

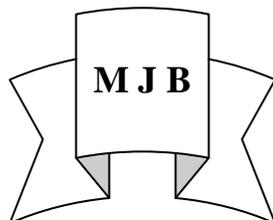
## Nephro-Protective Effect of *Punica granatum* in Gentamicin-Induced Nephrotoxicity in Rats

Nidhal AK Mohammed Ali

Shatha Z Saeed\*

Dept. of Pharmacology, College of Medicine, Hawler Medical University, Erbil, Iraq.

\*Dept. of Pathology, College of Medicine, Hawler Medical University, Erbil, Iraq.



### Abstract

The aqueous extract of *Punica granatum* (AEPF) was investigated for its protective effects on gentamicin-induced renal toxicity in rats. Nephrotoxicity was induced by intraperitoneal injection of gentamicin (100 mg/kg) for 8 days. The effect of AEPF (100mg/kg) given orally either with gentamicin or after 8 days of gentamicin administration was assessed by biochemical and histopathological changes of kidneys.

AEPF protected the rats from alteration in serum levels of urea, creatinine, uric acid, sodium, potassium and chloride better when co-administered with gentamicin than when given after induced gentamicin nephrotoxicity and also through reversing the mild tubular necrosis than severe tubular necrosis.

### الخلاصة

تم دراسته تأثير المستخلص المائي لـ *Punica granatum* (AEPF) الوقائي للتسمم الكلوي المحدث بالجنتاميسين في الجرذان. تم استحداث التسمم الكلوي باعطاء الجنتاميسين (١٠٠ ملغم/كغم) عن طريق الخلب لمدة ثمانية ايام. تم تقييم تأثير الـ AEPF المعطى عن طريق الفم اما معا او بعد ثمانية ايام من اعطاء الجنتاميسين عن طريق الفحوصات البايوكيميائية والنسجية المرضيه للكلية. اظهر الـ AEPF تأثيرا وقائيا لكلى الجرذان من التغييرات في مستويات اليوريا والكرياتينين وحمض البوريك والصوديوم واليوتاسيوم والكلوريد في مصل الدم افضل عند اعطائه سويا مع الجنتاميسين مما اذا اعطي بعد حدوث التسمم الكلوي بالجنتاميسين وكذلك من خلال عكس النخر الانبوبي الكلوي المعتدل اكثر من النخر الانبوبي الكلوي الوخيم.

### Introduction

Phytotherapy is considered as a complementary approach for preventing and treating simple disease. Although it often lacks proper scientific validation but it is well grounded in medical tradition [1]. In addition to its ancient historical uses, recently many scientific studies have reported that the chemical constituents of different parts of *P. granatum* are used for their potential for prevention and treatment of many diseases. The peel, seeds, leaf and flower contain phenols (flavonoids, and tannins) that

possess diverse pharmacological properties [2, 3].

The major components of *P. granatum* (polyphenols) show remarkable antioxidant proprieties [1, 4] for cardiovascular health benefits that may in part be related to the ability of polyphenols to inhibit platelet function [5], in cancer prevention [6], in skin protection [7]. Pomegranate has also immunosuppressive activity [8], antidiabetic effect [9], hepatoprotection [10], anti-inflammatory [11], and antimicrobial effect [13-16].

The antibiotic, gentamicin is usually claimed to induces nephrotoxicity through the generation of oxidative radicals. It is actively transported into the proximal tubules and thereby causes tubular injury and a reduction in the glomerular filtration.

The nephrotoxicity caused by gentamicin is related to its high concentration attained in the cells of proximal tubules compared to the serum concentrations [17-19] and its tendency to bind strongly to the components of the brush border membrane of the proximal tubule leading to the most common clinical presentation of acute renal failure characterized by the presence of enzymuria that is represented by the elimination in the urine of fragments of brush border membrane or lysosomal enzymes [20- 24]

Therefore the objective of this study was to investigate the prophylactic effect of aqueous extract of *P. granatum* in gentamicin- induced renal oxidative damage in rats either administered with gentamicin at the same time or administered after inducing nephrotoxicity by gentamicin through determination of kidney function parameters.

## **Materials and Methods**

### **Plant material and preparation of plant extract**

Fresh Pomegranate fruit was collected from Iraqi Kurdistan region (Shaqlawa region) and was defined *punica granatum L.* in the department of plant, College of Agriculture, University of Salahaddin.

