

Methanolic Leaf Extract of *Azadirachta indica* Ameliorates Gentamicin-induced Nephrotoxicity in Male Wistar rats

¹G.O. Adunmo, ²A.A. Adesokan, ³O.A. Aribisala, ⁴I.W. Sabinari

1.Department of Medical Laboratory Science, University of Ilorin, Ilorin, Nigeria.

2.Department of Medical Biochemistry, University of Ilorin, Ilorin, Nigeria.

3.Department of Medical Biochemistry, Ladoke Akintola University of Technology, Ogbomosho, Nigeria.

4.Department of Physiology, University of Ilorin, Ilorin, Nigeria.

Abstract

This study investigated the possible protective effect of methanolic leaf extract of *Azadirachta indica* on gentamicin-induced nephrotoxicity in male Wistar rats. Male Wistar rats (average weight= 184.05g) were divided into five (5) groups, with five (5) rats each receiving vehicle (normal saline, *p.o*), GEN (21.16 mg/kg Gentamicin, *i.p*), AZI (200 mg/kg methanolic leaf extract of *Azadirachta indica*, MEAI, *p.o*), GEN+AZI (Gentamicin with 200 mg/kg and 400 mg/kg of AZI), 5 minutes after administration of Gentamicin for 7 days. Serum total protein, albumin, creatinine, urea, sodium, potassium and MDA levels were assayed using standard procedures. Renal histopathological study was also conducted. All data were expressed as means $\pm$ SEM and significance were accepted at $p < 0.05$.

Gentamicin caused impaired renal function with increased serum urea, creatinine and MDA, decreased sodium, potassium, total protein and albumin levels. Histopathology revealed altered renal histoarchitecture. However, treatment with MEAI especially the 400 mg/kg dose, improved renal function, restored total protein and decreased MDA in Gentamicin-induced Nephrotoxicity.

Taken together, MEAI shows capacity to improve renal function and histology by attenuating lipid peroxidation in Nephrotoxicity induced by gentamicin.

Keywords: Nephrotoxicity, Gentamicin, *Azadirachta indica*, Malondialdehyde

Introduction

Nephrotoxicity is a major cause of acute kidney injury (AKI), a condition that affects about 13.3 million people globally.^{1,2,3} It is usually characterized with impaired glomerular filtration, electrolyte imbalance, and distorted renal architecture.

The pathological mechanisms underlying these perturbations have been shown to include microvascular dysfunction, chronic inflammation, oxidative stress and tubular necrosis.^{4,5,6}

Drug-induced renal failure is a well-recognized clinical phenomenon,⁷ although the incidence of drug – induced renal disease is not well defined. Previous reports suggest that between 17- 26 percent of cases of AKI are directly attributed to drugs and chemicals.^{8,9} Because the kidneys provide the final common pathway for the excretion of drugs and their metabolites, they are exposed to high concentrations of potentially toxic substances.¹⁰ Many groups of drugs precipitate renal dysfunction as side effects, generally affecting the renal tubular cells and renal papillae.^{11,12}

Gentamicin is an amino glycoside antibiotic that is commonly used as first-line treatment of life-threatening infections. However, gentamicin treatment has been associated with adverse renal conditions particularly nephrotoxicity in clinical and experimental animal cases.^{13,14,15} While the cause of gentamicin-induced nephrotoxicity is yet to be fully elucidated, decreased urea/creatinine clearance, accumulation of reactive oxygen species (ROS) and pro-inflammatory cytokines were previously reported.^{15,16}

The relevance of herbal medicine has continued to grow due to the bio-effectiveness of plants of ethnobotanical origin in the management of infections and chronic diseases at lesser cost and easier accessibility, especially to low resource people.¹⁷ *Azadirachta indica* (Neem) is a member of the Meliaceae family found in Africa, Asia and Arabian countries. Its leaves, bark and fruits are attributed with numerous therapeutic benefits against malaria, infections, hyperglycemia, inflammation, gastric ulcer and tumors.¹⁸ The flavonoids constituents are appreciated for the potency of *Azadirachta indica* to prevent microbial growth,¹⁹ scavenge free radicals,²⁰ decrease angiogenesis²¹ and induce cancer cells apoptosis.²² Previous experimental investigations observed that extract of *Azadirachta indica* ameliorated neurotoxicity²³ and cisplatin –induced renal dysfunction.²⁵ This study sought to investigate the potential of MEAI in ameliorating nephrotoxicity-linked impaired renal function caused by gentamicin exposure in male Wistar rats.

