Comparative effects of L-arginine and vitamin C on gentamicin-induced alterations in some biochemical indices of kidney function

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Abstract
Kidney injury is an adverse effect of gentamicin which limits its use as an antimicrobial agent. Finding an antidote for this adverse effect will reduce the number of cases of kidney injury that accompany the use of gentamicin. This study compares the effects of L-arginine and vitamin C on gentamicin-induced kidney injury in Wistar rats. Rats were assigned to 5 groups (n=6). Groups I to V were treated respectively with distilled water, gentamicin, L-arginine and gentamicin, vitamin C and gentamicin, and L-arginine, vitamin C and gentamicin. After 14-day treatment, the animals were euthanized and plasma creatinine, plasma urea, plasma protein, plasma potassium, urinary protein, kidney malondialdehyde (MDA), Kidney superoxide dismutase (SOD), and kidney glutathione peroxidase (GPx) were analyzed. Administration of gentamicin increased the levels of plasma creatinine, plasma urea, urinary protein, urinary potassium, and MDA significantly ($p < 0.05$) compared to the distilled water-treated group. The levels of plasma protein, SOD and GPx were significantly reduced ($p < 0.05$). Treatment with L-arginine, vitamin C, and combined L-arginine and vitamin C significantly reduced ($p < 0.05$) creatinine, urea, urinary protein, plasma potassium and MDA. Plasma protein, SOD, and GPx were significantly raised compared with gentamicin control. The results indicate that vitamin C is more effective than L-arginine in reversing gentamicin-induced biochemical changes, but combined vitamin C and L-arginine produced even more ameliorative effect. Therefore, taking L-arginine and vitamin C during gentamicin therapy may reduce the incidence of kidney injury.

Keywords: Gentamicin, vitamin C, L-arginine, nephrotoxicity, oxidative stress

Introduction

The kidney filters the blood and removes substances that can cause cell damage from the body. Such substances include toxic chemicals and waste products of metabolism. The kidney is highly perfused and is thus prone to the effects of many of these toxic xenobiotics circulating in the blood (George *et al*., 2017; Radi, 2019). Among these toxic chemicals are many therapeutic agents which induce nephrotoxicity and are associated with renal failure. Aminoglycosides is an example of drugs known to be toxic to the kidney (Krause *et al*., 2016). These drugs are widely prescribed for the treatment of infections

caused by many gram-negative bacteria. However, drugs in the group induce nephrotoxicity in quite a number of individuals. Self-medication and misuse of gentamicin, an aminoglycoside, has led to many cases of renal failure and other adverse effects among rural and urban dwellers (Jado *et al*., 2020). Even when patients comply with physician's prescription, the use of gentamicin may still induce nephrotoxicity. In spite of this limiting factor, gentamicin is still widely prescribed because of its high therapeutic efficacy in many bacterial infections. Since its introduction into clinical practice in 1963, gentamicin has remained an effective antimicrobial agent for the treatment of many gram-negative bacterial infections (Krause *et al*., 2016). In many cases, it remains the effective drug of choice for many strains of bacteria that are resistant to other antimicrobials.

Therefore, it is important to find a remedy for gentamicin-induced nephrotoxicity so that cases of renal problems can be minimized among patients who may need to take the drug. It has been reported that gentamicin causes renal damage by stimulating the production of reactive oxygen species that triggers oxidative stress (Mishra *et al*., 2021). Oxidative stress is widely recognized for its role in the development of many diseases, including those of the kidney (Pizzino *et al*., 2017). Free radicals cause cellular damage that eventually results in various diseases by enhancing the synthesis of cytokines, which mediate the process of inflammation (Valko *et al*., 2015). Enhancing the activities of the antioxidant system results in decreased oxidative stress and inflammation, and probably arrests disease progression. Antioxidants can also repair the damage already caused by oxidative stress (Forman and Zhang, 2021). The protection offered by antioxidants involves endogenous and exogenous components. These two components function synergistically to mop up free radicals from the body (Mehdi *et al*., 2020). In view of the vital role they play in preventing and repairing oxidative stress-induced damage, administration of exogenous antioxidants with gentamicin may likely minimize its adverse effects on the kidney.

