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Gender differences in response to vitamin E and C in gentamicin induced nephrotoxicity in Wistar rats

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ABSTRACT

Background: Nephrotoxicity is the most recognized side effect of gentamicin. Vitamin E and vitamin C demonstrate their effective role in the prevention of nephrotoxicity. Likewise, previous studies have suggested that women have low risk of end-stage renal disease at premenopausal period.

Objectives: This study aims to investigate the possibility of any gender difference in response to antioxidant effects vitamins E and C in gentamicin-induced nephrotoxicity.

Materials and Methods: Wistar rats were randomly assigned to 6 groups each including both male and female rats. The first and second groups received saline (control group) and almond oil, the third group received gentamicin. The fourth group received a regular dose of gentamicin + vitamin E. Similarly, the fifth group received a regular dose of gentamicin + vitamin C. The sixth group received a dose of gentamicin + vitamin C and E simultaneously constantly. This protocol continued for 9 days.

Results: Gentamicin increased significantly urea, creatinine (Cr) and malondialdehyde (MDA), but it decreased superoxidase dismutase (SOD) level ($P<0.05$). Treatment with antioxidant vitamins improved urea, creatinine, MDA, and SOD serum level significantly in both genders ($P<0.05$). Likewise, kidney MDA level enhanced significantly ($P<0.05$) and treatment with antioxidant vitamins reduced MDA level too ($P<0.05$). Gentamicin decreased kidney SOD activity in male and female rats ($P<0.05$). However, treatment with antioxidant vitamins did not improve its level in male rats, while in female rats, vitamins E and C compensated for kidney SOD activity.

Conclusions: Antioxidant vitamins modified gentamicin-induced nephrotoxicity in both genders, with some difference in response to vitamins E and C between the genders, that was higher in female rats.

Implication for health policy/practice/research/medical education:
In this experimental study, 92 adult Wistar rats made the subject of the study. Antioxidant vitamins modified gentamicin-induced nephrotoxicity in both genders. However, it is noteworthy that this effect is higher in female rats.

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Original Article

1. Background

Nephrotoxicity is the most recognized side effect of aminoglycosides (1-3). They are a group of antibiotics that are administered against sever gram-negative bacterial infections (4,5). Gentamicin is an aminoglycoside that is very effective for the treatment of bacterial gram-negative infections such as pseudomonas, proteus, and serratia. It also cheap and available (6-8). It is a strongly cationic drug (9-11) that is accumulated in epithelial cells lining in

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the S1 and S2 segments of proximal tubule which cause damage to brash borders leading to the loss of brash borders integrity and nephrotoxicity (12,13). Gentamicin nephrotoxicity is manifested with lower glomerular filtration rate (GFR), renal blood flow (RBF), increasing serum creatinine (Cr), blood urea nitrogen (BUN), and tubular necrosis (9-11). Previous studies have shown that free radicals and decrease of antioxidant defense have essential roles in gentamicin nephrotoxicity (4,14,15). It enhances hydrogen peroxidase, oxygen-free radicals by mitochondria causing increase in lipid peroxidation (16-19). Therefore, renal damage related to gentamicin induces lipoproxidation (20) nitrotyrosin formation (21) and protein oxidation (22).

Recently, numerous agents have been adminisered to reduce gentamicin nephrotoxicity including pomegranate extract (23), calcium (24), pyridoxal phosphate (25), selenium (26) and fish oil (27). Vitamin E is a natural antioxidant (28) which has been proved to have protective effect against nephrotoxicity induced by cyclosporine (29) and cisplatin (30,31). On the other hand, vitamin C is a water soluble vitamin. Meanwhile, its antioxidant effects were observed in different diseases. It inhibits lipid peroxidation and restoration endothelial functions (32). Vitamin E and C are considered among the most common antioxidants. These antioxidant vitamins have been investigated in different experimental models to find out their possible effective roles in the prevention of cisplatin-induced nephrotoxicity (32,33). These antioxidant vitamins increase tissue antioxidant activity and decline free radicals, which in turn have some possible important roles in protection against gentamicin nephrotoxicity (34). Moreover, they increase superoxidase dismutase (SOD) activity and decreased lipid peroxidation in rats (34).

