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Evaluation of preventive effects of Zinc, Vitamin D and their combination against Nephrotoxicity induced by Gentamicin in rats

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Abstract
The kidneys are key organs with important functions in regulating of the hydro-electrolyte equilibrium, in elimination of the waste products from the blood, and in maintaining of the organism's acid-base balance. In acute renal failure, the kidney filtration capacity is altered, which results in an accumulation of the excessive amounts of wastes and also in a marked deterioration of renal structure with cell necrosis and apoptosis. Zinc (Zn) and Vitamin D (VD) are micronutrients implicated in many diseases. Our study was to evaluate the preventive effects of Zinc and Vitamin D in nephrotoxicity induced by gentamicin in rats. A total of seventy-eight albino rats were divided into thirteen groups of six male rats for each group according their body weight. Nephrotoxicity were induced by gentamicin injection after seven days treatment. The rats were treated respectively with 25 and 50 mg / kg of zinc and 6, 12 and 24 µg / kg of Vitamin D and also with combination of zinc and Vitamin D at the same concentrations. There was a significant increase in serum of Urea, Creatinine and Low Total Protein level in the serum of rats treated with Gentamicin compared to rats in the control lot. However, Zinc and Vitamin D supplementation caused a significant decrease in serum of Urea and Creatinine activity with elevation of Total Protein concentration in the serum of rats due to their combination. This study revealed that zinc (25 to 50 mg / kg) and Vitamin D (6; 12 and 24µg / kg) attenuate Gentamicin effect in rats. The best protective combination of kidney was 50 mg / kg Zinc combined with 12 and 24 µg / kg of Vitamin D (Zn 50 + 12 VD et Zn 50 + 24 VD). This study suggests that zinc combined with Vitamin D could be a good protective against nephrotoxicity induced by Gentamicin.

Keywords: nephroprotective, zinc, Vitamin D, nephrotoxicity, gentamicin, rat

Introduction

The kidneys are key organs with important functions in regulating of the hydro-electrolyte equilibrium, in elimination of the waste products from the blood, and in maintaining of the organism's acid-base balance (Michalek, 2016) [51]. In acute renal failure, the kidney filtration capacity is altered, which results in an accumulation of the excessive amounts of wastes and also in a marked deterioration of renal structure with cell necrosis and apoptosis. The development of chronic renal failure occurs as a result of severe metabolic changes in the body, and it is associated with kidney histopathological changes, characterized by hypertrophy of the basement membrane, glomerular endothelial cell proliferation, interstitial edema, intertubular capillary congestion (Mrozikiewicz-Rakowska *et al.*, 2015; Alarifi *et al*., 2012) [52, 53].

Gentamicin is usually used for the treatment of infections caused by gram-negative bacteria. (Tavafi *et al*., 2012; Randjelovic *et al*., 2012) [36-38, 39]. Gentamicin is one of the aminoglycosides. However; its clinical use is limited by its nephrotoxicity (Sahar, 2010; Maldonado *et al*., 2003) [54, 55]. Gentamicin leads to lipids peroxidation and damages to cellular and intracellular membranes (Jabbari *et al*., 2011; Lee *et al*., 2012) [41, 11]. It has been estimated that up to 30% of patients treated with aminoglycosides for more than seven days show some signs of nephrotoxicity (Pani *et al*.,2011) [56].

Zinc (Zn) is an essential trace element that plays pivotal roles in cellular integrity and biological functions related to cell division, growth and development (Wessels *et al*., 2017) [43], because more than 300 enzymes depend on zinc for its function. It also plays an important role in nucleic acid replication, transcription and protein synthesis, cell division and differentiation (Croxford *et al*., 2011) [5] and plays an important role regeneration of the damaged cells. Moreover, zinc is suggested as an important component of the antioxidant defense system (Özaslan *et al*., 2005) [28,44], and it was also revealed to inhibit generation of reactive oxygen species and enhance the activity of antioxidant pathways (Zhou *et al*.,2005). Zinc (Zn) has many biologically significant interactions with hormones (Abdella *et al.*, 2011) [1, 40].

