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ASCORBIC ACID;
RENOPROTECTIVE EFFECTS IN GENTAMICIN INDUCED RENAL INJURY IN RATS

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ABSTRACT... Objectives: Investigating the Renoprotective effects of Ascorbic acid in gentamicin induced renal injury (GIRI) in male Wistar rats. **Study design:** Experimental study. **Place and Duration:** Animal house of Al-Tibri from September 2016 to December 2016. **Methodology:** 60 male Wistar rats were selected by non-probability purposive sampling, and were divided into three groups; Group A- control, Group B- Gentamicin induced renal injury (GIRI) (70 mg/kg/bwt i.m) daily , and Group C- GIRI + AA (Ascorbic acid - 0.2 mg/kg/bwt) daily. Cardiac puncture was performed by a Disposable Syringe for blood sampling. Sera were used for biochemical testing. Renal tissue was stained with H & E for histological examination. Statistix 8.1 software (USA) was used for data analysis at $P \leq 0.05$. **Results:** Blood urea and serum creatinine were elevated in the group B (GIRI) compared to GIRI+AA and the controls ($P=0.0001$). Serum superoxide dismutase, glutathione peroxidase, and catalase were low in GIRI compared to GIRI+AA and control groups. Renal tissue in ascorbic acid treated rats showed improved tissue architecture. **Conclusion:** It is concluded that the Ascorbic acid exerts Renoprotective effects in gentamicin induced renal injury in rat model.

Key words: Gentamicin, Renal injury, Renal Function, Antioxidants Status.

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INTRODUCTION

Gentamicin belongs to the aminoglycoside family of drugs. It is a well established antibiotic for the gram negative infections.[1] Gentamicin is being used clinically since 40 years. It is effective against the Staphylococci, and Enterococci, especially in synergy with β-lactams antibiotics. Gentamicin is a first line antibiotic for a number of severe infections. Drug resistance is low, and also costs less. Gentamicin is safe with low risk of Clostridium difficile associated pseudo membranous colitis compared to cephalosporins and Quinolones. However; its side effects of ototoxicity and nephrotoxicity are notorious. Nephrotoxicity and ototoxicity have compromised its clinical use. Reported prevalence of gentamicin induced renal injury (GIRI) varies from as low as 1.2% to a peak of 55%. Average rate ranges between 8% and 26%.[1,2] Nephrotoxicity has limited its therapeutic use. In renal failure, its use is contraindicated. In older age groups, gentamicin is used cautiously. In patients with compromised renal function, the dose and duration of gentamicin use necessitates substantial reduction. Proximal renal tubule is the site of accumulation of gentamicin, whereby it induces the nephrotoxicity.[1,2] Mechanisms of GIRI is not well established. Free radical formation is a postulated mechanism. Oxidative stress is a recognized contributing factor in the pathogenesis of GIRI, hence anti oxidants may help neutralize the effects.[3,4] The antioxidant have proven role against the prevention of nephrotoxicity. The present study investigated the possible oxidative load induced by gentamicin that caused renal injury. Also the microscopy was performed to localize the site of renal injury by gentamicin by histopathological examination.[5,6] Ascorbic acid is a powerful anti-oxidant vital amine. Antioxidants are capable of preventing cellular injury by scavenging the free radicals.[7,8] Ascorbic acid

is a natural antioxidant vitamin present in citrus fruits. Its role in the oxidative stress is already established. It functions as a redox pair in the body.[9,10]

The present in-vivo experimental study investigated the possible renoprotective effects of Ascorbic acid in gentamicin induced renal injury in Rats. The present study hypothesized that the ascorbic acid has no effect against the gentamicin induced oxidative stress and renal injury in male Wistar albino rat model at animal house of Isra University.