L-arginine, an amino acid, is a precursor for nitric oxide (NO) synthesis. Both L-arginine and its product, NO have been shown to possess anti-stress properties (Abu-Serie *et al*., 2015). L-arginine has also been reported to induce antioxidant response and suppress oxidative stress (Liang *et al*., 2018). Likewise, Vitamin C is widely recognized as an antioxidant. Vitamin C functions as cofactor for many enzymes such as prolyl 4-hydroxylase and lysyl hydroxylase (Carr and Maggini, 2017). It also shields cell components against the deleterious effects of free radicals generated from metabolic processes (Padayatty and Levine, 2016). In this study, a comparative assessment of the effects of L-arginine and Vitamin C, and their combination, on gentamicin-induced nephrotoxicity in rats was investigated.

Materials and Method

Experimental animals

Thirty healthy adult Wistar rats of both sexes weighing 180 ± 20 gram were used for the study. They were obtained from the Animal holding unit of the Department of Pharmacology and Therapeutics, LAUTECH, Ogbomoso. The animals were allowed to acclimatize for 7 days in a well-ventilated area of the laboratory in the Department. They were fed standard commercial animal feed (Ladokun Feeds, Ibadan) and were allowed free access to clean water. The standard guidelines for laboratory animal use were followed when handling the rats (National Institute of Health, 1985)

Experimental procedure

Rats were assigned to 5 groups of 6 per group and treated intraperitoneally as follows:

Group I: Distilled water (10 mL/kg b.w); Group II: Gentamicin (100 mg/kg b.w); Group III: Gentamicin (100 mg/kg b.w) + L-arginine (500 mg/kg b.w); Group IV: Gentamicin (100 mg/kg b.w) + Vitamin C (500 mg/kg b.w); Group V: Gentamicin (100 mg/kg b.w) + L-arginine (500 mg/kg b.w) + Vitamin C (500 mg/kg b.w).

All drugs were administered daily for 14 consecutive days. The animals were euthanized on the 15th day, blood samples and kidney tissues were collected for determination of organ weight relative to body weight and analysis of biochemical parameters.

Biochemical analyses

Plasma urea, creatinine, protein, potassium, and urinary protein

Creatinine and urea levels in plasma samples separated from the blood were analyzed. Using commercial kits (Biolabo SA, Maizy, France), plasma creatinine was determined by the modified kinetic Jaffe reaction (Labbé *et al*., 1996; Mohamed and Lasheen, 2014). Estimation of plasma urea was done by Urease-Berthelot method (Searcy *et al*., 1967; Kolawole *et al*., 2013) using kits procured from Randox Laboratory Ltd. UK. Plasma protein was determined by the method of previously described (Cannon et al., 1974; Kholif *et al*., 2014). Plasma levels of potassium and sodium were analyzed by Humalyte plus, an automated electrolyte analyzer (Human Diagnostics Worldwide) (Timerga *et al*., 2020).

Malondialdehyde, superoxide dismutase, and glutathione peroxidase

Kidney tissues kept at -80 ^{o}C were homogenized and the homogenates were centrifuged for 10 min at 3000 rpm. MDA in the supernatant obtained was estimated by the method of Esterbauer and Cheeseman (1990). In this method, MDA in the sample reacts with thiobarbituric acid and the colour generated was read at 535 nm. The result was expressed as nmol/mg wet tissue. The method described by Rotruck *et al*. (1973) was used to determine the activity of glutathione peroxidase. Superoxide dismutase was estimated by the method of Kakkar *et al*. (1984).

Statistical analysis

Data were expressed as mean ± SEM and subjected to one-way analysis of variance (ANOVA) and then Student's t- test using GraphPad Prism version 5.0. Values were considered significant at $p < 0.05$.