The whole fruit including the peel was washed with tap water and cut into tiny bits of about 2 cm. Fifty grams of the whole fruit was immersed in 200 ml distilled water and subsequently was mixed using a blinder machine . The mixture was then filtered with a No 1 Whatman filter paper and the

filtrate was concentrated to dryness in vacuo at 40°C using a rotary evaporator to obtain the crude extract.

### **Animals**

Male Albino rats weighing between 170-190  $\pm$ 20 g aged 12 weeks were used in the present study. The rats were obtained from the animal house of the Hawler Medical College. The animals were kept under standard laboratory conditions of light/dark cycle (12/12h) and temperature (25 $\pm$ 2°C). The rats were allowed to food and water *ad libitum*. They were provided with a nutritionally adequate standard laboratory diet.

### **Experimental design**

The rats (n=24) were randomly allocated to four groups of six rats each: Group 1 (C) received only distilled water orally and by IP rout throughout experiment period. Group 2 (G) received single intraperitoneal (IP) injection of gentamicin daily at a dose of 100 mg/kg body weight.

Group 3 (G+AEPF) received gentamicin (IP) at 100 mg/kg concurrently with aqueous extract of *P. granatum* fruit (AEPF) given by oral gavage once daily for eight days at a dose of 100 mg/kg body weight. Group 4 (G-AEPF) received once daily IP injection of gentamicin for 8 days and a single oral dose of AEPF (100 mg/kg body weight) on day 8 of the experiment.

Twelve hour after the last treatment, all rats were anesthetized by IP injection of ketamine (35mg/kg) and blood samples were immediately obtained by intracardiac aspiration. The serum was rapidly separated and processed for determination of urea, creatinine, uric acid, potassium, sodium and chloride using commercially ARKRAY kits

After the animals were sacrificed, postmortem examination was performed. The rat kidneys were identified and carefully dissected out *en bloc* for histopathological

examination. Pieces of kidney from each group were fixed immediately in 10% neutral formalin for a period of at least 24 h, dehydrated in graded (70–100%) alcohol, embedded in paraffin, cut into 4–5  $\mu\text{m}$  thick sections, stained with Hematoxylin–eosin and finally observed under a light microscope. The sections were evaluated for the pathological changes of nephrotoxicity mainly in proximal tubules such as: swelling, loss of brush borders, desquamation and intraluminal cast formation

#### **Statistical data analysis**

The results were given as the mean  $\pm$  SEM. One-way analysis of variance (ANOVA) were used to compare data between groups for changes in serum levels of creatinine, BUN, potassium, sodium, and chloride using SPSS, version 11. The level of significance was set at 0.05.

#### **Discussion**

Urea, creatinine, uric acid and electrolytes are markers of kidney function. The alterations in the levels of these markers in gentamicin treated group shown in this study have also been reported by others when toxic doses of gentamicin are administered [28, 29]. Gentamicin induces oxidative stress through induction of reactive oxygen species such as free radical, superoxide, hydroxyl radical anion and hydrogen peroxide [30-34].

When ROS are generated as a consequence to tissue injury induced by gentamicin and is not eliminated, it will 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 eventually acute renal failure [35-38]. This side effect was evident in the present study by the significant alteration in urea, creatinine, uric acid and electrolytes

serum levels of this group. Such effects have also been documented previously [39, 40].

Furthermore the histo-pathological changes seen in the kidneys section in gentamicin treated group supports the concept of gentamicin-induced renal toxicity and explains the significant alteration in kidney function tests found in this study. As the proximal tubule accumulates this antibiotic highly because of its physio-chemical properties therefore it is the main part of kidney affected by gentamicin toxicity which was obvious in the sections of kidneys of gentamicin treated groups. The histo-pathological changes were similar to others findings [41, 42].

The co-treatment of AEPF attenuated gentamicin induced renal oxidative damage in rats. These effects were evident from the significant decrease in serum levels of urea, creatinine, uric acid and potassium compared to gentamicin and they were close to those in the control untreated group. These effects are assumed to be related to the antioxidant property of pomegranate through scavenger of free oxygen radical released as a consequence of oxidative damage as reported in numerous studies [43, 44].

The antioxidants, polyphenols are rich in pomegranate and they are more potent, on a molar basis, than many other antioxidants, like vitamins C and E and coenzyme Q10 and in other natural juices, such as blueberry, cranberry, and orange, as well as in red wine [45, 46].