MATERIAL AND METHODS

An in-vivo experimental study was designed to investigate the effects of ascorbic acid against the GIRI. The research took place at the Animal house of Al-Tibri from September 2016 to January 2017. Sixty adult male Wistar albino rats were selected through non-probability (purposive) sampling according to inclusion criteria of; body weight 200- 250 grams, male rats, Wistar strain, age 9-12 weeks, looking healthy, feeding well and actively moving. Lazy and sick male rats, female rats, different body weights and age were excluded. Housing of rats was in accordance to the Guidelines of NIH and Ethics guidelines of institute. Animals were housed for 10 days acclimatization. Optimal temperature was 22- 25 ^{0}C and optimal humidity (55- 60%). Dark/light cycle of 12/12 hours each was strictly followed. Clean tap H_2O and chow diet was available ad-libitum. 60 animals were divided (random selection) into 3 groups A, B and C. Grouping and treatment schedule was followed for 28 days (4 weeks) as; Group A (n=20): Control rats – receive 0.9% (0.3 ml) NaCl mixed in water, Group B (n=20): Gentamicin induced renal injury (GIRI) (Gentamicin given at dose of 70 mg/kg/bwt i.m) daily,[11] and Group C (n=20): GIRI (70 mg/kg/bwt i.m) + Ascorbic acid (AA- 0.2 mg/kg/bwt) orally daily. Gentamicin (Genticyn ® - Reckitt & Colman, Pakistan) and Ascorbic acid (Abbott) 500 mg was purchased from Pharmacy. For the formulation preparation, Tweens 80 was used in sterile water (H_2O). Blood samples were drawn by 5 ml sterilized Disposable syringes (BD, USA). These syringes are well known of their precision and sterility. 18 hours of post experiment period, blood samples were drawn by cardiac puncture. Blood samples were stored in the gel tubes. Tubes were centrifuged at 4000 rpm (10 minutes). Separated sera were stored at -20^{0}C in deep freezers (Dawalance). Blood urea and serum creatinine were analyzed by the colorimetric Jaffé method (Roche Cobas e 411 analyzer). Serum superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) were detected by ELISA kits (Fortress Diagnostics). Animals were sacrificed by cervical dislocation as cited.[12] Ketamine and Xylazine were used for the animal anesthesia purpose. A senior trained veterinary's person performed the laparotomy and kidneys were removed by fine surgical dissection. Tissue sections were embedded in paraffin blocks. Tissue sections (3-5µ) were prepared and stained with Hematoxylin and Eosin (H & E stain). Tissue architecture details were examined by light microscopy. Research protocol was approved by the ethical review committee for the study of animal research. Statistix 10.0 software (USA) package was used for the Biostatistial analysis. One-way-ANOVA was for the statistical group comparisons. Post Hoc Tukey Cramer analyzed the statistical differences between groups. Results were presented as "mean ± SD". Level of statistical significance was $P < 0.05$ (95% confidence interval).

RESULT

Blood urea and serum creatinine were improved in the group C (GIRI+ AA) compared to B (GIRI). Means± SD blood urea in GIRI and GIRI+AA were noted as 35.5±12.13 and 28.25±5.31 compared to controls 21.3±2.45 mg/dl (P=0.0001). Means± SD blood urea in GIRI and GIRI+AA were noted as 2.1±1.5 and 1.54±0.5 compared to controls 0.71±0.21mg/dl (P=0.0001). Serum SOD in GIRI and GIRI+AA were noted as 77.5±12.91 and 130.83±12.10 compared to controls 132.41±31.62 mg/dl (P=0.0001) (U/ml). Serum GPX in GIRI and GIRI+AA were noted as 87.23±21.31 and 125.9±9.18 compared to controls 133.31±32.35 mg/dl (P=0.0001) (nM/min/mL). Serum CAT in GIRI and GIRI+AA were noted as 171.7±92.3 and 266.32±31.15 compared to controls 401.5±67.31 mg/dl

(P=0.0001) (nM/min/mL). Histopathological examination showed normal looking glomeruli (g), proximal tubule (p), distal tubule (d) and normal interstitium in the control (Group A) (Figure-1). Damaged glomeruli, complete glomerular and tubular atrophy, interstitial hemorrhage (arrow) & edema, necrosis, and desquamation of tubular epithelium were noted in the GIRI (Group B) (Figure-2). Renal tissue showed visible glomeruli (g), glomerular corpuscle (g), renal tubule (t), less interstitial edema, and hemorrhage in the ascorbic acid treated GIRI (Group C) (Figure-3).