Results

Administration of gentamicin caused significant increase in the relative weight of kidney compared to the control (Figure 1). Gentamicin also raised the levels of plasma creatinine, plasma urea, urinary protein, urinary potassium, and kidney malondialdehyde (MDA) significantly ($p < 0.05$) compared to the distilled water-treated control. The levels of plasma protein, superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the kidney were also significantly reduced ($p < 0.05$) in gentamicin-treated rats compared to control. Co-administration of gentamicin with L-arginine, vitamin C, and combined L-arginine and vitamin C caused significant changes in these parameters. Plasma creatinine, plasma urea, and urinary protein were significantly reduced ($p < 0.05$), while plasma protein was significantly raised compared to rats treated with gentamicin only. Plasma creatinine was reduced by 18.60, 37.21, and 43.60 % in groups treated with L-arginine, vitamin C, and L-arginine and vitamin C combined respectively (Table 1). The changes observed in the other biochemical parameters follow

the same pattern, with vitamin C and the combination of vitamin C and L-arginine having greater potentials in mitigating the derangement caused by gentamicin. Urinary potassium was significantly reduced compared to gentamicin control, but sodium concentration was not significantly altered (Figure 2). Kidney level of MDA also decreased significantly ($p < 0.05$) compared with gentamicin treated rats (Figure 3). Following treatment with L-arginine and vitamin C, Plasma protein, SOD, and GPx were significantly raised ($p < 0.05$) compared with gentamicin control as shown in Figure 4

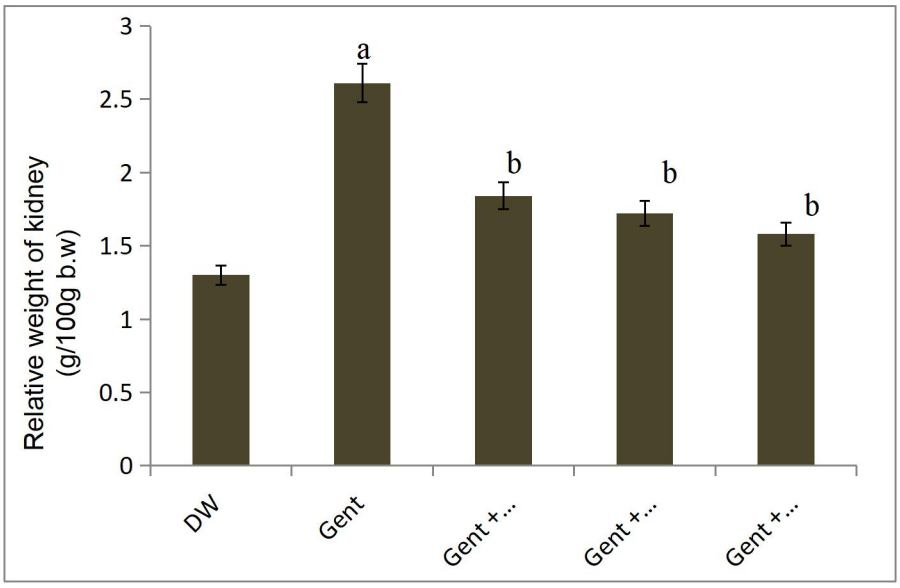

Figure 1: Effects of gentamicin, vitamin C, and L-arginine on relative weight of kidney in rats
[a]$p < 0.05$ compared with distilled water treated group, [b]$p < 0.05$ compared with gentamicin-treated group. DW = Distilled water- treated control group, Gent = gentamicin only, Gent + Arg = gentamicin and L-arginine, Gent + Vit C = gentamicin and vitamin C, Gent + Arg + Vit C = gentamicin,L- arginine, and vitamin C

Table 1: Effects of L-arginine and Vitamin C on plasma creatinine, plasma urea, plasma protein and urinary protein in gentamicin-treated rats

Groups	Plasma Creatinine (mg/dl)	Plasma Urea (mg/dl)	Plasma Protein (g/dl)	Urinary Protein (g/24 h)
DW	0.88±0.02	25.44±3.53	9.14±1.05	0.08±0.01
Gent	1.72±0.21[a]	46.10±5.71[a]	5.62±0.94[a]	0.32±0.04[a]
Gent + Arg	1.40±0.11	34.05±4.05[b]	7.04±1.02[b]	0.20±0.02[b]
Gent + Vit C	1.08±0.07[b]	32.20±4.90[b]	8.22±1.11[b]	0.15±0.01[b]
Gent + Arg + Vit C	0.97±0.03[b]	28.51±3.62[b]	8.73±1.42[b]	0.12±0.01[b]

