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Molecular Mechanisms of the Modulatory Effect of Vitamin E on Tacrolimus (FK506)-Induced Renal Injury in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ESA, AAHAA and AB designed the study. Author HA performed the experiments. Authors HA and MNED carried out the statistical analysis. Authors ESA and AB wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: This study was designed to evaluate the possible modulatory effect of vitamine E on tacrolimus (FK506)-induced renal injury in rats.
Methods: Twenty-four male Wistar rats (6 animals in each group) were used in this study. The first group (Control) received normal saline intraperitoneal (i.p.) daily for 21 days. The second group received FK506 (1 mg/kg/day i.p.) daily for 21 days. The third group was administered Vitamin E (250 mg/kg/day by oral gavage) 5 days before and concurrently during FK506 administration daily for 21 days. The fourth group received Vitamin E alone (as previously described in the third group).
Results: Administration of FK506 significantly increased blood urea nitrogen and serum creatinine

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levels. In addition, FK506 has also reduced the renal content of reduced glutathione, as well as the enzymatic activities of superoxide dismutase and catalase. Furthermore, these effects were associated with an increase in lipid peroxidation, inducible NO-synthase (iNOS), and NF-κB expression. Moreover, histopathological examinations showed severe damage of the renal tissues in animals treated with FK506. Interestingly, it was found that concomitant administration of vitamin E along with FK506 ameliorated all these parameters and improved renal function. Furthermore, the immunosuppressive effect of FK506 was not affected by vitamin E.

Conclusion: The findings of the present study suggest that concomitant use of vitamin E might be useful in reducing nephrotoxicity induced by FK506.

Keywords: Tacrolimus; Vitamin E; Nephrtoxicity; ROS; iNOS; NF-κB.

1. INTRODUCTION

Solid organ transplantation has become one of the most important fields in modern medicine due to its contribution in decreasing the mortality rate of patients with organs failure. However, the risk of transplant rejection threatens the success of the transplantation procedure. Transplant rejection can be prevented by the use of an immunosuppressive agent to suppress the reaction of the immune system to the transplanted tissue. Tacrolimus (FK506) is one of the calcineurin inhibitors that suppress T-cell activation by inhibiting the cellular phosphatase calcineurin [1,2]. The mechanism involves binding of FK506 to the FK binding protein 12 (FKBP12). The tacrolimus-FKBP12 complex then binds to calcineurin and mediates inhibition of phosphatase activity of calcineurin. This inhibition prevents the dephosphorylation and nuclear translocation of the nuclear factor of activated T cells (NFAT) and subsequent transcription of IL-2 and other cytokines. Calcineurin inhibitors (CNI) are among the most efficient immunosuppressive agents and therefore are widely used in organ transplantation and for the treatment of many inflammatory diseases. However, the clinical use of the calcineurin inhibitor FK506 is strongly limited by acute and chronic nephrotoxicity which remains a major clinical problem [2-4]. The mechanisms of FK506-induced renal injury are not fully elucidated. However, one of the possible mechanisms of FK506-induced nephrotoxicity is thought to be over production of reactive oxygen species (ROS) and a consequent imbalance between oxidants and endogenously produced antioxidants [4,5]. The harmful effects of ROS induced by FK506 can be antagonized by using a powerful antioxidant agent [4,5]. One of the most important natural antioxidants is vitamin E (Vit E) [6,7]. Vit E has eight naturally occurring components including α, β, γ, and δ derivatives of tocopherol and tocotrienol. In 1989, it has been reported that α-tocopherol has high activity as a chain-breaking antioxidant [8]. Furthermore, α-tocopherol has been demonstrated to be the most abundant lipid-soluble antioxidant in humans and it has been detected in cellular and sub-cellular membranes [9]. Thus it was interesting to investigate the potential protective role of Vit E against FK506-induced nephrotoxicity in rats.