Since gentamicin nephrotoxicity is a reversible process or partially reversibly depending on the level of kidney damage and gentamicin concentration in kidney cells and blood [47], this explains that co-treatment of AEPF (100 mg/kg) with gentamicin reversed the mild kidney damage in G+AEPF group when the levels of

gentamicin in kidney tissues were not too high while when the processes of kidney degeneration is progressing (group G-AEPF) by the administration of gentamicin for a long time (8 days) and at toxic high dose, the kidneys function fails more and less gentamicin is excreted which leads to a higher concentration of gentamicin in the bloodstream that may further damage the kidneys. The effect of AEPF (100 mg/kg) after 8 days of administration of toxic doses of gentamicin although revealed significant decrease in urea, creatinine and uric acid serum levels compared to gentamicin group but it showed less protection of kidney damage than the G+AEPF group (compared to control and G+AEPF) indicating the lower potency of AEPF to protect the kidney from oxidative damage when the process of tubular necrosis is moderate rather than mild (group G+AEPF) as demonstrated by the histo-pathological sections.

The non significance differences of serum levels of uric acid and chloride between control and G-AEPF probably is due to the small number of studied rats.

Therefore, it can be assumed that the nephroprotection shown by AEPF in gentamicin-induced nephrotoxicity, in the dose used in the present study could be mediated through its potent antioxidant effects that help to preserve intracellular levels of biological pathways that supportively enhance excretion of toxic levels of gentamicin. Whereas when kidney damage is profound, the antioxidant effect of AEPF was not enough to reverse the moderate kidney damage. Probably higher dose is required to reduce ROS and protect the cells from oxidative damage and prevent the moderate kidney damage.

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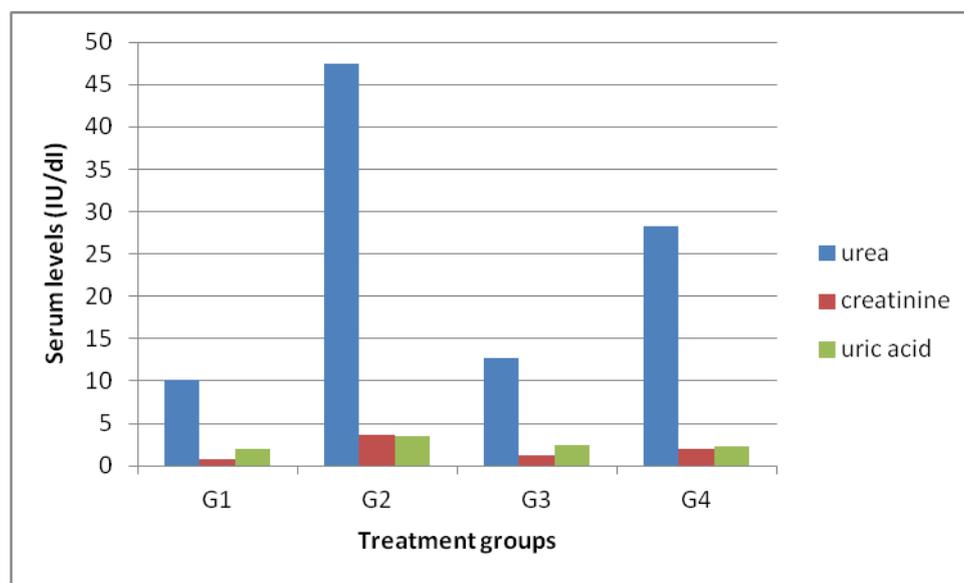
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**Table 1** The effect of aqueous extract of *P. granatum* on serum levels of urea, creatinine and uric acid in different groups. (G: control, G2: gentamicin only, G3: both AEPF and gentamicin (G+AEPF), G4: gentamicin then AEPF (G-AEPF)).

Biochemical parameters	Experimental group	Mean (±SD )	P* value	Significance between groups**
Urea	G1	10.11 ( ±2.13 )	< 0.001	G1XG2 G1XG4 G2XG3 G2XG4 G3XG4
	G2	47.44 (±12.96)		
	G3	12.73 (±1.18 )		
	G4	28.29 (±5.05 )		
Creatinine	G1	0.78 (± 0.12 )	< 0.001	G1XG2 G1XG4 G2XG3 G2XG4 G3XG4
	G2	3.66 (±0.93 )		
	G3	1.29 (± 0.19 )		
	G4	2.03 (± 0.59 )		
Uric acid	G1	1.92 (± 0.24 )	0.001	G1XG2 G2XG3 G2XG4
	G2	3.53 (± 1.01)		
	G3	2.40 (± 0.39 )		
	G4	2.32 (±0.46 )		