[a]p < 0.05 compared with distilled water treated group, [b]p < 0.05 compared with gentamicin-treated group. DW = Distilled water- treated control group, Gent = gentamicin only, Gent + Arg = gentamicin and L-arginine, Gent + Vit C = gentamicin and vitamin C, Gent + Arg + Vit C = gentamicin, L- arginine, and vitamin C

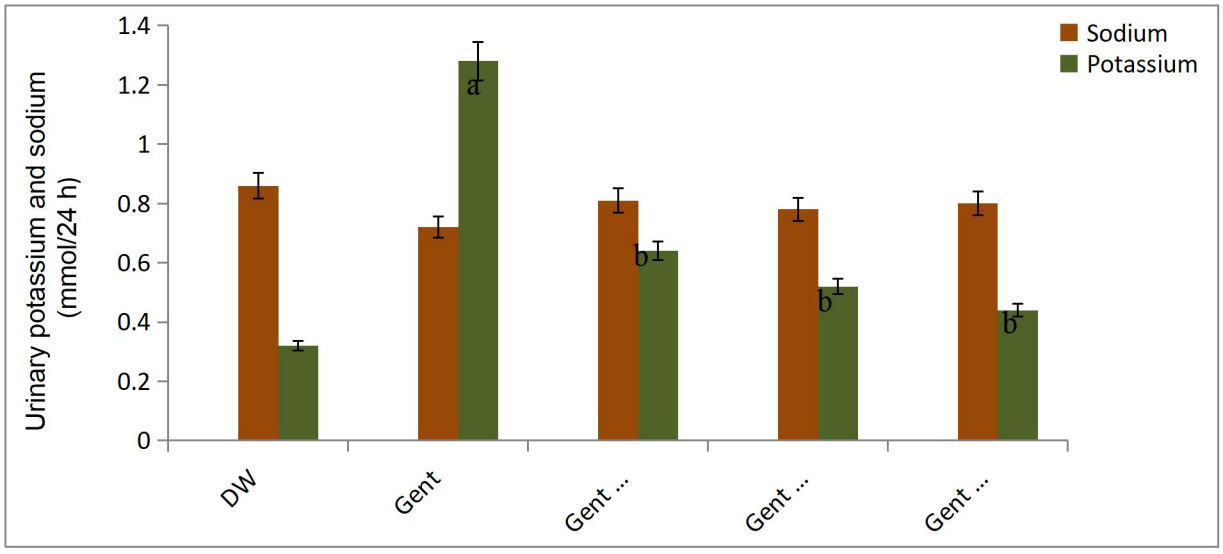

Figure 2: Effects of vitamin C and L-arginine on urinary potassium and sodium in gentamicin-treated rats

[a]p < 0.05 compared with distilled water treated group, [b]p < 0.05 compared with gentamicin-treated group. DW = Distilled water- treated control group, Gent = gentamicin only, Gent + Arg = gentamicin and L-arginine, Gent + Vit C = gentamicin and vitamin C, Gent + Arg + Vit C = gentamicin, L- arginine, and vitamin C