Vitamin D is a fat soluble steroid hormone, and may be found in two forms, as ergocalciferol (vitamin D2) produced by plants and fungi and as cholecalciferol (vitamin D3: 1,25Dihydroxyvitamin D3 (1,25(OH)2D3) produced by the animal tissue and the cutaneous synthesis under the action of ultraviolet light at 7dehydrocholesterol present in the skin (Zhang *et al*., 2015) [42].

Although vitamin D is synthesized in the skin from 7-dehydrocholesterol exposed to UVB radiation, it also may be obtained from the diet what is particularly important to people who have limited exposure to the sunlight. The main dietary sources of this vitamin include oily fish, egg yolk and supplemented milk (Ding *et al*., 2012) [6].

The aim of this study was to evaluate preventive effects of zinc and vitamin D in prevention of gentamicin-induced nephrotoxicity in rats.

Materials and Methods

Animal material

The animal species chosen for this study was the male Wistar albino rat. The rats were bred in the animal facility of Higher Normal School (ENS) at room temperature. In these premises, the photoperiod was 12 hours and the animals had free access to water and food. 114 male rats, aged 7-8 weeks, weighing approximately 100 g to 230 g were used for the experiments. The selected rats were nulliparous, non-pregnant and had not been the subject of previous studies.

Chemicals

- Gentamicin (PHILOGENTA, Nigeria, 12-2019).
- Zinc was purchased from Walmark (France, 02-2019) in the form of a bottle containing 30 tablets of 15 mg of Zinc.
- Vitamin D Uvedose were obtained from CRINEX (France, 05-2018)
- NaCl [0,9%] (Baxter, Belgique)

Methods

Preparation of solutions

- Gentamicin was administrated intraperitoneally by injection at a single dose of 80 mg/kg.
- 15 mg tablet was dissolved in 5 mL distilled water per kilogram bodyweight (Piao *et al*., 2016) administered by gavage.
- Vitamin D (Uvedose) 2 mL were dissolved in olive oil with 12 µg/kg bodyweight (Claudia *et al*.,2016) administered by gavage.

Animals' treatment

Animals were divided into 13 lots of 6 rats each (n= 6). The duration of experiment was for 14 days. The treatments are carried out every day at the same hour during the experimental period.

Treatment of control lots

- **Lot 1**: received NaCl 9 ‰ at 1 mL/rat by gavage for fourteen days (Normal control).
- **Lot 2**: were administered with Gentamicin (80mg/kg) intraperitoneally (ip) on the fourteenth day of treatment (Negative Control).

Treatment with different doses of zinc (25 and 50 mg/kg bw) by gavage follow by Gentamicin injection

- **Lot 3**: received 25 mg/kg of zinc bw by gavage for fourteen days followed by Gentamicin (80 mg/kg bw) intraperitoneally for fourteenth day of treatment (Zn 25 + Ge)
- **Lot 4**: received 50 mg/kg bw of zinc by gavage for fourteen days followed by Gentamicin (80 mg/kg bw) for fourteenth day of treatment (Zn 50 + Ge)

Treatment with different doses of vitamin D (6, 12 and 24 µg/kg bw) by gavage follow by Gentamicin injection

- **Lot 5**: received 6 µg/kg bw of vitamin D by gavage for fourteen days followed by Gentamicin (80 mg/kg bw) intraperitoneally for fourteenth day of treatment (6VD + Ge)
- **Lot 6**: received 12 µg/kg bw of vitamin D by gavage for fourteen days followed by Gentamicin (80 mg/kg bw) intraperitoneally for fourteenth day of treatment (12VD + Ge)
- **Lot 7:** received 24 µg/kg bw of vitamin D by gavage for fourteen days followed by Gentamicin (80 mg/kg bw) intraperitoneally for fourteenth day of treatment (24VD + Ge)

