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Impact of colistin-induced nephrotoxicity on renal oxidant/antioxidant status in male rats

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ABSTRACT

Colistin, a glycopeptide antibiotic, is only used as an emergency treatment in case of multidrug-resistant bacterial infections. Due to its propensity for causing nephrotoxicity, colistin has a limited range of applications. In this study, the effect of colistin administration on kidney functions and oxidant/antioxidant status were investigated in rats. Twenty-four male rats were divided randomly into three equal groups. Control group; Colistin 7 group: received 300,000 IU colistin intraperitoneally twice daily for 7 days; Colistin 14 group: received 300,000 IU colistin twice daily for 14 days. The obtained results demonstrated a significant increase in serum urea, creatinine, uric acid, and renal Malondialdehyde (MDA) concentrations with marked decrease in serum albumin, renal glutathione (GSH) concentrations, and superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in both colistin-administered groups when compared to the control. Additionally, colistin administered for 14 days exhibited significant alterations in the biochemical parameters compared to colistin 7 days administered rats' group. In conclusion, colistin alters the renal oxidant/antioxidant status and elevates kidney function tests that are deteriorated by a long treatment period.

1. INTRODUCTION

Antimicrobial resistance threatens the efficient prevention and treatment of many illnesses on the rise (WHO, 2015). Antimicrobial resistance is a hazard on a worldwide scale, and novel resistance mechanisms are developing quickly. Previously authorized for use in medicine, colistin is a polymyxin antibiotic. This glycopeptide antibiotic is used as a last resort. Commercially, it is marketed as colistin sodium (CMS, colistin sodium methane- sulfonate), which is meant to be administered parenterally and inhaled, as well as colistin sulphate, which is applied topically and taken orally (Li et al., 2006). However, due to the prevalence of nephrotoxicity, colistin use was largely curtailed (Li et al., 2006). According to a meta-analysis, 36.2% of patients who received colistin experienced nephrotoxicity. Despite the significant nephrotoxicity concerns that have been identified, colistin is again being used as an emergency treatment because multidrug-resistant bacterial infections are now diffuse and are related to a higher death rate. Nephrotoxicity brought on by colistin has the potential to result in irreversible kidney damage (Javan et al., 2015). Clinical signs include elevated serum creatinine levels, proteinuria, or oliguria (Hartzell et al., 2009).

Nephrotoxicity is one of the significant side effects limiting colistin use. The length of time and dosage of colistin are factors in nephrotoxicity. Colistin-induced renal failure is hypothesized to impact the proximal tubules. Numerous experimental studies have demonstrated that the development of nephropathy and its reversibility can be attributed to oxidative stress and apoptotic activity (Ozkan et al., 2013).

Reactive oxygen species (ROS), which antioxidants can neutralize under normal circumstances, can be released by cells during oxidative metabolism. However, in pathological circumstances, ROS can harm lipids, proteins, and DNA in cells, altering their structure and function (Rao and Ramesh, 2015). Additionally, research has revealed that stress caused by oxidation eventually leads to cell death and is essential for the emergence of colistin-induced kidney injury (Edrees et al., 2018). Colistin-induced nephropathy is thought to be exacerbated by oxidative stress (Ozkan et al., 2013). Renal cell apoptosis and renal failure are caused by reactive oxygen species that are generated in the mitochondria (Lopez-Novoa et al., 2011). Therefore, the current study was designed to investigate the effect of colistin administration in two duration protocols on renal antioxidant status.

2. MATERIAL AND METHODS

2.1. Experimental animals:

Twenty-four male albino rats aged 7-8 weeks old weighing 160 - 220 g were used in the current study. Rats were

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obtained from the Laboratory Animal Research Center, Faculty of Veterinary Medicine, Benha University. In cages, the animals were kept with bedding made of hardwood shavings under proper living circumstances (21 to 24 °C, 12 h light/dark cycle, not exceeding 60% humidity), supplied with a standard meal, and given access to fresh water for one week before to their use. The Animal Care and Use Committee, Faculty of Veterinary Medicine, Benha University, Egypt, approved the experimental protocols (BUFVTM 09-09-22).

2.2. Chemicals:
Colistin (each 1 g contains colistin sulphate 245.555 mg equivalent to colistin base 5 M.I.U), was obtained from ADWIA Pharmaceutical Company (Egypt).