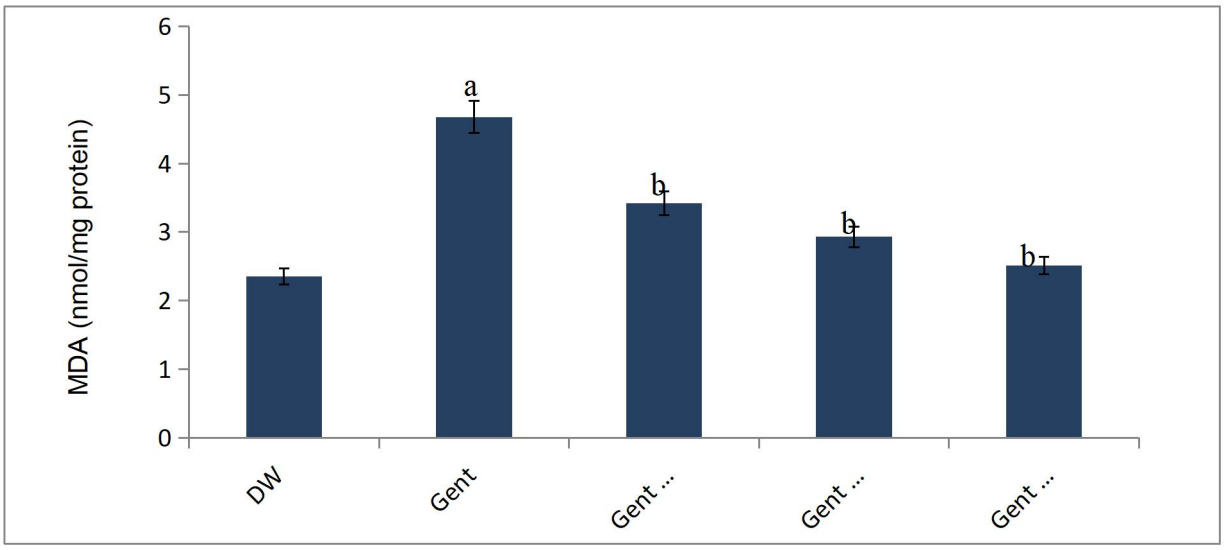

Figure 3: Effects of vitamin C and L-arginine on malondialdehyde level in the kidney of gentamicin-treated rats

[a]p < 0.05 compared with distilled water treated group, [b]p < 0.05 compared with gentamicin-treated group. DW = Distilled water- treated control group, Gent = gentamicin only, Gent + Arg = gentamicin and L-arginine, Gent + Vit C = gentamicin and vitamin C, Gent + Arg + Vit C = gentamicin, L- arginine, and vitamin C, MDA = malondialdehyde

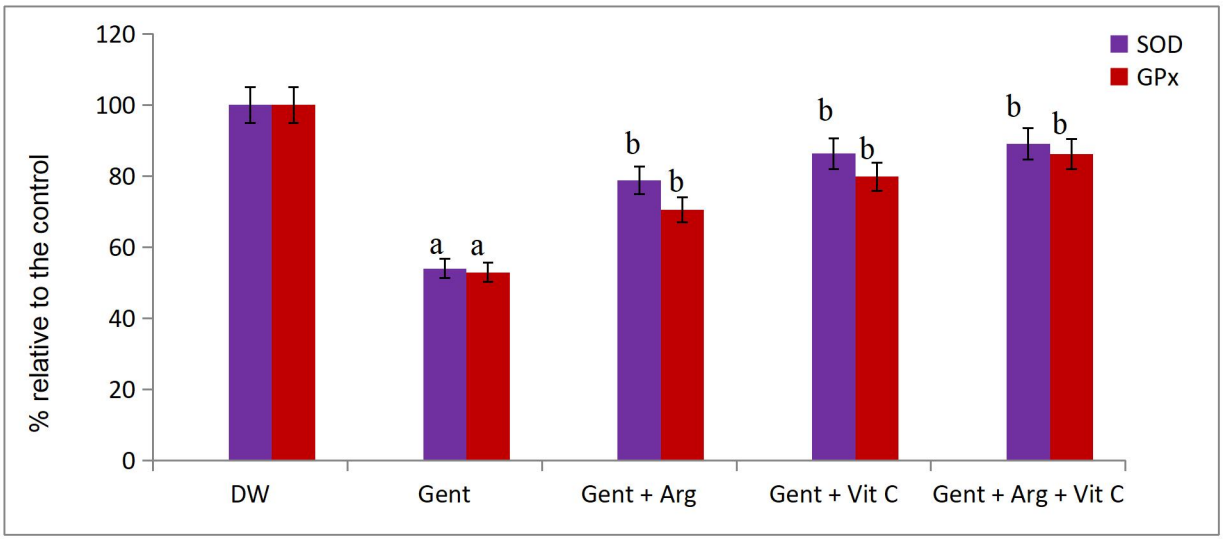

Figure 4: Effects of vitamin C and L-arginine on superoxide dismutase and glutathione peroxidase levels in the kidney of gentamicin-treated rats
[a]p < 0.05 compared with distilled water treated group, [b]p < 0.05 compared with gentamicin-treated group. DW = Distilled water- treated control group, Gent = gentamicin only, Gent + Arg = gentamicin and L-arginine, Gent + Vit C = gentamicin and vitamin C, Gent + Arg + Vit C = gentamicin, L- arginine, and vitamin C