Correspondence to:

Dr Adunmo G.O.

Department of Medical Laboratory Science,
Faculty of Basic Clinical Science,
University of Ilorin,
PMB 1515, Nigeria.
Email: godwinadunmo@yahoo.com
Phone: +2348037902903

Methodology

Experimental Animals:

Twenty-five (25) male Wistar rats (mean weight: 184.05g) were purchased from a commercial breeder in Ilorin, Kwara State and kept in the animal house of the Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The animals were separated into five (5) groups (n=5). They had access to feed and tap water and were maintained under standard environmental conditions of temperature, relative humidity, and 12-h dark/ light cycle. The NIH Guide for the care and use of Laboratory animals was adhered to. Animals were euthanized, blood samples collected and centrifuged for 10 min at 3000 rpm to separate serum. The kidneys were excised and fixed in 10% formal saline prior to hematoxylin and eosin staining.

Identification of Plant Material

The leaves of *Azadirachta indica* were collected in its natural habitat in and around Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Identification and botanical authentication of plant was carried out in the Department of Pure and Applied Biology of the institution given voucher number LAU 979.

Preparation of Methanolic Leaf Extract of *Azadirachta indica* (MEAI)

The leaves of *Azadirachta indica* were macerated and shade dried at room temperature. Fine powder was made using grinder mixture. 200g powder material of *Azadirachta indica* was mixed with 600ml of 95% methanol at room temperature for 72 hours. After 3 days, the mixture was sieved and supernatant collected. The solvent was extracted from the mixture using distillation separation unit. A semi solid extract was obtained after complete elimination of alcohol. The residue was kept in the refrigerator for further use.

Gentamicin

Commercial gentamicin (80mg) was obtained from Akol Pharmacy, Ogbomoso. And intraperitoneal route of administration was adopted.

Experimental Design

Control group received vehicle, while GEN group was administered with gentamicin (21.16 mg/Kg, *i.p*). MEAI200mg/kg group received 200mg/kg body weight of *Azadirachta indica*, while GEN+MEAI200mg/kg and GEN+MEAI_{400mg/kg} received treatment with 200mg/kg and 400 mg/kg respectively of MEAI orally.

Biochemical Assays

Renal function test.

Serum urea, creatinine, protein and albumin were determined colorimetrically using reagent kit from Randox Diagnostica. Serum Sodium and Potassium levels were determined colorimetrically using reagent kits from Teco.

Assessment of Lipid Peroxidation

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances [TBARS] produced during lipid peroxidation as described by Niehaus and Samuelson (1968).

Statistical Analysis.

Result Values were expressed as mean $\pm$ SEM (standard error of mean) for five rats in each group. The comparison of data within groups was performed by one-way analyses of variance. Significant difference between groups was assessed by Duncan's multiple range tests using $p < 0.05$.

Results

Effect of MEAI on serum urea, creatinine and malondialdehyde in Gentamicin-induced Nephrotoxicity in male Wistar rats.

Administration of gentamicin significantly increased serum urea, creatinine and MDA levels compared with control. While administration of 200mg/kg of MEAI in normal rats did not alter serum urea and MDA level but creatinine level was markedly decreased compared with control. Treatment of gentamicin-induced rats with 200mg/kg of MEAI significantly elevated serum urea level, but reduced creatinine level. However, higher dose of MEAI (400mg/kg), significantly reduced urea and creatinine

Table 1: Showing the effect of MEAI on serum urea, creatinine and malondialdehyde (MDA) in Gentamicin-induced Nephrotoxicity in male Wistar rats