\*ANOVA test, and \*\*LSD test (Post Hoc test) with significant P value < 0.05

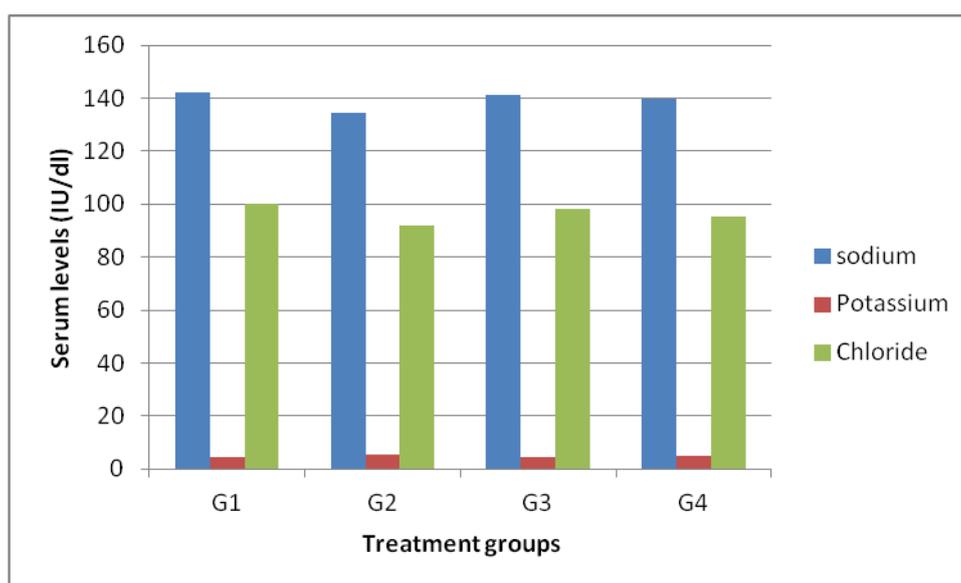


**Figure 1** Effect of aqueous extract of *P. granatum* (AEPF ) on serum levels of urea, creatinine and uric acid in different treatment groups. {G1: Control, G2: Gentamicin only, G3: Both AEPF and gentamicin (G+AEPF), G4: Gentamicin then AEPF (G-AEPF)}.

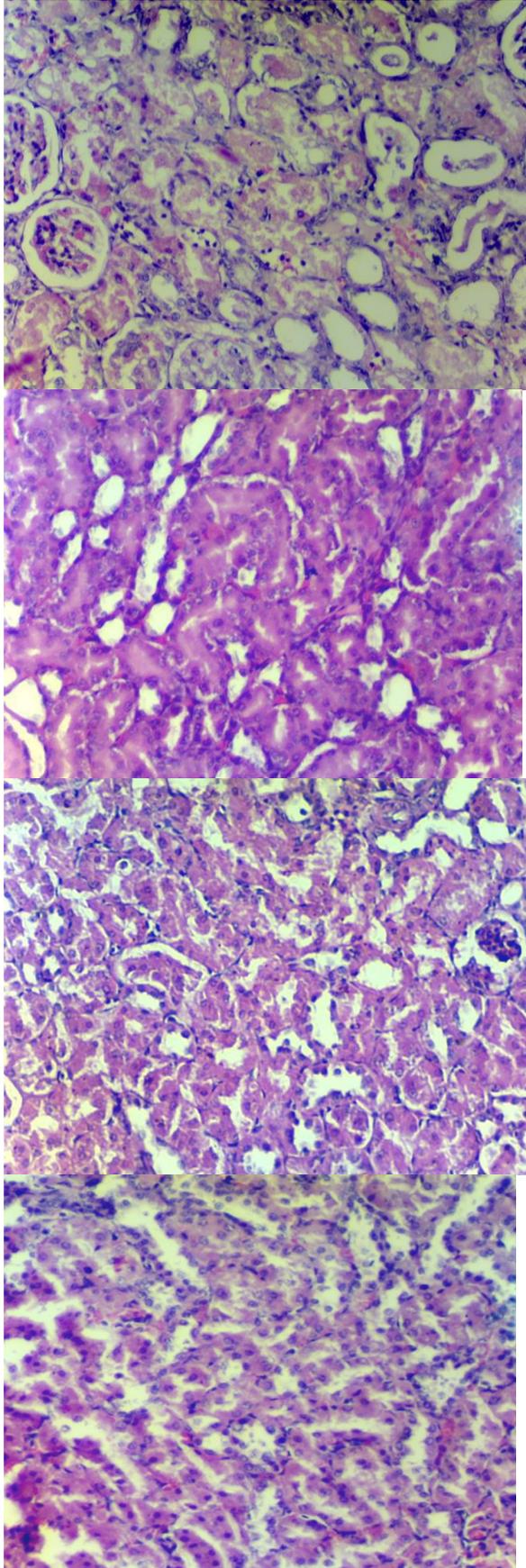
**Table 2** The effect of aqueous extract of *P. granatum* on serum levels of sodium, potassium and chloride in different groups. (G: control, G2: gentamicin only, G3: both AEPF and gentamicin (G+AEPF), G4: gentamicin then AEPF (G-AEPF)).