According to epidemiologic studies, there are gender differences in renal diseases, and women have lower risk of end-stage renal disease at premenopausal period (35,36). The prevalence and progression of renal diseases are influenced by gender (37). Probably, sex steroids like estradiol have some roles in gender differences. These findings have been approved by ovarectomized studies and hormone replacement therapy (38). Furthermore, there is evidence that estradiol has some renal protective roles, because it is an anti-inflammatory factor which inhibits apoptosis (39,40).

2. Objectives

With regard to gender differences in kidney disease and the possible roles of vitamin E and C in the improvement of kidney function; in this way, a question is raised about the effect of gender on response to antioxidant vitamins. Hence, the aim of this study is to investigate the possibility of gender difference in response to antioxidant vitamins in gentamicin-induced renal toxicity.

3. Materials and Methods

One hundred and four adult Wistar rats were the subject of this study. They included females weighing 178.0 ± 2.5 g and male ones weighing 197.4 ± 7.2 g on average. In this research, the rats were used from the Animal Centre of Zahedan University of Medical Sciences, Zahedan, Iran. The animals were housed at a temperature of 23–25°C with free access to water and rat chow. The rats were acclimatized to their diet for at least 1 week prior to the experiment.

Rats were randomly apportioned to six groups each including both male and female ones, to be under experiment for duration of 9 days. The first group (n = 9) received saline (control group), the second group received almond oil (n = 9). The third group (n = 9) got gentamicin, 80 mg/kg (19). Similarly, the fourth group (n = 9) got a regular dose of gentamicin + vitamin E, 1 g/kg/d (20). The fifth group (n = 8) obtained a continuous dose of gentamicin + vitamin C, 250 mg/kg/d (21). The sixth group (n = 8) obtained a dose of gentamicin + vitamin C and E simultaneously. Gentamicin was purchased from Caspian Company in Iran, vitamins E and C were purchased from Sigma St. Louis, MO, USA and Scharlab S.L, Spain, respectively.

On the next day after the final day of drug administration, blood samples were taken from the heart of each animal. The levels of urea and Cr were measured using quantitative diagnostic kits (Pars Azmoon, Iran), malondialdehyde (MDA) serum levels and the homogenized tissue supernatant were calculated based on the manual methodology (23, 41). At the end of the process SOD activity in serum and supernatant was measured using a colorimetric assay kit (ZelBio, Germany). During the study, the animals were weighed and recorded daily. At the end of the experiment, histopathological examinations were performed on the left kidney that were fixed in 10% formalin solution, sunk in paraffin for histopathological staining. The hematoxylin and eosin (H&E) stains were applied to examine the tubular damage. The presence of acute tubular injuries such as tubular dilation and simplification, tubular cells swelling, necrosis, tubular casts, and intra luminal cell debris with inflammatory cells infiltration were examined. The assessment was conducted by two pathologists blindly. The pathologic injuries of the

kidneys (kidney tissue damage score, KTDS) were recorded using grading scale of 0-4, which was based on subjective impression of the extent of cortical changes as follows:

0 = Indistinguishable from control
1 = Minimal, ≤25% cortex affected
2 = Mild > 25% and ≤50% cortex affected
3 = Moderate > 50% and ≤75% cortex affected
4 = Sever ≥ 75% cortex affected

This grading scale is adapted from Goering et al with minor modification (41).

3.1.Ethical issues

The research followed the tenets of the Declaration of Helsinki. This project was approved by Ethics Committee of Zahedan University of Medical. Prior to the experiment, the protocols were confirmed to be in accordance with the guidelines of Animal Ethics Committee of Zahedan University of Medical Sciences.

3.2. Statistical analysis

Data are expressed as mean ±SEM. The levels of urea, Cr, MDA, and SOD and kidney weights were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test. These parameters were compared in each group between male and female by t-Student test. The groups were compared by the Kruskal-Wallis or Mann-Whitney U tests with regard to the KTDS. P values less than 0.05 were considered statistically significant using SPSS version 16 for the data analysis.

4-Results

4.1. The effect of gentamicin on kidney weight

In male and female rats, the use of gentamicin does not have any significant effect on kidney weight. Similarly, the administration of vitamin E and C with gentamicin either alone or in co-administration has no effect on kidney weight (Figure 1).