Parameters	Control	GEN	MEAI _{200mg/kg}	GEN+MEAI _{200mg/kg}	GEN+ MEAI _{400mg/kg}
Urea (mg/dL)	30.37 $\pm$ 1.86 ^a	143.96 $\pm$ 8.01 ^{b,c}	31.48 $\pm$ 4.49 ^a	152.08 $\pm$ 9.67 ^c	114.65 $\pm$ 21.73 ^b
Creatinine (mg/dL)	1.22 $\pm$ 0.33 ^{a,b}	2.70 $\pm$ 0.37 ^c	0.87 $\pm$ 0.18 ^a	2.00 $\pm$ 0.27 ^{b,c}	1.37 $\pm$ 0.31 ^{a,b}
MDA(μ mol/g)	1.40 $\pm$ 0.00 ^a	2.03 $\pm$ 0.03 ^c	1.42 $\pm$ 0.12 ^a	1.80 $\pm$ 0.00 ^b	1.52 $\pm$ 0.10 ^a

Data were expressed as mean $\pm$ SEM. Mean that does not share the same letters in the same line are significantly different. Statistical significance was considered at $p < 0.05$.

Table 2: Showing effect of MEAI on serum total protein and albumin in Gentamicin-induced Nephrotoxicity in male Wistar rats

Parameters	Control	GEN	MEAI _{200mg/kg}	GEN+MEAI _{200mg/kg}	GEN+ MEAI _{400mg/kg}
Total protein (g/dL)	7.02±0.56 ^a	6.08±0.36 ^{a,b}	6.52±0.38 ^a	5.32±0.36 ^b	6.50±0.88 ^a
Albumin (g/dL)	3.27±0.14 ^a	2.38±0.09 ^b	3.21±0.16 ^a	2.28±0.36 ^b	2.82±0.19 ^a

Data were expressed as mean ±SEM. Mean that does not share the same letters in the same line are significantly different. Statistical significance was considered at $p < 0.05$.

Table 3: Showing effect of MEAI on serum sodium and potassium in Gentamicin-induced Nephrotoxicity in male Wistar rats

Parameters	Control	GEN	MEAI _{200mg/kg}	GEN+MEAI _{200mg/kg}	GEN+ MEAI _{400mg/kg}
Sodium (mmol/L)	136.57±11.70 ^a	114.78±5.54 ^b	147.58±2.85 ^a	132.84±1.49 ^a	130.52±4.12 ^{a,b}
Potassium (mmol/L)	4.50±0.48 ^a	5.18±0.30 ^a	5.78±0.63 ^b	5.90±0.58 ^{a,b}	6.90±0.24 ^b

Data were expressed as mean ±SEM. Mean that does not share the same letters in the same line are significantly different. Statistical significance was considered at $p < 0.05$.

levels while both doses of MEAI significantly attenuated MDA level.

Effect of MEAI on serum total protein and albumin in Gentamicin-induced Nephrotoxicity in male Wistar rats.

Administration of Gentamicin caused significant decreases in serum total protein and albumin levels when compared with control. Normal rats administered with 200mg/kg of MEAI showed significant decrease serum total protein level compared with control, while albumin level did not change. Treatment of gentamicin-induced rats with 200mg/kg of MEAI led to a significant decrease in serum level of total protein but not albumin, however, treatment with 400mg/kg of MEAI did not increase serum total protein and albumin levels.

Effect of MEAI on serum sodium and potassium concentration in Gentamicin-induced Nephrotoxicity in male Wistar rats.

Gentamicin significantly reduced serum level of sodium, but increased potassium level when compared with control. While administration of 200mg/kg of MEAI in normal rats significantly increased serum sodium and potassium level. Treatment of Gentamicin-induced rats with both 200mg/kg and 400mg/kg of MEAI restored serum sodium level but further elevated serum potassium level.

