract.

Conclusion

The present study suggest that pomegranate extracts, especially methanol peel extract is a potent nephroprotective agent on chronic renal failure rat model induced by adenine. This renoprotective effect of pomegranate extracts can be attributed to its high phenolic content and the mechanism of action may be through induction of various antioxidant enzymes and scavenging reactive oxygen species. Furthermore, another mechanism may be through anti-inflammatory and different signaling pathways, which need further investigation to elucidate this mechanism.

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