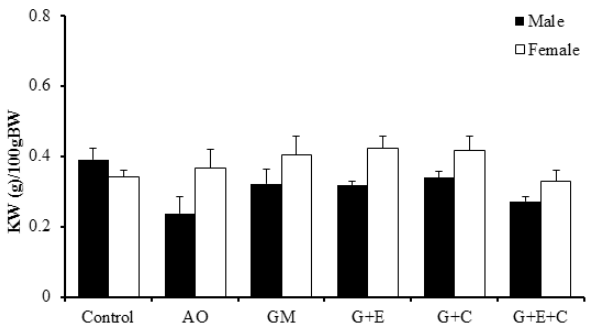

Figure 1. Kidney weight per 100 gram of body weight (KW/100 (g) BW). The groups received saline (control group), almond oil (AO) gentamicin 80 mg/kg/d (GM), GM+ vitamin E,1 g/kg/d (G +E), GM +vitamin C, 250 mg/kg/d (G +C) and GM + vitamin E and C (G + E +C) for 9 days.

4.2. The effect of gentamicin on serum urea, Cr, MDA level, SOD activity and KTDS

The measurement of urea and Cr in both sexes shows that the administration of gentamicin increased urea and Cr significantly ($P<0.05$). The presence of antioxidant vitamins reduces levels of urea and Cr. It means that the administration of vitamins E and C individually decreased urea in male rats; while their co-administration has no effect on urea level (Figure 2). In female rats, vitamin C, either individually or together with vitamin E, has a stronger effect in reducing urea levels (Figure 2).

Meanwhile, vitamins E and C, either alone or in combination, could significantly decrease Cr level at $P<0.05$ in both genders (Figure 2).

It is worth mentioning that the increase of Cr level in female rats is higher than that of male rats at the statistically significant level of $P<0.05$. This finding is in accordance with KTDS pathological findings (Figures 3 and 4).

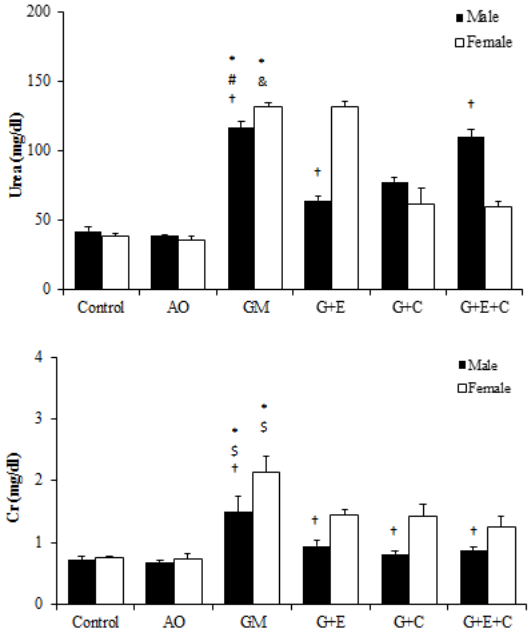

Figure 2. Blood urea and Cr level. The groups received saline (control group), almond oil (AO) gentamicin 80 mg/kg/d (GM), GM+ vitamin E, 1 g/kg/d (G +E), GM +vitamin C, 250 mg/kg/d (G +C) and GM + vitamins E and C (G + E +C) for 9 days. The symbols indicate significant difference; * from control or AO groups, # from G+E or G+C groups, & from G+C or G+E+C groups, $ from G+E, G+C or G+E+C groups in the same gender ($P<0.05$). The symbol † indicates significant difference from female rats in the same group ($P<0.05$).

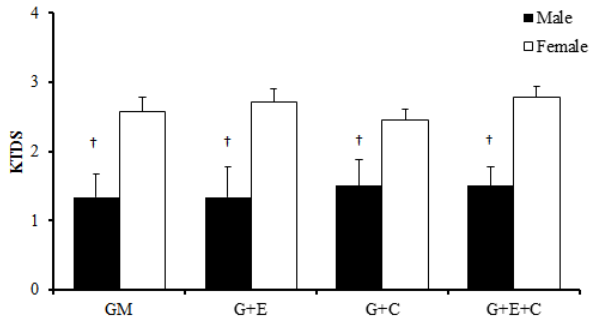

Figure 3. Kidney tissue damage score (KTDS). The groups received gentamicin 80 mg/kg/d (GM), GM+ vitamin E, 1 g/kg/d (G +E), GM +vitamin C, 250 mg/kg/d (G +C) and GM + vitamin E and C (G + E +C) for 9 days. The symbol † indicates significant difference from female rats in the same group ($P < 0.05$).