Treatment with zinc and vitamin D by gavage followed by Gentamicin injection

- **Lot 8**: received 25 mg/kg bw of zinc + 6 µg/kg bw of vitamin D by gavage for fourteen days followed by Gentamicin (80 mg/kg bw) intraperitoneally for fourteenth day of treatment (Zn 25 + VD 6 + Ge)
- **Lot 9**: received 25 mg/kg bw of zinc + 12 µg/kg bw of vitamin D by gavage for fourteen days followed by Gentamicin (80 mg/kg bw) intraperitoneally for fourteenth day of treatment (Zn 25 + VD 12 + Ge)
- **Lot 10**: received 25 mg/kg bw of zinc + 24 µg/kg bw of vitamin D by gavage for fourteen days followed by Gentamicin (80 mg/kg bw) intraperitoneally for fourteenth day of treatment (Zn 25 + VD 24 + Ge)
- **Lot 11**: received 50 mg/kg bw of zinc + 6 µg/kg bw of vitamin D by gavage for fourteen days followed by Gentamicin (80 mg/kg bw) intraperitoneally for fourteenth day of treatment (Zn 50 + VD 6 + Ge)
- **Lot 12**: received 50 mg/kg bw of zinc + 12 µg/kg bw of vitamin D by gavage for fourteen days followed by Gentamicin (80 mg/kg bw) intraperitoneally for fourteenth day of treatment (Zn 50 + VD 12 + Ge)
- **Lot 13**: received 50 mg/kg bw of zinc + 24 µg/kg bw of vitamin D by gavage for fourteen days followed by Gentamicin (80 mg/kg bw) intraperitoneally for fourteenth day of treatment (Zn 50 + VD 24 + Ge)

Biochemical Study

Blood samples collected in anticoagulant-free tubes were centrifuged at 3000 rev / min for 15 minutes. The collected sera were used to measure the biochemical parameters namely: Urea, Creatinine and Total Protein with the *Cobas Integra 400 Plus* (ROCHE DIAGNOSTIC, Germany)

Statistical Analysis

Graph pad 5.1 was used for statistical analyses. Data were expressed as mean ± ET. Mean values of the different lots were compared using a one-way analysis of variance (ANOVA) with Dunnett test. If $p < 0.05$, the difference between the values was considered significant.

Result and Discussion

Results

Effects of zinc and Gentamicin injection on kidney markers

The figures (1A and 1B) show that respectively Gentamicin administration in rats induced significant augmentation ($p<0,05$) of urea (1,64 ± 0,26) and creatinine (49,07±27,33) report to control rats (0,24 ± 0,04 et 2,5 ± 0,17. However high concentration of zinc (25 and 50 mg/kg), renal makers augmentation by gentamicin injection was significantly inhibit with low concentration of urea (0,80±0,19 et 0,78±0,24) and creatinine (14,33 ± 5,56 et 17,33 ± 4,86) comparatively to gentamicin group (figures 1A et 2A). Totals proteins lowed in the group of gentamicin but no significantly ($p>0,05$) in comparison to others (figure 1C).

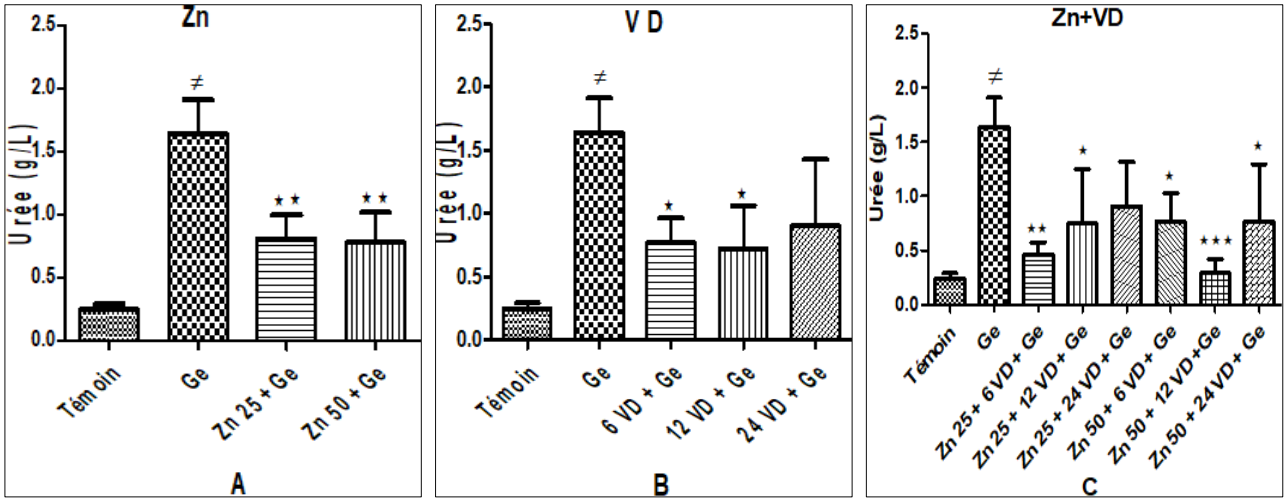
Effects of vitamin D and Gentamicin injection on kidney markers