2. MATERIALS AND METHODS

2.1 Animals

Male Wistar albino rats weighing 200-230 g were housed in a 12 h dark/light cycle animal facility with controlled humidity and constant temperature. The animals were fed a standard diet and water was supplied *ad libitum*. The animals were kept under observation for one week before the treatments for adaptation. The experimental protocol used in this study was approved by the Institutional Animal Ethics Committee.

2.2 Drugs and Chemicals

FK506 was purchased from Astellas Pharma Inc., Japan. Vit E was purchased from Pharco Pharmaceuticals, Alexandria, Egypt. Nuclear factor kappa-B (NF-κB), inducible NO-synthase (iNOS) and catalase (CAT) ELISA kits were purchased from EIAab Science Co., Ltd., China. Superoxide dismutase (SOD), reduced glutathione (GSH), and thiobarbituric acid reactive substances (TBARS) assay kits were purchased from Trevigen (USA), Oxford biomedical research (Oxford MI, USA), and Cell Biolabs (USA) respectively. Interleukin-2 (IL-2) ELISA kit was purchased from Uscn Life Science Inc, Wuhan, China. Creatinine and blood urea nitrogen (BUN) assay kits were purchased from Bioassay system (USA).

2.3 Experimental Design

Twenty-four male Wistar albino rats were used in this study. The animals were randomly divided into four groups, 6 animals in each. The first group (Control) received normal saline intraperitoneal (i.p.) daily for 21 days. The second group received FK506 (1 mg/kg/day i.p.) [10] daily for 21 days. The third group was administered Vit E (250 mg/kg/day by oral gavage) [11] 5 days before and concurrently during FK506 administration daily for 21 days. The fourth group received Vit E alone (as previously described in the third group). 24 h after the last treatment, blood samples were collected for the determination of serum levels of creatinine, BUN as well as IL-2. After terminal bleeding, animals were sacrificed by cervical dislocation. The left kidney was dissected immediately after death, washed with ice cold phosphate buffered saline (PBS) and kept at -20ºC till used. The right kidney was fixed in 10% neutral-buffered formal saline for histopathological investigation.

2.4 Assessment of BUN and Serum Creatinine

The serum levels of BUN and creatinine were determined by commercial kits according to the manufacturer's instructions (Bioassay system, USA).

2.5 Assessment of Serum Cytokine

The serum level of IL-2 was determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Uscn Life Science Inc, Wuhan, China).

2.6 Determination of Oxidative Damage Markers

2.6.1 Determination of lipid peroxides

The by-product of lipid peroxidation malondialdehyde (MDA) in renal tissues was measured using a TBARS assay kit according to the manufacturer's instructions (Cell Biolabs, Inc., USA). Briefly, Tissue samples were homogenized in ice-cold PBS containing 1X BHT (Butylated hydroxytoluene) and centrifugated at 10,000 g for 5 min at 4℃ to collect the supernatant. The unknown MDA containing samples or MDA standards were first reacted with thiobarbituric acid (TBA) at 95℃. After a brief incubation, the samples and standards can be read spectrophotometrically. The MDA content in unknown samples was determined by comparison with the predetermined MDA standard curve.

2.6.2 Determination of iNOS expression

The protein level of iNOS in renal tissues was determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (EIAab Science Co., Ltd., China).

2.6.3 Determination of NF-κB expression

Total NF–κB level in renal tissues was determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (EIAab Science Co., Ltd., China).

2.7 Determination of Endogenous Antioxidants

2.7.1 Determination of GSH content

Renal content of GSH was determined by colorimetric assay according to the manufacturer's instructions (Oxford biomedical research, Oxford MI, USA). Briefly, the method is based on the reaction of GSH with Ellman's reagent [5, 5'-dithiobis-2-nitrobenzoic acid (DTNB)] which gives rise to a product that can be quantified spectrophotometrically at 412 nm.

2.7.2 Determination of SOD activity

Renal SOD activity was determined by assay kit according to the manufacturer's instructions (Trevigen, USA). In Trevigen's superoxide dismutase assay, superoxide radicals generated from the conversion of xanthine to uric acid and hydrogen peroxide by xanthine oxidase, converts nitroblue tetrazolium (NBT) to NBT-diformazan. NBT-diformazan absorbs light at 550 nm. SODs reduce superoxide radical concentrations and thereby lower the rate of NBT-diformazan formation. The extent of reduction in the appearance of NBT-diformazan is a measure of SOD activity.