Examining the results of KTDS, after gentamicin administration, did not show significant differences between gentamicin group with other groups. Likewise, vitamins E and C did not have any significant effect on KTDS. Examining KTDS shows a significant difference of kidney damage which is observed to be significantly higher among female rats than male ones at $P < 0.05$ (Figure 3).

After treatment with gentamicin, in male and female rats, MDA level of serum increased significantly. Also, treatment with antioxidant vitamins improved MDA serum level significantly in both of gender at $P < 0.05$; but in female rats vitamin C individually has no effect on MDA level (Figure 5).

Considering the activity of SOD of serum, this activity is reduced in male rats following gentamicin administration when compared with vehicle groups at the statistically significant level (Figure 5). The administration vitamins E and C increased SOD activity of serum significantly (Figure 5). In female rats, gentamicin decreased SOD activity. This difference was significant in comparison with control and AO groups. On the other hand administration of

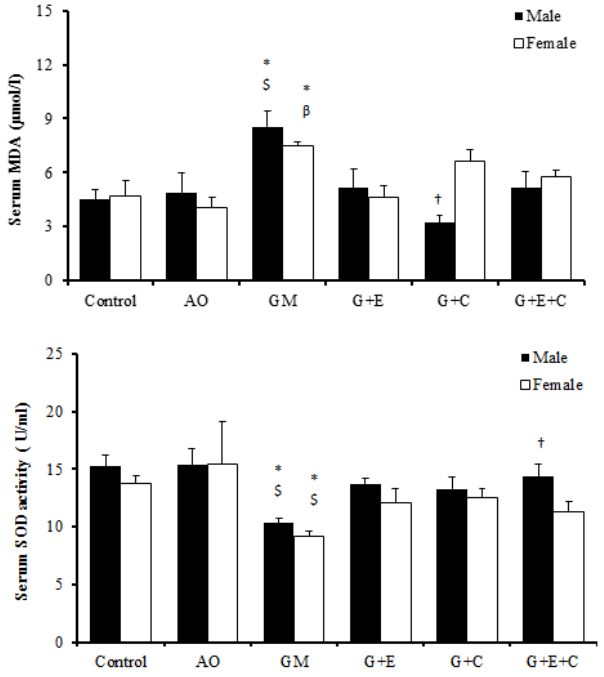

Figure 5. Serum malondialdehyde (MDA) and superoxidase dismutase (SOD) activity. The groups received saline (control group), almond oil (AO) gentamicin 80 mg/kg/d(GM), GM+ vitamin E, 1 g/kg/d (G +E), GM + vitamin C, 250 mg/kg/d (G +C) and GM + vitamin E and C (G + E +C) for 9 days. The symbols indicate significant difference; * from control or AO groups, β from G+E and G+E+C groups and $ from G+E, G+C or G+E+C groups in the same gender ($P < 0.05$). The symbol † indicates significant difference from female rats in the same group ($P < 0.05$).

