

## POMEGRANATE ATTENUATES ACUTE GENTAMICIN-INDUCED NEPHROTOXICITY IN SPRAGUE-DAWLEY RATS: THE POTENTIAL ANTIOXIDANT AND ANTI-INFLAMMATORY EFFECTS

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### ABSTRACT

**Objectives:** Nephrotoxicity is a renal-specific situation in which the excretion of toxic metabolites is reduced due to toxic agents and drugs. Gentamicin is an antibiotic belongs to aminoglycoside group which may induce nephrotoxicity due to induction of oxidative stress. Pomegranate is a component of the traditional medicine called *Punica granatum* with significant nephron-protective effect. Therefore, the objective of the present study was to evaluate the nephronprotective effect of pomegranate in gentamicin-induced nephrotoxicity.

**Methods:** A total of 30 male Sprague-Dawley rats were used which divided into Group 1 (n=10): Rats treated with distilled water 5 ml/kg plus normal saline 5 ml/kg for 12 days, Group 2 (n=10): Rats treated with distilled water 5 ml/kg plus gentamicin 100 mg/kg for 12 days, and Group 3 (n=10): Rats treated with pomegranate 100 mg/kg plus gentamicin 100 mg/kg for 12 days. Blood urea, serum creatinine, malondialdehyde (MDA), kidney injury molecule (KIM-1), and cystatin-C were measured in both control and experimental groups.

**Results:** Rats treated with gentamicin showed nephrotoxicity as evident by significant elevation in serum creatinine, blood urea, serum creatinine, KIM-1, MDA, and cystatin-C sera levels. Pomegranate leads to significant reduction of blood urea and serum creatinine compared to gentamicin group,  $p < 0.05$ . Pomegranate also reduced MDA, KIM-1, and cystatin-C sera levels significantly compared to gentamicin group,  $p < 0.01$ .

**Conclusion:** Pomegranate produced significant nephroprotective effect on gentamicin-induced nephrotoxicity through modulation of oxidative stress and inflammatory biomarkers.

**Keywords:** Nephrotoxicity, Gentamicin, Pomegranate.

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### INTRODUCTION

Nephrotoxicity is a renal-specific circumstance due to toxic agents and drugs. About 20% of nephrotoxicity is induced and caused by drugs; this fraction is augmented in the old age due to ascend in the life span [1].

Gentamicin is an antibiotic of aminoglycoside group, used for the treatment of dissimilar bacterial infections. 90% of administrated gentamicin is excreted unchanged in the proximal renal tubule that leads to a necrosis at a higher dose [2].

An excess in the generation of reactive oxygen species (ROS) and free radicals is the main mechanism of gentamicin-induced nephrotoxicity. Absolutely, gentamicin induces the expression of transporter proteins at proximal renal tubules causing free radical generations [3]. As well, gentamicin-induced nephrotoxicity is a versatile incident which previously allied to the oxidative stress only.

Chronic and high dose of gentamicin initiates *in vitro* and *in vivo* free radical productions and induction of oxidative stress. Gentamicin elicits mitochondrial superoxide anions that cause the generation of hydroxyl radicals [4].

Hence, antioxidant agents reveal a renoprotective effect in gentamicin-induced nephrotoxicity. Pomegranate peel contains 50% of bioactive constituents including flavonoids, phenolics, proanthocyanidin, and ellagitannins with different minerals. Pomegranate edible part contains 10% seeds and 40% arils, arils mainly enclose anthocyanins, while the seeds contain mainly anthocyanin and glucosides. The most important phytochemical in the pomegranate juice is an ellagitannin which also named punicalagin which inhibits peroxidation through scavenging of free radicals [5].

Pomegranate effect on free radicals is happened by different mechanism, it scavenges various types of free radicals such as ROS and reactive nitrogen species, and also, it inhibits ROS-generating enzymes including cyclooxygenase, lipoxygenase, and xanthine hydrogenase/oxidase [6].

Therefore, the objective of the present study was to evaluate the nephroprotective effect of pomegranate on gentamicin-induced nephrotoxicity.