2.3. Experimental Design:
For the experiments, rats were randomly distributed into three groups (8 rats/group). Control group: didn't receive any treatment; Colistin 7 group: received 300,000 IU colistin for 7 days; Colistin 14 group: received 300,000 IU colistin for 14 days; colistin was intraperitoneally injected twice daily (Yavuz et al., 2021).

2.4. Sampling:
Blood samples
Under the influence of sodium pentobarbital anesthesia (60 mg/kg b.wt), blood samples were collected by ocular vein puncture of the medial canthus of the eye in clean tubes and allowed to clot for 30 minutes. Serum was separated by centrifugation at 3000 r. p. m for 15 minutes. Serum was taken by automatic pipettes and received in sterile tubes, then kept in deep freeze at -20 °C until use for subsequent biochemical analysis. All sera were analyzed for the determination of the following parameters: urea, creatinine, uric acid, and albumin.
Tissue specimens (Renal tissue)
Briefly, 0.5 g of renal tissues were cut, weighed, minced into small pieces, and homogenized in a Teflon-glass homogenizer with 9 volumes of 1.15% KCl buffer to make 10 % homogenates. The homogenates were centrifuged at 6000 r. p. m for 15 minutes at 4°C, then the resultant supernatant was used for the determination of MDA, SOD, GPx, and GSH.

2.5. Biochemical analysis:
Serum urea and creatinine concentrations were determined by the methods of (Kaplan, 1984) and (Schirmeister et al., 1984), respectively. Moreover, uric acid and albumin concentrations were spectrophotometrically determined according to the methods described by (Young et al., 1975) and (Kaneko, 1997), respectively. Moreover, MDA, SOD, GPx and GSH were determined according to the methods described by (Esterbauer and Cheeseman, 1990), (Sun et al., 1988), (Lawrence and Burk, 1976), and (Sedlak and Lindsay, 1968) respectively.

2.6. Statistical analysis:
Data were analyzed using one-way analysis of variance (ANOVA), and Duncan was used as the post-hoc test by SPSS 25 (SPSS Inc., Chicago, USA). Data was presented as the mean± standard error (SEM). P values < 0.05, the data were statistically significant.

3. RESULTS
Effect of colistin administration on Kidney Functions
Intraperitoneal injection of colistin twice daily for 7 days exhibited an elevation in serum levels of urea (1.7 folds), creatinine (1.53 folds), and uric acid (1.52 folds) in comparison with control group (Figure 1a-c). On the other hand, albumin level was significantly lowered (39.38%) as compared to control (Figure 1d). Additionally, colistin administration for 7 days significantly increased MDA level (1.73 folds) (Figure 2a), decreased GSH (52.20%) (Figure 2d) and antioxidant enzyme activities (SOD: 49.23%; GPx: 52.6) in kidney tissue compared to control levels (Figure 2b, c).
On the other hand, rats injected with colistin for 14 days showed more deterioration in the examined parameters than that of colistin 7 group. Serum levels of urea, creatinine, and uric acids were significantly elevated (4.48 – 4.02 – 2.43 folds respectively) in colistin 14 group than in control (Figure 1a-c). Moreover, serum albumin decreased by 62.72% compared to control (Figure 1d). At cellular level, renal MDA level was significantly increased (2.80 folds), GSH level and antioxidant enzyme activities of SOD and GPx were decreased by (72.86% - 76.20 – 71.85 %, respectively) (Figure 2b-d).

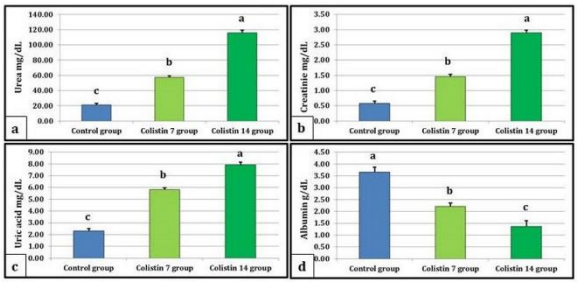

Figure 1 Effect of colistin administration on kidney functions in rats.
a: Serum Urea level (mg/dL); b: Serum Creatinine level (mg/dL); c: Serum Uric acid level (mg/dL); d: Serum Albumin level (g/dL).