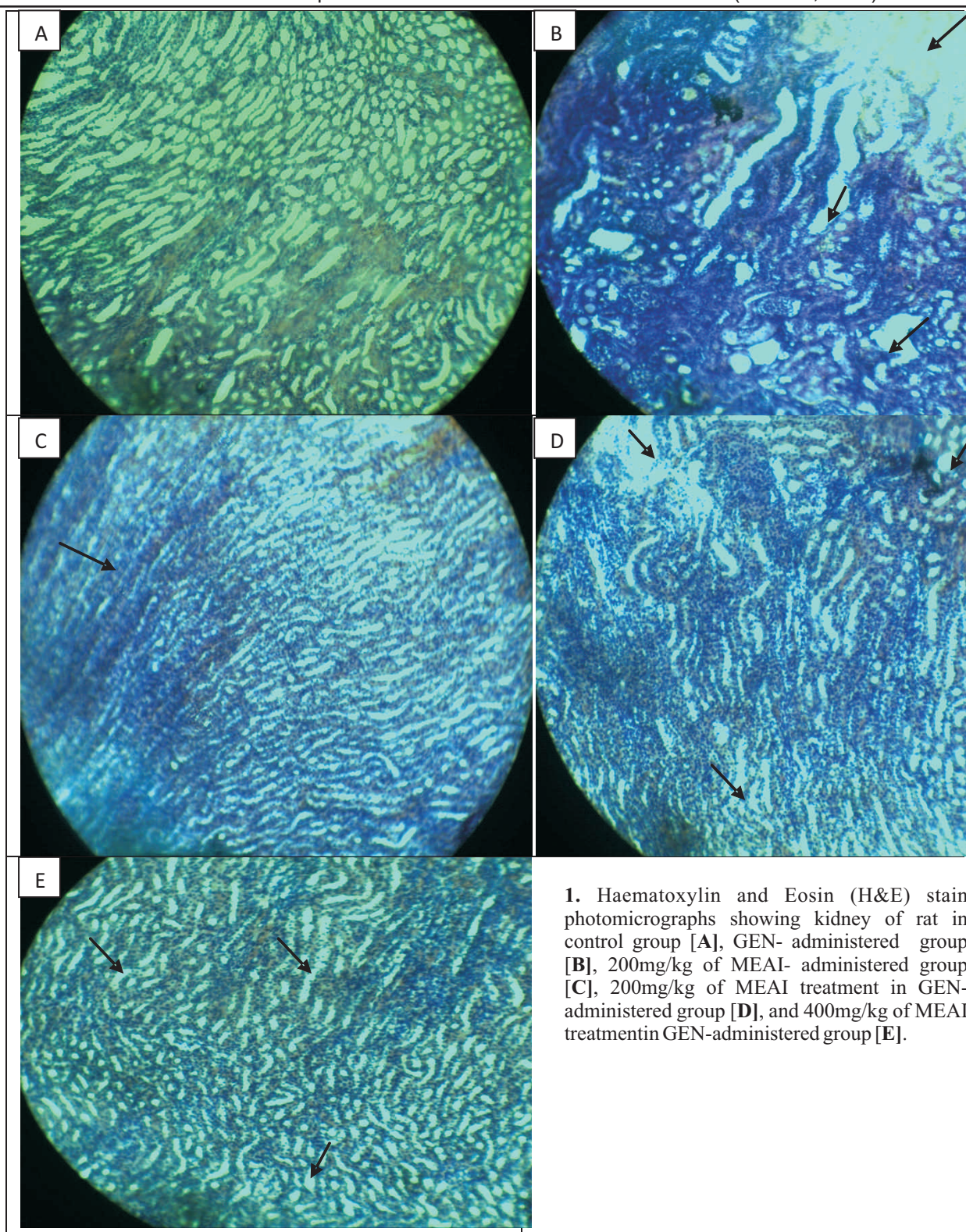
Histopathological Evaluations

Control group revealed kidney tissue having normal histoarchitecture (A). GEN distorted kidney

structure with severe necrosis of convoluted tubules and collecting ducts (B). The stroma was edematous and tissue was infiltrated by numerous chronic inflammatory cells suggesting severe tubular necrosis. Normal rats receiving 200mg/kg of MEAI, histopathological examination (C) show partially normal kidney architecture but there is compression of the collecting tubules, junction of glomerular capsule and proximal convoluted tubules. In GEN+200mg/kg of MEAI group, there was mild interstitial edema, mild degree of glomerular congestion and few congested blood vessels (D). The tissue was sparsely infiltrated by chronic inflammatory cells with the visceral and parietal layers of the glomerular capsules are impaired, showing moderate tubular necrosis. GEN+400mg/kg of MEAI group, histopathological examination (PE) show mild interstitial edema and moderate degree of glomerular congestion indicating moderate tubular necrosis with significant reversal of inflammatory changes and hemorrhage.