Discussion

Gentamicin has proved very useful in the treatment of many infectious diseases, but its adverse effects on the kidney remains an unpleasant drawback (Randjelovic *et al*., 2017). Finding treatment regimens that significantly reduce the incidence of nephrotoxic effect of gentamicin will greatly enhance its therapeutic benefits. In recent years, the usefulness of L-arginine and vitamin C in ameliorating drug–induced toxicity has been investigated and reported by many researchers (Bautista-Ortega and Ruiz-Feria, 2010; Hassan *et al*., 2020). The present study compares the effects of L-arginine and vitamin C treatment on some kidney function indices in gentamicin-induced toxicity in rats. Reports have shown that increase in the weight of kidney is positively correlated with kidney damage (Murawski *et al*., 2010). Therefore, increase in kidney weight observed in this study suggests that administration of gentamicin caused kidney injury. Treatment with L-arginine, vitamin C, and their combination significantly reduced the relative kidney weight from 2.61±0.32 to 1.84±0.20, 1.72±0.27, and 1.58±0.15 respectively, suggesting that vitamin C is more effective than L-arginine in ameliorating gentamicin-induced renal toxicity. The levels of superoxide dismutase and glutathione peroxidase increased significantly in groups

treated with vitamin C and L-arginine, compared with the gentamicin control. Gentamicin accumulates in the cells of proximal tubules and induces nephrotoxicity by activating the intrinsic apoptosis, decreasing the synthesis of ATP, and inducing oxidative stress by generating superoxide anions and hydroxyl radicals in the kidney (Randjelovic *et al*., 2017). Increased level of oxidative stress within renal cells results in kidney injury. Suppressing oxidative stress is thus a promising therapeutic approach to prevent or reduce gentamicin-induced nephrotoxicity (Jaikumkao *et al*., 2016). In the absence of timely therapeutic intervention, kidney damage leads to alterations in tissue, urinary, and plasma levels of biochemical parameters like creatinine, urea, malondialdehyde, and enzymatic antioxidants (Al Asmari *et al*., 2017).

In this study, L-arginine and vitamin C significantly reduced the derangements in biochemical indices of kidney function caused by gentamicin. This indicates that L-arginine and vitamin C inhibited the generation of free radicals and suppressed oxidative stress within the renal tissue. The results agree with previous studies which reported suppressive effects of vitamin C and L-arginine on oxidative stress (Mohamed and Lasheen, 2014; Yan *et al*., 2012). L-arginine was earlier reported to increase renal blood flow and rate of glomerular filtration, which in turn decreases the concentration of gentamicin in the kidney and reduces its harmful effect on the organ (Persson *et al*., 2017). Both vitamin C and L-arginine increased the activities of superoxide dismutase and glutathione peroxidase, although the effect of vitamin C was more pronounced. This is consistent with previous studies which reported their antioxidant and ameliorative effects in renal damage caused by various insults (Zhu *et al*., 2016; Liang *et al*., 2018). Vitamin C also inhibited lipid peroxidation better than L-arginine as shown by their effects on kidney MDA. The results agree with previous studies on the inhibitory effects of both L-arginine and vitamin C on lipid peroxidation (Lubec et al., 1995; Huang *et al*., 2002). The significant reduction in urinary potassium and protein, as well as the increased plasma protein following treatment with vitamin C and L-arginine suggest that both were able to attenuate renal injury induced by gentamicin (Başhan *et al*., 2014; Ismaeil and Emara, 2019).

Conclusion

From the results of this study, we conclude that both vitamin C and L-arginine ameliorated kidney injury induced by gentamicin in rats. Vitamin C showed better effects than L-arginine, but combined L-arginine and vitamin C is more effective than when the drugs were administered individually. The use of L-arginine and vitamin C as supplements with gentamicin may be a therapeutic approach in reducing or even preventing the incidence of gentamicin-induced kidney damage.

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