2.7.3 Determination of CAT activity

Renal CAT activity was determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (EIAab Science Co., Ltd., China).

2.8 Histopathological Examination

Kidney specimens from all animals were immediately dissected and fixed in 10% neutral-buffered formal saline for at least 72 hours. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 6µm thick were cut and stained with Haematoxylin and eosin [12] for histopathological investigation.

2.9 Statistical Analysis

Results are expressed as means ± SD. Statistical analysis was performed using one way ANOVA followed by Turkey-Kramer as a post-hoc test. *P*-values below 0.05 were considered as indication for statistically significant differences between conditions compared.

3. RESULTS

3.1 Effects of FK506 and/or Vit E on Serum Creatinine and BUN Levels

Treatment of rats with FK506 produced a significant increase in BUN (Fig. 1A) and serum creatinine levels (Fig. 1B). However, concomitant administration of Vit E along with FK506 significantly reduced the levels of BUN (Fig. 1A) and serum creatinine (Fig. 1B) as compared to FK506 alone treated animals. No significant changes were observed in rats treated with Vit E alone.

3.2 Effects of FK506 and/or Vit E on Oxidative Damage Markers

3.2.1 Effects of FK506 and/or Vit E on lipid peroxides

Administration of FK506 significantly induced lipid peroxidation as indicated by an increase in the by-product of lipid peroxidation MDA. On the other hand, lipid peroxidation is significantly attenuated in animals treated with FK506 in combination with Vit E as compared to FK506 alone treated animals (Fig. 2A). No significant changes were observed in animals treated with Vit E alone.

3.2.2 Effects of FK506 and/or Vit E on iNOS expression

As demonstrated in Fig. 2B, administration of FK506 significantly induced iNOS expression. However, concomitant administration of Vit E along with FK506 significantly reduced iNOS expression as compared to FK506 alone treated animals. The basic level of iNOS expression was not changed in rats treated with Vit E alone.

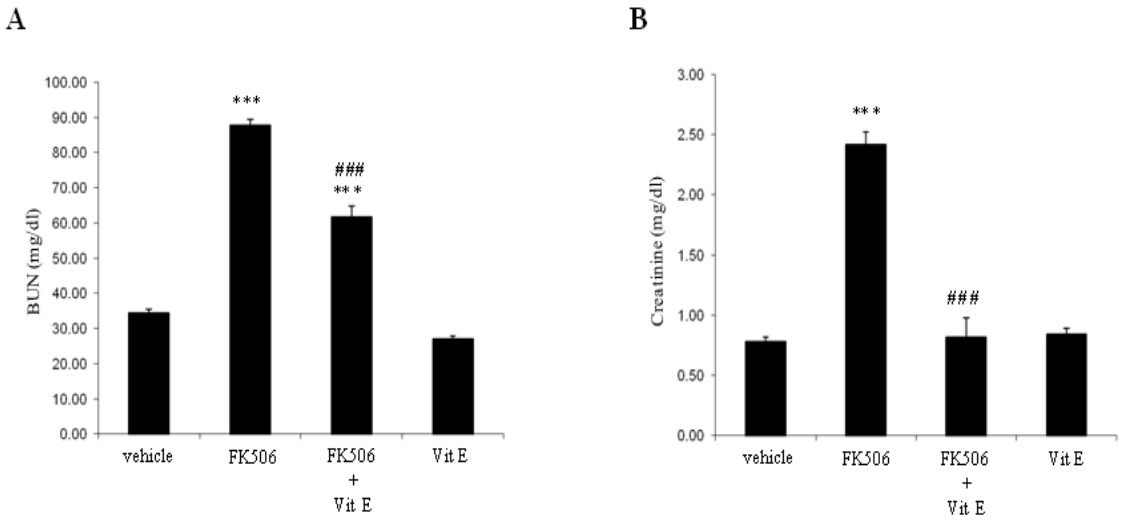

Fig. 1. Effects of FK506 and/or Vit E on BUN (A) and serum creatinine (B) in male Wistar albino rats