Gentamicin administration for seven days significantly

increased (p<0,05) kidneys markers as urea (1,64 ± 0,26) and creatinine (49,07±27,33) compared to control group respectively (0,24 ± 0,04 and 2,5 ± 0,17) (figures 2A and 2B). Otherwise, pre-treatments with diverse concentration of vitamin D (6; 12 and 24 µg/kg) associated to Gentamicin injection decreased significantly (p<0,05) creatinine (12,03 ± 4,53; 14,03 ± 7,80 and 15,40 ± 9,93) activity. But, only 6 and 12 µg/kg of vitamin D decreased significantly (p<0,05) urea (0,77± 0,19 et 0,72 ± 0,34) activity in serum (figures 2A and 2B). In treated group, Totals proteins concentration was reduced comparing to gentamicin group (figure 2C).

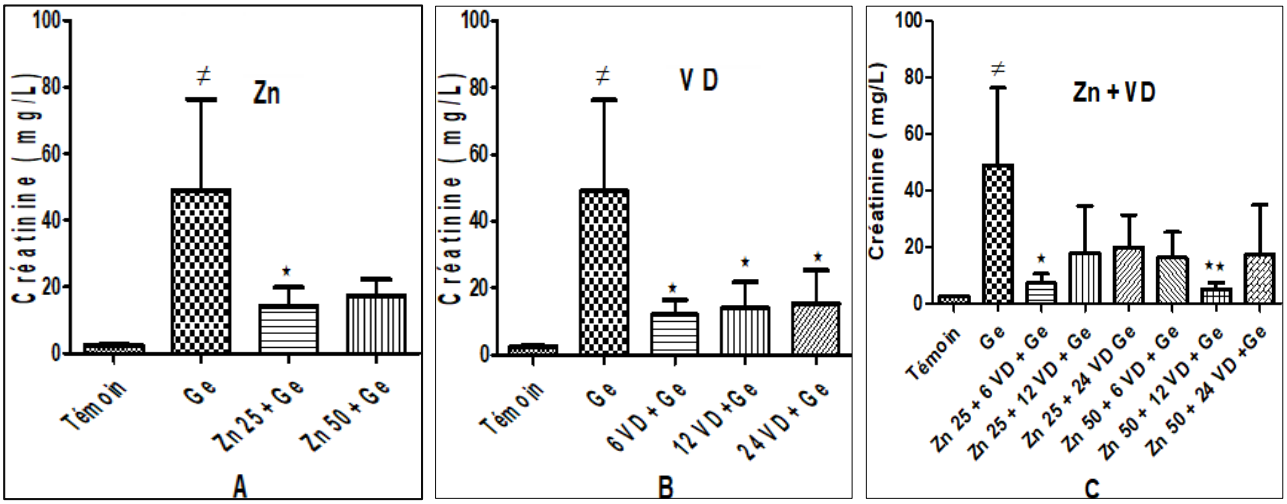
Zinc and vitamin D combination effect on kidney markers

Combination done with zinc and vitamin D showed the evolution of kidney markers (Urea and Creatinine) report to control group. Gentamicin injection increased urea and creatinine concentrations. A diminution of Totals Proteins was observed in gentamicin group compared to all groups. In addition, (Zn25 + 6 VD; Zn 50 + 12 VD) attenuated significantly (p<0,05) Urea (0,46 ± 0,11 and 0,29 ± 0,12) and Creatinine (7,13 ± 3,22 et 5,13 ± 2,15) in reference to Gentamicin group. These groups were the better combination of zinc and vitamin D (figure 1C and 2C). Concerning totals proteins, their concentration decreased after gentamicin injection in rats, it has been shown a diminution (62,63 ± 2,80) reported to those of control rats (68,23 ± 3,91) and others groups. The groups Zn 50 +12VD and Zn50+ 24VD respectively increased significantly (p<0,05) Totals proteins concentrations (72,83 ± 4,45 et 76,40 ± 3,65) compared to control group. The best combinations for totals proteins were (Zn 50 + 12 VD et Zn 50 + 24 VD) (figure 3C).