Discussion

Despite its therapeutic benefit against bacterial infections, the adverse effects of gentamicin are well reported in humans and experimental animals.^{13,15} Nephrotoxicity represents the most common side effect of gentamicin treatment that worsens renal function and cause tubular epithelial damage.^{1,26,27} Data from this study reveal that gentamicin administration caused nephrotoxicity accompanied by elevated serum urea and creatinine, electrolyte disturbance, lipid peroxidation and reduced protein and albumin levels in male Wistar rats. However, aqueous leaf extract of



1. Haematoxylin and Eosin (H&E) stain photomicrographs showing kidney of rat in control group [A], GEN- administered group [B], 200mg/kg of MEAI- administered group [C], 200mg/kg of MEAI treatment in GEN-administered group [D], and 400mg/kg of MEAI treatment in GEN-administered group [E].

MEAI, especially the maximum dose (400 mg/kg), improved renal dysfunction in gentamicin-exposed male Wistar rats.

Urea and creatinine serum levels are commonly accepted clinical measures of renal function. Altered renal clearance of both urea and creatinine is associated with impaired renal function.^{29,30} From our data, gentamicin administration

caused elevated serum levels of urea and creatinine. Healthy rats given 200mg/kg of MEAI had no change in serum urea level, but creatinine level shows reduction. However, treatment with 200mg/kg of MEAI raised serum urea level but reduced creatinine level in gentamicin-induced rats, while 400mg/kg of the extract attenuated both urea and creatinine levels. These results are in agreement with findings from a

recent study.²⁴

Sodium and potassium are key ions necessary for osmotic balance of body fluid. Drug-induced renal dysfunction is associated with electrolytes disturbance.^{32,33} Gentamicin administration in this study caused significant decrease in serum sodium level but a marked increase in potassium level. Our observations suggest that impaired renal function by gentamicin administration engender ionic imbalance, particularly, hyperkalemia with increased urinary sodium excretion that is usually accompanied with fluid loss. In the study of Medic *et al.*,²⁸ unchanged serum sodium ion level was reported following gentamicin administration. From our data, 200mg/kg of MEAI administration in healthy rats, elevated serum sodium and potassium levels compared with control. However, both doses of MEAI restored serum sodium level towards control but serum potassium level was raised above control level in gentamicin-exposed rats taking 400mg/kg of MEAI showing that at maximum dose, the extract could worsen hyperkalemia. Normally, elevated circulating potassium would elicit β -adrenergic response as a short term regulatory mechanism to drive K^+ into the cells through the Na^+/K^+ ATPase pump,³¹ although this wasn't verified in this study.

Oxidative stress results from excessive ROS production in organs with corresponding failure of their clearance by innate antioxidants.^{34,35,36} ROS may produce cellular injury and necrosis via mechanisms including lipid peroxidation, protein denaturation and DNA damage. In the present study, a marked increase in lipid peroxidation (MDA) was observed following gentamicin administration. This observation is supported by the findings of Medic *et al.*,²⁸ Gentamicin-induced generation of MDA could alter renal function and speed up renal injury progression,^{37,38} because the nephrons are delicate structures that are easily predisposed to damage of any sort. In this study, healthy rats that were administered 200mg/kg of MEAI did not show changes in MDA level. However, treatment of gentamicin-administered rats with both 200mg/kg and 400mg/kg of MEAI significantly decreased MDA level. The report of Moneim *et al.* showed that MEAI ameliorated lipid peroxidation in Cisplatin-induced nephrotoxicity,²³ which is in support of the antioxidant or anti-peroxidant potential of the extract in our model.

Proteinuria is an early sign of kidney disease that most often reflects abnormal glomerular impermeability to filtration of plasma proteins. In the present study, serum total protein and albumin levels were found to be significantly decreased in gentamicin-induced rats when compared with control suggestive of cyto/histoxic effect of excessive MDA generated from gentamicin which could damage nephrons and thereby cause loss of protein from blood. In healthy rats, 200mg/kg of MEAI decreased serum total protein

and did not change albumin level. Gentamicin-induced rats given 200mg/kg of MEAI exhibit decreased serum total protein level, but did not alter albumin level, while treatment with 400mg/kg of MEAI did not restore total protein and albumin levels.