	Group A (Controls)	Group B (GIRI)	Group C (GIRI+ AA)	P-value
Blood urea (mg/dl)	21.3±2.45	35.5±12.13	28.25±5.31	0.001
Serum Creatinine (mg/dl)	0.71±0.21	2.1±1.5	1.54±0.5	0.001
Serum SOD (U/ml)	132.41±31.62	77.5±12.91	130.83±12.1	0.0001
Serum GPX (nM/min/mL)	133.31±32.35	87.23±21.31	125.9±9.18	0.0001
Serum Catalase (nM/min/mL)	401.5±67.31	171.7±92.3	266.32±31.15	0.0001

Table-I. Liver enzymes and anti oxidant enzymes in controls and experimental rats (n=60)
GIRI- Gentamicin induced renal injury, AA- Ascorbic acid

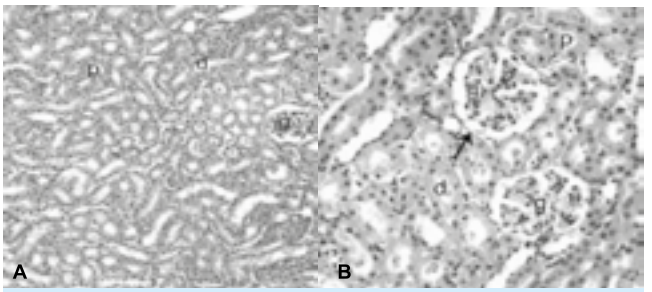

Figure-1. Control- Renal tissue A. (H & E x100) B. (H & E x400) showing glomeruli (g), proximal tubule (p), distal tubule (d) and normal interstitium

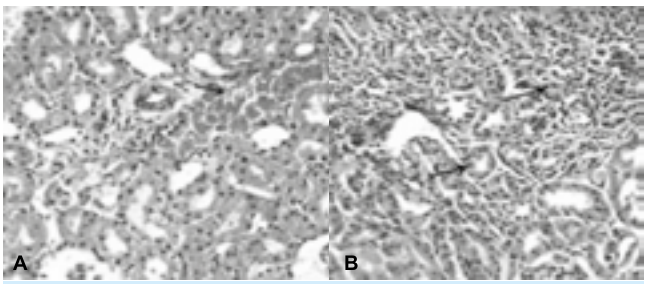

Figure-2. GIRI (Gentamicin induced renal injury) Renal tissue A & B. Damage glomeruli, complete atrophy, interstitial hemorrhage (arrow) & edema, necrosis, and desquamation of tubular epithelium is visible (H & E x400)

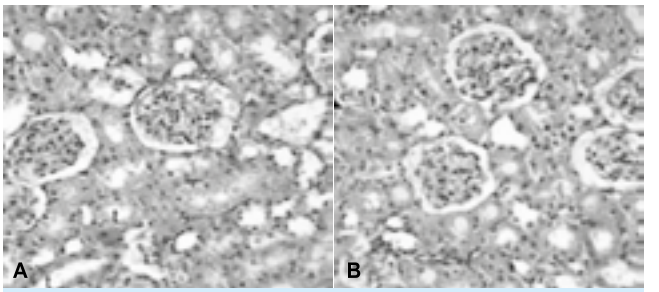

Figure-3. GIRI + AA- A & B. Renal tissue- showing glomeruli (g), glomerular corpuscle (g), renal tubule (t), interstitial edema, and hemorrhage. (H & E x400)

DISCUSSION

The present gentamicin induced renal injury (GIRI) research analyzed the possible renoprotective effects of an easily available, inexpensive and simple drug – the Ascorbic acid.