Biochemical parameters	Experimental group	Mean (±SD )	P * value	Significance between groups**
Sodium	G1	142.3 (±2.33)	0.01	G1XG2 G2XG3 G2XG4
	G2	134.5 (±6.56 )		
	G3	141 (± 1.41 )		
	G4	139.6 (±1.86 )		
Potassium	G1	4.32 (± 0.28)	< 0.001	G1XG2 G1XG4 G2XG3 G3XG4
	G2	5.43 (±0.54 )		
	G3	4.6 (±0.32 )		
	G4	5.09 (±0.36 )		
Chloride	G1	100.2 (±4.66 )	0.086	G1XG2 G2XG3
	G2	91.8 (±7.33 )		
	G3	98.2 (± 4.11 )		
	G4	95.2 (± 5.74 )		

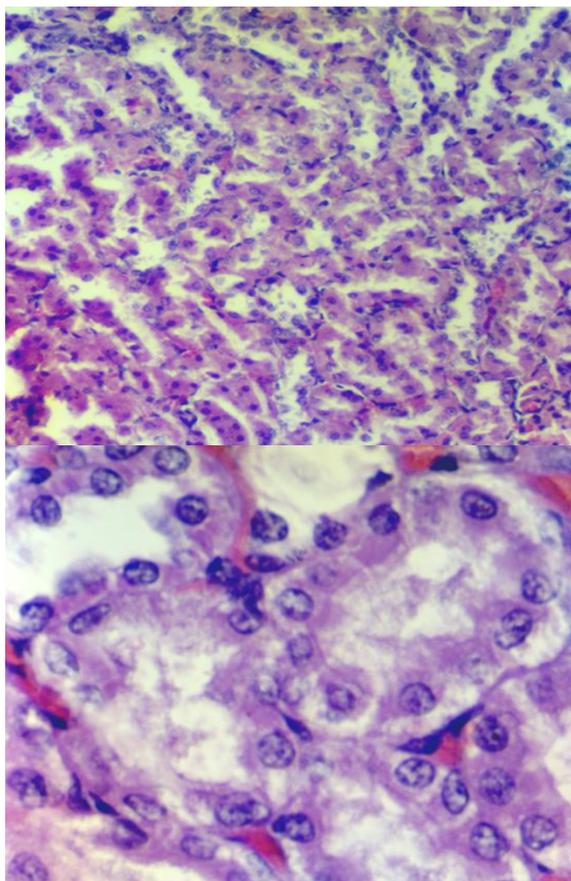
\*ANOVA test, and \*\*LSD test (Post Hoc test) with significant P value < 0.05



**Figure 2** Effect of aqueous extract of *P. granatum* (AEPF) on serum levels of sodium, potassium and chloride in different treatment groups. {G1: Control, G2: Gentamicin only, G3: Both AEPF and gentamicin (G+AEPF), G4: Gentamicin then AEPF (G-A



**Figure 3** Nephroprotective effect of *P. granatum* extract on gentamicin induced kidney damage. Histological finding of (A) normal kidney (Control Group), (B) Group2 (G) received gentamicin showing sever tubular necrosis and intraluminal cast (C) in Group 3(G+AEP) showing normal renal tubules with mild swelling compared with (D) in Group 4 (G-AEPF) showing more tubular necrosis. (H and E, original magnification x100).



**Figure 4** Nephroprotective effect of *P. granatum* extract on gentamicin induced kidney damage. (A) Histological finding of Group 2 (G) received gentamicin showing sever tubular necrosis and intraluminal cast. (B) Histological finding of Group 3(G+AEP) showing mild tubular swelling. (H and E, original magnification x400).