Gentamicin-induced renal damage ranges from alterations in tubular reabsorption, loss of renal microstructure to necrosis of proximal tubule cells.^{40,41,42} In this study, gentamicin caused tubular epithelial damage with intense granular degeneration. The presence of the tubular necrosis, epithelial loss and degeneration of glomeruli has been previously reported.⁴³ Cellular injury could lead to infiltration with numerous inflammatory cells causing fluid accumulation, cellular congestion and kidney hypertrophy. Moreso, the mesangial cell network, which maintains the renal vasoelasticity is disrupted by gentamicin. Healthy rats administered with 200mg/kg of MEAI only displayed mild changes in renal architecture without any severe damage. Following treatment of gentamicin-induced rats with both 200 mg/kg and 400 mg/kg of MEAI treatment, mild recoveries were observed, although micrograph from the 400 mg/kg dose shows improved recovery when compared with that of the lower dose. This suggests that treatment with methanolic leaf extract of *Azadirachta indica* ameliorated nephrotoxicity-associated histopathological alterations which is in line with the work of Abireh *et al.*²⁴

Conclusion

Summarily, treatment with MEAI in gentamicin-induced nephrotoxic rats improved renal function and histoarchitecture. Marked ameliorative effects were observed in gentamicin-induced that received maximum dose of MEAI (400mg/kg) compared with the lower dose (200mg/kg) of the extract. Existing studies have attributed the protective activity of MEAI to the antioxidant and anti-inflammatory phytochemical constituents. Findings in this study justify the potency of the extract to significantly attenuate lipid peroxidation, restore blood proteins and enhance renal function in the MEAI extract-treated rats.

Acknowledgement

We thank the staff of Biochemistry department, Ladoke Akintola University, Ogbomoso for their assistance during this research

References

1. Al-Azzam SI, Abdul-Razzak KK, Jarada MW. The nephroprotective effects of pioglitazone and glibenclamide against gentamicin-induced nephrotoxicity in rats: a comparative study. *J. Chemother.* 2010; **22**(2), 88–91.
2. Lewington AJ, Cerdá J, Mehta RL. Raising