The Gentamicin is a widely used antibiotic of aminoglycosides for the gram negative infections. The present study proved the blood urea and serum creatinine were raised and antioxidant enzymes- the SOD, GPX and CAT were low in the GIRI rats (Table-I), and histopathological examinations showed damage to the renal tissue (Figure-2). Gentamicin is water soluble, filtered through the glomeruli but a fraction is reabsorbed by the proximal renal tubule. Gentamicin accounts for a large number of nephrotoxicity and renal failure cases in the medical wards.[13,14] Various mechanisms have been proposed for the GIRI. But the accepted mechanisms include the oxidative stress which plays major role in the renal tissue injury. Low antioxidative enzymes (SOD, GPX and CAT) of present study confirm the mechanism of GIRI. The findings are in agreement with previous studies.[15-17] In the present study the kidney tissue destruction was noted (Figure-2), these

findings are in agreement with recently published studies.[11,18,19] GIRI rats showed widespread damage to the renal tissue architecture. Changes in the glomeruli, proximal tubule, distal tubule, interstitium and capillaries were markedly noted in the GIRI rats (Figure-2). Proximal and distal tubules showed cell disruption, necrosis and degenerative changes. Serum SOD, GPX, and CAT are natural antioxidant enzymes and their role is evident as shown in the Table-I. Our findings are in agreement with previous studies.[20-22] GIRI is induced by the reactive oxygen species (ROS) free radicals which are highly reactive. They react with the cell parts – including, phospholipids, proteins, carbohydrates, RNA and DNA, oxidize them resulting in cell injury. Serum and tissue SOD, GPX, and CAT play major role in handling, and neutralizing the ROS. The Ascorbic acid is itself an antioxidant which helps in the scavenging of ROS free radicals. It improved the biochemical markers – antioxidant enzyme as shown in Table-I and tissue architecture as shown in Figure-3. Our evidence based findings are in agreement with previous studies.[11,23,24] Our findings are in keeping with a recent study[11] which reported similar Renoprotective effects of Ascorbic acid in gentamicin induced renal injury in rat model. They reported the co-administration of Ascorbic acid with Gentamicin minimized the oxidative stress and improved the renal tissue injury. These findings support our present study. Another study[25] used Gentamicin (100 mg/kg/day) and found significant decrease in the antioxidant enzymes – the GPX, GSH, SOD and CAT in rat study. These findings corroborate with our present research study. They reported increased lipid peroxidant marker the malondialdehyde (MDA), this finding is inconsistent to present study as we could not detected MDA due to funding issues. A previous study[26] injected gentamicin (80 mg/kg/day) in rats and reported the GPX was reduced and kidney tissue architecture showed destruction. The findings of above study corroborate with our present study. A previous study[27] reported significant decrease in GPX, SOD, GR and CAT in GIRI guinea pigs experimental study. Our present study confirms the oxidative stress in the GIRI rats, ascorbic acid showed excellent free radical scavenging activity. These findings are in agreement with previous studies.[28,29] A previous study[30] analysed the renoprotective effects of ascorbic acid (0.5 mg/kg/day) against chromium-induced nephrotoxicity. The histopathological improvement of renal tissue by the ascorbic acid is consistent with previous studies.[26,30] The findings support our present research study. The present study concludes that the ascorbic acid improves the gentamicin induced renal injury and may be prescribed in the susceptible patients who are risk.

CONCULSION

The present study concludes that the Gentamicin induced renal injury occurs by the generation of free radical reactive oxygen species (ROS). Ascorbic acid shows renoprotective potential against oxidative stress. As the Ascorbic acid is a simple, inexpensive and easily available drug, hence it may be utilized clinically to save the kidneys of patients prone to gentamicin induced renal failure.

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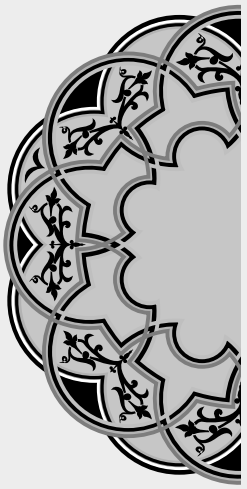

"No deal is better than the bad deal."

Unknown

AUTHORSHIP AND CONTRIBUTION DECLARATION

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2	Umair Ali Soomro	Statistical analysis, Manuscript write up, Proof Reading Correspondence.	
3	Shomail Saeed Siddiqui	Drug dose, drug toxicity, dose calcualtion, Manuscript write up, Proof reading	
4	Kashif Rasheed Shaikh	Concept, Materials handing, Collection of Biopsy materials, Statisining microscopy, Compilation of resutls, Statistical analysis, Manuscript write up, Correspondence.	
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