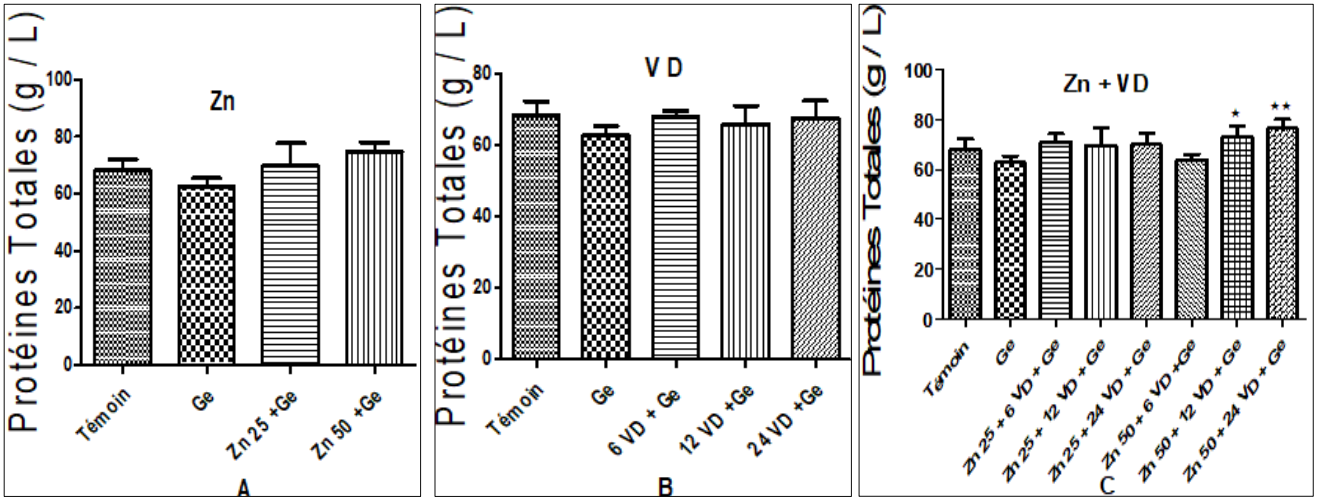
Ge: gentamicine, Zn: zinc, n=6/lot; VD: Vitamine D
≠, *: (P < 0,05): Différence significative ≠: (p < 0,05) vs témoin *: (p < 0,05) vs gentamicine

Fig 1: Effect of Zinc, Vitamin D and Gentamicin administration on Urea level in rat's serum
(A) effect of Zinc on Urea, **(B)** effect of Vitamin D on Urea, **(C)** effect of Zinc and Vitamin D combination on Urea



Ge: gentamicine, Zn: zinc, n=6/lot; VD: Vitamine D
≠, *: (P < 0,05): Différence significative ≠: (p < 0,05) vs témoin *: (p < 0,05) vs gentamicine

Figure 2: Effect of Zinc, Vitamin D and Gentamicin administration on Creatinine level in rat's serum
(A) Effect of Zinc on Creatinine, **(B)** effect of Vitamin D on Creatinine, **(C)** effect of Zinc and Vitamin D combination on Creatinine



Ge: gentamicine, Zn: zinc, n=6/lot; VD: Vitamine D
≠, *: (P < 0,05): Différence significative ≠: (p < 0,05) vs témoin *: (p < 0,05) vs gentamicine

Figure 3: Effect of Zinc, Vitamin D and Gentamicin administration on Total Protein level in rat's serum
(A) effect of Zinc on Total protein, **(B)** effect of Vitamin D on Total protein, **(C)** effect of Zinc and Vitamin D combination on Total protein