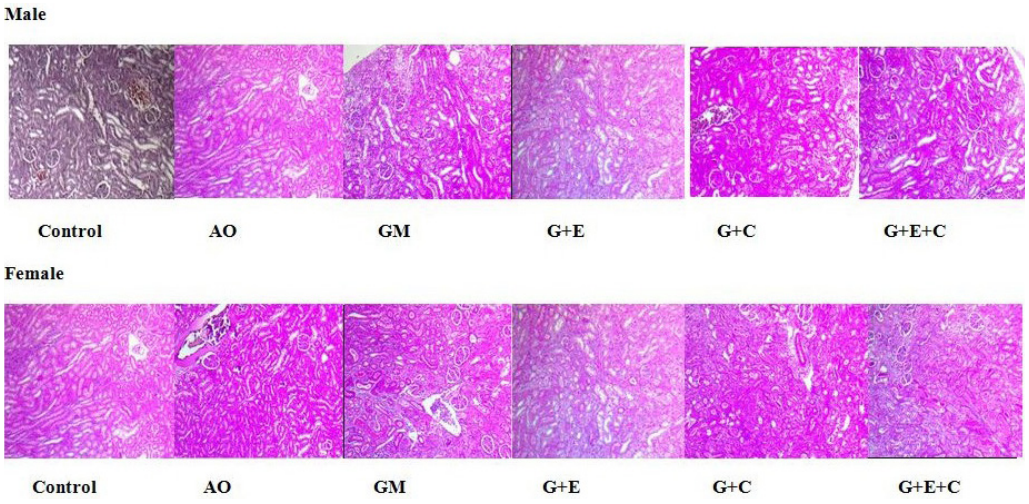

Figure 4. The pathology images (×100) of kidney tissue in male and female experimental groups, rats received saline (control group), almond oil (AO) gentamicin, 80 mg/kg/d (GM), GM + vitamin E, 1 g/kg/d (G +E), GM +vitamin C, 250 mg/kg/d (G +C) and GM + vitamin E and C (G + E +C) for 9 days.

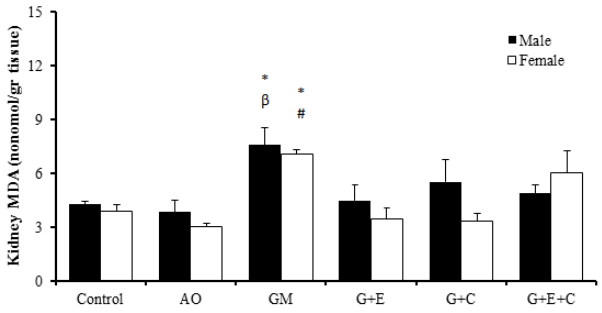

Figure 6. Kidney malondialdehyde (MDA) level. The groups received saline (control group), almond oil (AO) gentamicin 80 mg/kg/d (GM), GM + vitamin E, 1 g/kg/d (G +E), GM + vitamin C, 250 mg/kg/d (G +C) and GM + vitamin E and C (G + E +C) for 9 days. The symbols indicate significant difference; * from control or AO groups, # from G+E or G+C groups and β from G+E or G+E+C groups in the same gender ($P<0.05$).

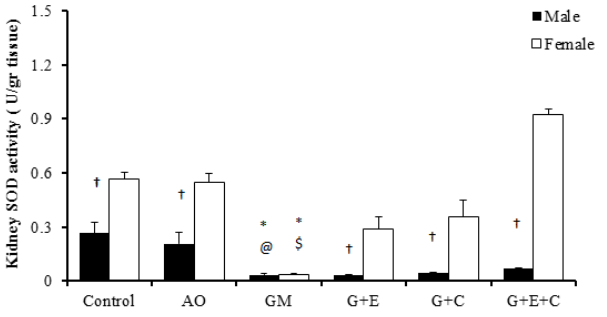

Figure 7. Kidney superoxidase dismutase (SOD) activity. The groups received saline (Control group), almond oil (AO) gentamicin 80 mg/kg/d (GM), GM+ vitamin E, 1 g/kg/d (G +E), GM + vitamin C, 250 mg/kg/d (G +C) and GM + vitamin E and C (G + E +C) for 9 days. The symbols indicate significant difference; * from control or AO groups, @ from G+E+C groups, and $ from G+E, G+C or G+E+C groups in the same gender ($P<0.05$). The symbol † indicates significant difference from female rats in the same group ($P<0.05$).

vitamins E and C improved SOD activity significantly (Figure 5).

4.3. The effect of gentamicin on kidney MDA level and kidney SOD activity

The analysis of kidney MDA level, in male and female rats, showed that gentamicin significantly enhanced MDA. Similarly, treatment with antioxidant vitamins significantly lowered it in male rats in all groups except G+C group (Figure 6). On the other hand, in female rats, vitamin C and E could each decrease MDA level significantly after gentamicin administration, while co-administration antioxidant vitamins did not have any effect on kidney MDA level (Figure 6). Kidney SOD activity is severely affected by gentamicin in male and female rats in a statistically significant level. However, in male rats, treatment with antioxidant vitamins individually did not improve SOD activity, but their co-administration enhanced SOD activity (Figure 7). It is worth mentioning that in female rats, vitamins E and C alone and in combination compensated for kidney SOD activity in a statistically significant level (Figure 7). Co-administration antioxidants vitamins have stronger effect in female rats than male rats ($P<0.05$).