- awareness of acute kidney injury: a global perspective of a silent killer. *Kidney international*, 2013; 84(3), pp.457-467.
3. Mehta RL, Cerdá J, Burdmann EA, Tonelli M, García-García G, Jha V, Susantitaphong P, Rocco M, Vanholder R, Sever MS, Cruz, D. International Society of Nephrology's Oby25 initiative for acute kidney injury (zero preventable deaths by 2025): a human rights case for nephrology. *The Lancet*, 2015; 385(9987), pp.2616-2643.
 4. Szczech LA, Granger CB, Dasta JF, Amin A, Peacock WF, McCullough PA, Devlin JW, Weir MR, Katz JN, Anderson Jr FA, Wyman A. CLINICAL PERSPECTIVE. *Circulation*, 2010; 121(20), pp.2183-2191.
 5. Ajami M, Egtesadi S, Pazoki-Toroudi, H, Habibey R, Ebrahimi SA. Effect of crocus sativus on gentamicin induced nephrotoxicity. *Biological research*, 2010; 43(1), pp.83-90.
 6. Wang YH, Zhang YG. Kidney and innate immunity. *Immunology letters*, 2017; 183, pp.73-78.
 7. Schetz M, Dasta J, Goldstein S, Golper T. Drug-induced acute kidney injury. *Current opinion in critical care*, 2005; 11(6), pp.555-565.
 8. Nash K, Hafeez A, Hou S. Hospital-acquired renal insufficiency. 2002; 39: 930-6
 9. Uchino S, Kellum JA, Bellomo R, et al; Beginning and Ending Supportive Therapy for the Kidney (BEST Kidney) Investigators. Acute renal failure in critically ill patients: a multinational, multicenter study. *JAMA*. 2005;294(7):813-818.
 10. Knights KM, Rowland A, Miners JO. Renal drug metabolism in humans: the potential for drug-endobiotic interactions involving cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) *British journal of clinical pharmacology*, 2013; 76(4), pp.587-602.
 11. Tavafi M, Ahmadvand H, Toolabi P. Inhibitory effect of olive leaf extract on gentamicin-induced nephrotoxicity in rats. *Iran J Kidney Dis*. 2012 Jan;6(1):25-32. PMID: 22218116.
 12. Mohana LS, Kiran Reddy UT, Sandhya Rani KS. A review on medicinal plants for nephroprotective activity. *Asian Journal of Pharmaceutical and Clinical Research*, 2012; 5(4):8-14.
 13. Selby NM, Shaw S, Woodier N, Fluck RJ, Kolhe NV. Gentamicin-associated acute kidney injury. *QJM: An International Journal of Medicine*, 2009; 102(12), pp.873-880.
 14. Eshraghi-Jazi F, Talebi A, Mirsaeedi FS, Ahmadian S, Moslemi F, Nematbakhsh M. Gentamicin Induced Nephrotoxicity: The Role of Sex Hormones in Gonadectomized Male and Female Rats. *Scientifica (Cairo)*. 2016;5025097. doi:10.1155/2016/5025097
 15. Jaikumkao K, Pongchaidecha A, Thongnak LO, et al. Amelioration of Renal Inflammation, Endoplasmic Reticulum Stress and Apoptosis Underlies the Protective Effect of Low Dosage of Atorvastatin in Gentamicin-Induced Nephrotoxicity. *PLoS One*. 2016;11(10):e0164528. Published 2016 Oct 11. doi:10.1371/journal.pone.0164528
 16. Pavlakou P, Liakopoulos V, Eleftheriadis T, Mitsis M, Dounousi E. Oxidative stress and acute kidney injury in critical illness: pathophysiologic mechanisms—biomarkers—interventions, and future perspectives. *Oxidative Medicine and Cellular Longevity*. 2017 Jan 1;2017.
 17. Zong A, Cao H, Wang F. "Anticancer polysaccharides from natural resources: a review of recent research," *Carbohydrate Polymers*, 2012; vol. 90, no. 4, pp. 1395–1410.
 18. Alzohairy MA. "Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment", *Evidence-Based Complementary and Alternative Medicine*, vol. 2016, Article ID: 7382506, 11 pages.
 19. Yerima MB, Jodi SM, Oyibo K, Maishanu HM, Farouq AA, Junaidu AU, Shinkafi AL. Effect of neem extracts (*Azadirachta indica*) on bacteria isolated from adult mouth. *Nigerian Journal of Basic and Applied Science*, 2012; 20(1), 64–67.
 20. Ghimeray AK, Jin CW, Ghimire BK, Cho DH. "Antioxidant activity and quantitative estimation of azadirachtin and nimbin in *Azadirachta indica* A. Juss grown in foothills of Nepal," *African Journal of Biotechnology* 2009; vol. 8, no. 13, pp. 3084–3091.
 21. Mahapatra S, Young CYF, Kohli M, et al., "Antiangiogenic effects and therapeutic targets of *azadirachta indica* leaf extract in endothelial cells," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012; Article ID 303019, 14 pages.
 22. Gunadharini DN, Elumalai P, Arunkumar R, Senthilkumar K, Arunakaran J. Induction of apoptosis and inhibition of PI3K/Akt pathway in PC-3 and LNCaP prostate cancer cells by ethanolic neem leaf extract. *Journal of ethnopharmacology*. 2011 Apr 12;134(3):644-50.
 23. Moneim AEA. *Azadirachta indica* attenuates cisplatin-induced neurotoxicity in rats. *Indian journal of pharmacology*, 2014; 46(3), p.316.
 24. Abireh IE, Ozioko OM, Ozor II, Finbarrs-Bello E, Ozioko US, Egbo F. *Azadirachta indica* (Neem) Leaf Extract Effect as an Option of Treatment of Ibuprofen-induced Nephrotoxicity. *Journal of Advances in Medicine and Medical Research*, 2020; pp.56-62.
 25. Abdel Moneim AE, Othman MS, Aref AM. *Azadirachta indica* attenuates cisplatin-induced nephrotoxicity and oxidative stress. *BioMed research international*, 2014.
 26. Edwards JR, Diamantakos EA, Peuler JD, Lamar PC, Prozialeck WC. A novel method for the evaluation of proximal tubule epithelial cellular necrosis in the intact rat kidney using ethidium homodimer. *BMC physiology*. 2007 Dec 1;7(1):1.