Discussion

This study was conducted to investigate the effects of zinc and vitamin D in prevention of gentamicin-induced nephrotoxicity in rats. Gentamicin is one of the antibiotics used in renal toxicity for induced nephropathy. After Seven (7) days administration of gentamicin, urea value (1,64 ± 0,26) and creatinine value (49,07 ± 27,33) significantly increased comparatively to control group (0,24± 0,04 et 2,5±0,17). Considerable increase in plasma creatinine and urea nitrogen is suggested as an index of gentamicin-induced nephrotoxicity (Tavasi et Ahmadvand, 2011) [17]. Gentamicin is actively reabsorbed in the proximal convoluted tubule and its concentration in the tubular cells damages the proximal tubule and disturbs renal blood circulation, which in turn results in a reduction in GFR glomerular filtration rate and an increase in the levels of plasma creatinine and urea nitrogen (Okokon *et al*., 2011) [14]. However, in serum of rats treated with gentamicin, totals proteins concentration decreased. Proteinuria, usually reflecting the loss of normal glomerular filtration permeability to plasma proteins is an early sign of kidney disease (Cohen et Lemann, 1991) [4]. Thus, detection of proteinuria is necessary for the recognition of most kidney disease (Lesely et Levey, 2005) [10]. Gentamicin-administered rats (negative control group) had encountered acute kidney dysfunction as evidenced by elevation of serum urea and creatinine with low total protein in serum. The results of our investigation is in conformity with previous reports attributing these changes to nephrotoxicity induced by gentamicin (Bamba *et al*., 2016; Javed *et al*., 2015; Reddy *et al*., 2011) [2, 15, 16].

In this study, intraperitoneal injection of gentamicin (80 mg/kg p.c) for seven days showed renal dysfunction. Many researchers believe that the increase in the production of ROS by gentamicin is the key to the toxicity resulted from its administration (Tavafi *et al*.,2012; Pai *et al*.,2012) [36-38]. It has been shown that oxidative e stress plays an important role in gentamicin nephrotoxicity (Morales *et al*., 2010) [26]. Anyway, the groups pretreated with 25 and 50 mg/kg of zinc (Zn25; Zn50), and inject with gentamicin showed significant decrease in serum of urea (0,80 ± 0,19 and 0,78 ± 0,24) and creatinine 14,33 ± 5,56 et 17,33 ± 4,86) when compared with gentamicin group. Gentamicin induces the production of high reactive oxygen species that can activate some pro-inflammatory and pro-apoptotic mediators, leading to renal injuries (Ozbeck *et al*., 2009) [27]. But zinc is known to influence cytokine production and to stabilize the cellular membrane, thus preventing inflammatory lesions (Kaur *et al*., 2014) [8]. According to some studies, zinc induces the decrease of reactive oxygen species and provides cellular membrane stability, showing a high protective effect against free radicals (Bicer *et al*., 2012) [3]. Our results are similar to those of Teslariu *et al*., in which zinc administration proved a protective role in experimental gentamicin-induced acute renal failure. The treatment with Zn significantly diminished the alteration of renal function, with an evident improvement of serum urea and creatinine levels and also of the urinary protein values (Teslariu *et al*., 2016) [20, 57]. Zinc pre-treatment can contribute to protect against nephrotoxicity induced by gentamicin.

Like Zinc pre-treatment, rats were also pre-treating with Vitamin D before Gentamicin injection. Therefore, rats were pre-treating with 6; 12 et 24 µg/kg (6VD; 12VD et 24 VD) of vitamin D before gentamicin injection. Groups received gentamicin increase significantly serum urea (1, 64 ± 0,26) and creatinine (49,07 ±27,33). But the pre-treatment groups (6VD and 12VD) show significant decrease of serum urea (0,77± 0,19 et 0,72 ± 0,34). Otherwise, the groups (6VD; 12VD and 24VD) also reduced significantly serum creatinine (12,03 ± 4,53; 14,03 ± 7,80 et 15,40 ± 9,93) in comparison with Gentamicin group. These results are confirmed by Paoulomi *et al*., who indicated that supplementation of *Aloe barbadensis* restored the increased levels of urea and creatinine in gentamicin-induced rats Paoulomi *et al*.,2012 [21]. Diminution of urea and creatinine concentration reveal kidney functions restauration. According to Parikh *et al.,* a resistance to vitaminD3 has been reported in CKD and is associated with progression of renal disease (Parikh *et al.,* 2015) [30]. The renal protective effect of vitamin D has been linked with inhibition of the renin-angiotensin system and NF-κB pathway (Li, 2010) [35]. Functionally, gentamicin-related nephrotoxicity is characterized by a decrease in glomerular filtration rate and high levels of serum creatinine and blood urea, indicating renal dysfunction ((Romero *et al*.,2009; Soliman *et al*.,2007) [18, 19]. Despite, dysfunction induced by gentamicin, it has been shown to have therapeutic potential in attenuating experimentally induced glomerular