*Data represent means ± S.D. (n=6), *** p < 0.001 versus control, ### p < 0.001 versus FK506 alone-treated animals*

3.2.3 Effects of FK506 and/or Vit E on NF-κB Expression

NF-κB expression was highly induced in animals treated with FK506 alone. On the other hand, concomitant administration of Vit E along with FK506 significantly reduced the expression of NF-κB as compared to FK506 alone treated animals (Fig. 2C). NF-κB expression was not altered in animals treated with Vit E alone.

3.3 Effects of FK506 and/or Vit E on Endogenous Antioxidants

3.3.1 Effects of FK506 and/or Vit E on GSH Content

As shown in Fig. 3A, treatment of animals with FK506 significantly reduced the renal content of GSH. However, GSH level in animals treated with FK506 in combination with Vit E was highly increased compared to FK506 alone treated animals. Renal GSH content in rats treated with Vit E alone significantly increased compared with control group.

3.3.2. Effects of FK506 and/or Vit E on SOD Activity

Administration of FK506 produced a significant reduction in SOD activity. On the other hand, concomitant administration of Vit E along with FK506 significantly increased SOD activity as compared to FK506 alone treated group (Fig. 3B). SOD activity in rats treated with Vit E alone significantly increased compared to control group.

3.3.3 Effects of FK506 and/or Vit E on CAT activity

Treatment of animals with FK506 significantly reduced CAT activity. However, CAT activity in rats treated with FK506 in combination with Vit E was significantly increased as compared to FK506 alone treated animals (Fig 3C). No significant changes were observed in animals treated with Vit E alone.