27. Stojiljkovic N, Stoilkovic M, Mihailovic D, Randjelovic P, Ilic S, Gocmanac-Ignjatovic M, et al. Beneficial effects of calcium oral coadministration in gentamicin-induced nephrotoxicity in rats. *Ren Fail.* 2012;34:622-7.
28. Medić B, Stojanović M, Rovčanin B, et al. Pioglitazone attenuates kidney injury in an experimental model of gentamicin-induced nephrotoxicity in rats. *Sci Rep.* 2019;9(1):13689. Published 2019 Sep 23. doi:10.1038/s41598-019-49835-1
29. Perez-Valdivieso JR, Bes-Rastrollo M, Monedero P, de Irala J, Lavilla FJ. Prognosis and serum creatinine levels in acute renal failure at the time of nephrology consultation: an observational cohort study. *BMC nephrology*, 2007; 8, 14.
30. Vanholder R, Gryp T, Glorieux G. Urea and chronic kidney disease: the comeback of the century?(in uraemia research). *Nephrology Dialysis Transplantation.* 2018 Jan 1;33(1):4-12.
31. Castañeda-Bueno M, Arroyo JP, Gamba G. Independent regulation of Na⁺ and K⁺ balance by the kidney. *Medical principles and practice*, 2012; 21(2), pp.101-114.
32. Singh NP, Ganguli A, Prakash A: Drug-induced kidney diseases. *JAPI*, 2003;51:970–979.
33. Evenepoel P. Acute toxic renal failure. *Best Practice & Research Clinical Anaesthesiology*, 2004; 18(1), pp.37-52.
34. EBSCO, CAM. Review Board: Conditions: Atherosclerosis and Heart Disease Prevention. 4. 2007.
35. Fetouh FA, Azab AE. Ameliorating Effects of Curcumin and Propolis against the Reproductive Toxicity of Gentamicin in Adult Male Guinea Pigs: Quantitative Analysis and Morphological Study. *American Journal of Life Sciences*, (2014); 2, 138-149
36. Balakumar P, Rohilla A, Thangathirupathi A. Gentamicin-induced nephrotoxicity: Do we have a promising therapeutic approach to blunt it? *Pharmacol Res*, 2010; 62: 179 ± 186. doi: 10.1016/j.phrs.2010.04.004
37. Bayerle-Eder M, Pleiner J, Mittermayer F, Schaller G, Roden M, Waldhäusl W, Bieglmayer C, Wolzt M. Effect of systemic vitamin C on free fatty acid-induced lipid peroxidation. *Diabetes & metabolism*, 2004; 30(5), pp.433-439.
38. Kao M, Ang D, Pall A, et al. Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options. *J Hum Hypertens* 2010; 24, 1–8.
39. Li J, Li QX, Xie XF, Ao Y, Tie CR, Song RJ. Differential roles of dihydropyridine calcium antagonist nifedipine, nitrendipine and amlodipine on gentamicin induced renal tubular toxicity in rats. *Eur J Pharmacol.* 2009;620:97–104.
40. Danaci M, Kesici GE, Kesici H, Polat C, Belet B. Coumadin-Induced Renal and Retroperitoneal Hemorrhage, *Renal Failure*, 2006; 28:2, 129-132.
41. Randjelovic P, et al. “Gentamicin nephrotoxicity in animals: Current knowledge and future perspectives.” *EXCLI journal*, 2017; vol. 16 388-399. 24, doi:10.17179/excli2017-165
42. Martinez-Salgado C, Lopez-Hernandez FJ, Lopez-Novoa JM. Glomerular nephrotoxicity of aminoglycosides. *Toxicol Appl Pharmacol.* 2007;223:86–98.
43. Randjelovic P, Veljkovic S, Stojiljkovic N, Velickovic L, Sokolovic D, Stoilkovic M, Ilic I. Protective effect of selenium on gentamicin-induced oxidative stress and nephrotoxicity in rats. *Drug Chem Toxicol.* 2012 Apr;35(2):141-8. doi: 10.3109/01480545.2011.589446. Epub 2011 Nov 18. PMID: 22091876.