diseases (Kuhlmann *et al.*,2004; Makibayashi *et al*., 2001; Panichi *et al*., 2001) [9, 24, 25]. Pre-treatment with different doses of vitamin D attenuated Gentamicin effects on rats. The present study is in accordance with those of Park *et al*., in which paricalcitol resulted in attenuation of GM induced renal fibrosis, which was related to the attenuation of inflammatory processes. The possible mechanism involving these anti-inflammatory or antifibrotic effects of paricalcitol may be the interruption of NF-B and ERK signaling pathways (Park *et al*.,2009). A recent experiment also suggested that paricalcitol is able to repress the NF-kB-mediated gene transcription in inflamed renal tubular epithelium (Tan *et al*., 2008) [23].

This study was realizing to prevent kidney toxicity. In fact, it was important to find the better combinations of zinc and vitamin D against kidney toxicity. That conduct us to combine zinc and vitamin D so that observe better combinations which had protected effects on kidney injury. Gentamicin injection to rats produced a renal dysfunction by elevation of urea (0,24 ± 0,04) and creatinine (2,50 ± 0,17) concentration in serum. The combinations of this investigation (Zn25 + 6VD and Zn50 + 12VD) respectively indicated significant reduction of urea (0,46 ± 0,11 and 0,29 ± 0,12) and creatinine (7,23 ± 3,22 et 5,13 ± 2,15) in plasma serum. These combinations attenuate gentamicin effects. This study is confirmed by Mehri *et al*., who revealed that the GM-induced renal toxicity, as measured by multiple functional, structural and enzymatic factors is significantly reduced by co-supplementation of vitamins C and E. The results of this study suggest the potential of antioxidant vitamins to protect against GM-induced nephrotoxicity (Mehri *et al.*, 2005) [13].

Gentamicin induction to rats decreased totals proteins concentration in serum. Therefore, zinc or vitamin D administration produced low and no significant augmentation of totals proteins in the present study. Though, it has been established that gentamicin causes inhibition of protein synthesis in renal cells with consequent abundance of amino acid in the kidney resulting in increased urea levels (Sundin *et al*., 1997) [31]. This observed change is in accordance with the report of El-Zawahry and Abu El Kheir who stated that gentamicin administration to rats is associated with the excretion of protein (El-Zawahry and Abu El Kheir,2007) [7].

In addition to individual action of zinc and vitamin D, some combinations with different doses of zinc and vitamin D were tested. The pre-treatment done with combinations Zn50 + 12VD et Zn50 + 24VD trained respectively a significant elevation rate of totals proteins (72,83 ± 4,45 et 76,40 ± 3,65) in serum report to control group (68,23 ± 3,91). This augmentation involve proteins synthesis rise of renal cells. Zn50 + 24VD combination effect was more significant than Zn50 + 12VD combination. Our results are similar to those of the pass in which in the past, vitamin D was shown as an effective drug on podocytes preventing proteinuria, regulate bone remodelling, regulate cell cycles, and the renin-angiotensin system (Yildirim *et al*., 2013) [32].

Conclusion

At the end of our study, it shows that zinc oral supplementation with 25 to 50 mg / kg and vitamin D (6; 12 et 24µg/kg) are beneficial against Gentamicin induced nephrotoxicity. This study showed that the best combination is obtained with 50 mg / kg of Zinc combined with 12, 24 µg / kg of Vitamin D. Finally, this study suggests that zinc combined with vitamin D provides good protection of kidney against Gentamicin toxicity.

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