5. Discussion

The most important results of this study were as follows: 1) The administration of gentamicin caused nephrotoxicity in male and female rats. This was approved by examining the changes in the levels of urea, Cr, MDA, SOD activity and renal damage. 2) In the presence of antioxidant vitamins, the above-mentioned parameters improved. 3) Renal damage in the presence of gentamicin was more among females than among males and the vitamins have good effect on gentamicin nephrotoxicity in both genders. Although this effect was more prominent in female rats.

A study conducted by Kadkhodaie et al suggested that renal functioning improved after vitamins E and C administration in male rats. This finding is compatible with the results of this study. They also showed that vitamin C alone inhibited the increase of urinary enzymes related to gentamicin nephrotoxicity but has no effect on glutathione (GSH) and GFR (34). Although vitamin E inhibited urinary enzymes, it prevented the loss of GSH and consequently modified the GFR (34). This study documented that the simultaneous administration of vitamins E and C have better effects on renal functions (34), while this effect was not observed in our study. In addition, other studies concordant with our study have demonstrated that treatment with antioxidants improved gentamicin nephropathy and treatments related to superoxide dismutase compensates renal function (21,42). Also in lead-induced oxidative stress reported after α tocopherol and ascorbic acid administration improved antioxidant enzymes, thiol components, and favourably decreased lipid peroxidation (43).

Vitamin E is the most important endogenous antioxidant that acts by neutralizing free radicals (44). Increased levels of free radicals reduce vitamin E and its co-administration with vitamin C helps to compensate for its shortage. However, it is noteworthy that high doses of antioxidants work as an oxidizing agent (44). Therefore, in this study, we used moderate doses of vitamins E and C.

Although antioxidant vitamins modified renal functions, according to our study antioxidant vitamins did not have any effect on renal damage. On the other hand, the severity of kidney damage was more in female rats than male ones. Studies conducted on sex hormones and their effects have shown that testosterone, in male-gonadectomized rats, did not have any effect on gentamicin- induced nephrotoxicity, while low doses of estrogen intensified the damage (39). However, estradiol (0.5 mg/kg) with and without progesterone improved KTDS in female rats (39), while estradiol (1 mg/kg) in presence or absence of progesterone increased renal damage (39). In a study on sexual hormones, Ali et al reported, testosterone does not have any effect on gentamicin nephrotoxicity in castrated rats (45); while another study documented that testosterone 10 mg/kg improved cisplatin nephrotoxicity (46). In addition, protective effects of testosterone were approved in renal ischemia-reperfusion model (47). Similarly, a recent study detected that estrogen 500 mg/kg decreased renal damage in ischemia-reperfusion model and its administration improved proteinuria and renal damage (48).

A robust finding of this study was the increase of renal SOD activity in female rats after the administration of the vitamins, while this effect was not observed in male rats. This phenomenon may be due to the possible interaction of female sex hormone with antioxidant vitamins. Estradiol is an antioxidant, similar to vitamin E, which can modify oxidative stress and renal toxicity (49,50). In another study, Nematbakhsh et al have shown that in the presence of estradiol, vitamin C cannot decrease cisplatin-induced nephrotoxicity. on the other hand, SOD level increases in ovarectomized female rats. This finding is consistent with our results (30). Also, in this study, SOD activity increased, in response to the simultaneous application of vitamins E and C (30), which is in agreement with our results among female rats.

However, Ulas et al have reported that SOD activity in diabetic model rats did not have any effect on ovarectomized rats which were treated with estradiol and vitamin E, while this supplementation decreased lipid peroxidation (49).

About antioxidants, other studies have shown a synergism effect on the administration of vitamin E with probucol (51) and selenium on gentamicin nephrotoxicity (25). Moorthy et al (50) documented that treatment with sildenafil decreased gentamicin nephrotoxicity and improved renal functioning. Another article reported that low doses of pomegranate flower extract improved gentamicin toxicity and decreased BUN urea and Cr (23).

6. Conclusions

Antioxidant vitamins modified gentamicin-induced nephrotoxicity in both genders, with some difference in response to vitamins E and C between the genders. It is noteworthy that, this effect is higher in female rats.

Authors' contribution

TS and MN designed, conducted, supervised and analyzed the research and prepared the first draft of article. SM, OGh and FF participated in the performance of the research and collected the data. AAN analysed the pathology data. HB participated in the writing and editing of the paper.

Conflicts of interest

The authors declared no competing interests.

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