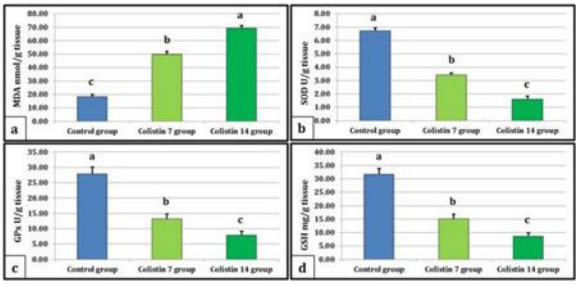

Figure 2 Effect of colistin administration on renal oxidative stress indices in rats
a: Tissue MDA level (nmol/g); b: Tissue SOD level (U/g); c: Tissue GPx level (U/g); d: Tissue GSH level (mg/g).

4. DISCUSSION
Although the efficacy of colistin as a gram-negative antibacterial, it is only prescribed to patients when there is no other therapy available. Colistin's nephrotoxicity is the leading cause of excluding this drug from the first-line options in the therapeutic plan. The current investigation confirmed the nephrotoxicity of colistin even in short duration. We can explain the elevation in serum parameters that reflect kidney function (Figure 1) by the alterations in renal antioxidant status (Figure 2). The elevation of renal MDA indicates loss of membrane integrity with subsequent

loss of renal function. It induces tubular lysis by causing damage to tubular epithelial cells that allow anion, cation, and water to enter. Tubule permeability is also increased by it (Gai et al., 2019). This was consistent with the previous results of (Ozkan et al., 2013), who attributed the nephrotoxicity following colistin administration to the oxidation stress. Colistin is filtered by renal tubules and secreted by glomeruli (Gales et al., 2001). Furthermore, colistin therapy is linked to nephrotoxicity because this medication is eliminated through the kidney, and its increased blood level may harm renal function (Ghlissi et al., 2014).

Renal failure is a result of the formation of many reactive oxygen species in mitochondria (Ozkan et al., 2013). When tissues and organs are exposed to damaging stimuli such as irritants, poisonous cellular components, or microbial infections, inflammation develops (Turner et al., 2014). Since one can readily be triggered by the other, inflammation and oxidative stress are pathological procedures strongly related to one another (Biswas, 2016). Increased ROS is thought to be a key factor in nephrotoxicity caused by colistin, which makes cell membrane damage and cellular death (Gai et al., 2019). Renal apoptosis has been discovered to be triggered by reactive oxygen species generated by the mitochondria (Lopez-Novoa et al., 2011). In a previous study on chronic renal failure induced by adenine, the increased serum level of urea, creatinine, and uric acid was explained on the basis endless cycle of oxidative stress, inflammation, and apoptosis (Said et al., 2019).

Similarly, (Ghlissi et al., 2014) reported the same findings and hypothesized that colistin treatment increases the release of ROS in addition to renal oxidative stress, mediates cell membrane damage, and results in cellular apoptosis; this oxidative stress may be the primary mechanism of the nephrotoxicity of colistin. Our findings support the hypothesis put forth by (Ceylan et al., 2018) that the oxidative stress caused by colistin would originate from excess production of free radicals and exhaustion of antioxidant enzymes like SOD (Figure 2), which would lead to nephrotoxicity. ROS also affects the mitochondria, resulting in mitochondrial malfunction and cell death by inducing an endogenous apoptotic cascade reaction that eventually results in renal failure (Dai et al., 2016). According to some theories, the membrane of tubular epithelial cells has a higher permeability, the enlargement and break down the tubular epithelial cell brought on by cation, anion and water input may affect the pathophysiology of colistin-induced nephrotoxicity (Javan et al., 2015).

5. CONCLUSION

In conclusion, colistin administration induced oxidative stress and renal damage via reduction of renal antioxidant defense system. The imbalance between oxidants and antioxidants in kidney tissue leads to renal impairment. Thus, consumption of natural antioxidant agents parallel with colistin administration may protect renal tissues, stop reactive oxygen species production and subsequently reduce the rate of colistin nephrotoxicity.

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