### METHODS

A total number of 30 Sprague-Dawley male rats were used, rats age ranges from 3 to 4 months and their body weight ranges from 200 to 400 g. The animals were placed at appropriate temperature of 22–25°C with 12/12 hrs, light-dark cycle. They left 1 week for adaptation without any intervention with free access to normal chow pellets and water. Humane care for animals was according to the guide to the care and the use of laboratory animal.

The rats were randomly divided into three groups, 10 rats in each group. Group 1 (n=10): Rats treated with distilled water (5 ml/kg, p.o) for 12 days, on day 6–12, they received an intraperitoneal (i.p.) injection of normal saline (5 ml/kg) daily. Group 2 (n=10): Rats treated with distilled water (5 ml/kg, p.o) for 12 days, and on day from 6 to 12, they received gentamicin 100 mg/kg, i.p. Group 3 (n=10): Rats treated with pomegranate (100 mg/kg, p.o) for 12 days, and on day 6–12, they received gentamicin 100 mg/kg, i.p. at an interval of 1 h. On the 13<sup>th</sup> day, rats were decapitated under light anesthesia, blood samples were obtained and serum was centrifugated at 3500 rpm/15 min. The method protocol was according to Singh *et al.* method [7].

**Table 1: Renal function and renal injury biomarkers in gentamicin-induced nephrotoxicity**

Variables	Control (n=10)	Gentamicin (n=10)	p
Blood urea (mg/dL)	41.83±7.46	56.87±9.33	0.007
Serum creatinine (mg/dL)	0.70±0.14	1.08±0.40	0.04*
MDA (ng/mL)	289.85±44.18	408.11±145.8	0.08
KIM-1 (pg/mL)	73.78±16.29	354.98±46.38	0.0001
Cystatin-C ng/ml	0.024±0.0005	0.028±0.001	0.0001

Data are expressed as mean±SD \*p<0.05; p<0.01, unpaired t-test, MDA: Malondialdehyde, KIM-1: Kidney injury molecule-1, Cys-C: Cystatin

**Table 2: Effect of pomegranate on the biochemical and renal injury biomarkers in gentamicin-induced nephrotoxicity**

Variables	Gentamicin (n=10)	Pomegranate (n=10)	p
Blood urea (mg/dL)	56.87±9.33	46.25±8.47	0.01*
Serum creatinine (mg/dL)	1.08±0.40	0.77±0.18	0.03*
MDA (ng/mL)	408.11±145.8	208.11±88.8	0.001
KIM-1 (pg/mL)	354.98±46.38	131.79±31.22	0.0001
Cystatin-C ng/ml	0.028±0.001	0.024±0.0004	0.0001

Data are expressed as mean±SD \*p<0.05; p<0.01, unpaired t-test, MDA: Malondialdehyde, KIM-1: Kidney injury molecule-1, Cys-C: Cystatin

All drug and herb were purchased from private pharmaceutical company. Gentamicin ampoule (Garamycin 80 mg Schering-Plough, USA) and pomegranate (Pomegranate extract 250 mg, 40% ellagic acid, Puritans Pride, USA).

Assessment of renal injury biomarkers: Blood urea and serum creatinine were assessed by autoanalyzer. Biomarkers of renal injury including malondialdehyde (MDA), kidney injury molecules (KIM-1), and cystatin-C were measured by ELISA kit methods according to the instruction of the manufacturer.

#### Statistics

Data of the present study were presented as mean±SD and the variables were tested using unpaired Student's t-test between control and treated groups. P value was regarded as statistically significant when it <0.05.

#### RESULTS

During gentamicin-induced nephrotoxicity, blood urea was increased significantly in gentamicin group up to 56.87±9.33 mg/dl compared to the control group 41.83±7.46 mg/dl (p=0.007), while serum creatinine in gentamicin group was increased significantly (1.08±0.40 mg/dl) compared to the control group (0.70±0.14 mg/dl), p=0.04. Regarding the oxidative stress and endogenous antioxidant capacity, there was insignificant increase in the MDA serum levels in gentamicin group (408.11±145.8 ng/ml) compare to the control group (289.85±44.18 ng/ml), p=0.08. Moreover, KIM-1 was significantly raised in gentamicin group (354.98±46.38 pg/ml) compared to the control group (73.78±16.29), p=0.0001. Definitely, cystatin-C serum level was significantly increased during induction of nephrotoxicity by gentamicin from 0.024±0.0005 ng/ml in the control group to 0.028±0.0016 ng/ml in the experimental group, p=0.01 (Table 1).

Pomegranate led to significant reduction of blood urea and serum creatinine compared to gentamicin group, p<0.05. Pomegranate also reduced MDA, KIM-1, and cystatin-C sera levels significantly compared to gentamicin group, p<0.01 (Table 2).

#### DISCUSSION

Gentamicin is a bactericidal antibiotic typified by chemical stability and quick antibacterial activity, which is widely used alone or in combination with β-lactam antibiotics for diverse and severe bacterial infections. In spite of these properties, gentamicin therapy leads to nephrotoxicity in about 30% of treated cases even with accurate monitoring [8].

The current study undoubtedly demonstrated that gentamicin was capable to induced experimental nephrotoxicity in rats which

presented via significant elevation in blood urea and serum creatinine which correspond with up to dated study [9].

It has been recognized by diverse studies that the creation of free radicals and initiation of oxidative stress is the major imperative pathway of gentamicin-induced nephrotoxicity. Overproduction of ROS is associated with depletion of proximal renal tubules antioxidant potential which developed into lipid peroxidation and tubular damages [10].

As a result, serum level of MDA is elevated in diverse models of gentamicin-induced nephrotoxicity as showed by Hajhashemi *et al.* study that established the protecting outcome of *Zataria multiflora* hydroalcoholic extract in the reduction of MDA with significant increasing effect of antioxidant enzyme activities [11].

The present study as well exemplified noteworthy effect of gentamicin in increasing KIM-1 levels as match up with Luo *et al.* study that demonstrated both KIM-1 and NGAL sera levels are perceptive and precise biomarkers during gentamicin-induced nephrotoxicity within 7 days. The rise in those biomarkers is time and dose reliant due to progressive gene expression of KIM-1 and NGAL [12].

Outstandingly, dissimilarity or discrepancy in the generation of free radicals and the deficiency to detoxify these free radicals by antioxidants lead to the induction of oxidative stress. Pomegranate has a higher antioxidant potential against free radicals due to phenolic and flavonoid compounds [13].

Concerning the effect of pomegranate on the oxidative stress and lipid peroxidation, pomegranate escorted insignificant effect in the reduction of lipid peroxidation marker (MDA) which was incoherent with different studies that established the antioxidant effect of pomegranate in the reduction of nephrotoxicity [14,15].

Besides, pomegranate appreciably reduced renal tubular injury biomarkers due to significant nephronprotective effect that attenuates gentamicin-induced nephrotoxicity as hold up by Kim *et al.* study that confirmed the administration of 25 mg/kg/day of pomegranate is talented to reduce the levels of KIM-1 and NGAL sera levels significantly in cadmium-induced nephrotoxicity due to the protective effect of pomegranate on renal tubules [16]. In addition, Wu *et al.* illustrated imperative effect of pomegranate in lessening of inflammatory and biomarkers of renal tubular damage during glycerol-induced acute nephrotoxicity [17].

Amusingly, the present study confirms the protective effect of pomegranate on the glomerular function through the reduction

of cystatin-C serum levels when coadministered with gentamicin. Pomegranate considerably reduces cystatin-C serum levels in both acute and chronic renal damage through modulation of glomerular blood flow and regulation of intraglomerular pressure and inflammations [18,19].

#### CONCLUSION

Pomegranate produced significant nephroprotective effect on gentamicin-induced nephrotoxicity through modulation of oxidative stress and inflammatory biomarkers.

#### AUTHORS' CONTRIBUTIONS

All authors contribute equally in data collection, experimental design, interpretation, statistical analysis, literature review, manuscript preparation, and review.

#### CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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