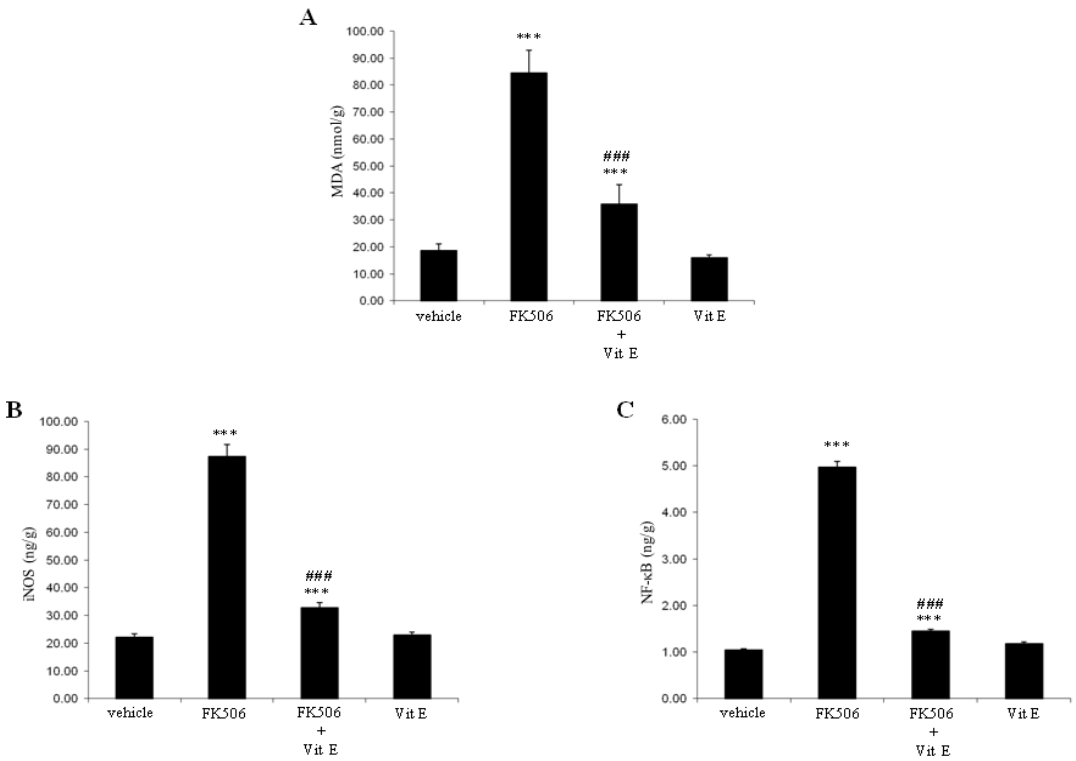

Fig. 2. Effects of FK506 and/or Vit E on renal content of MDA (A) as well as iNOS (B) and NF-κB Expression (C) in male Wistar albino rats

*Data represent means ± S.D. (n=6), *** p < 0.001 versus control, ### p < 0.001 versus FK506 alone-treated animals*

3.4 Histopathological Examination

As shown in Fig. 4, histopathological examinations showed sever damage of the renal tissue in FK506 alone-treated rats as indicated by necrotic damaged tubule & glomerular tufts segmentation (Fig. 4B). However, concomitant administration of Vit E along with FK506 improved the histopathological changes to a great extent as indicated by improvement in the tubular damage (Fig. 4C).

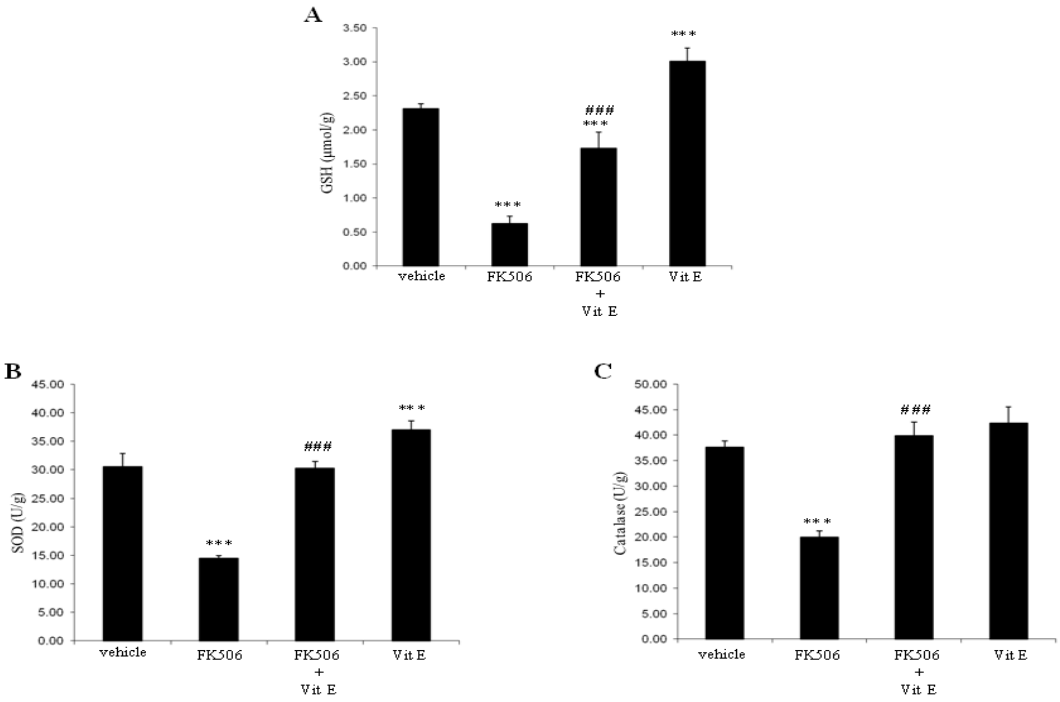

Fig. 3. Effects of FK506 and/or Vit E on renal content of GSH (A) as well as renal enzymatic activities of SOD (B) and CAT (C) in male Wistar albino rats

*Data represent means ± S.D. (n=6), *** p < 0.001 versus control, ### p < 0.001 versus FK506 alone-treated animals*

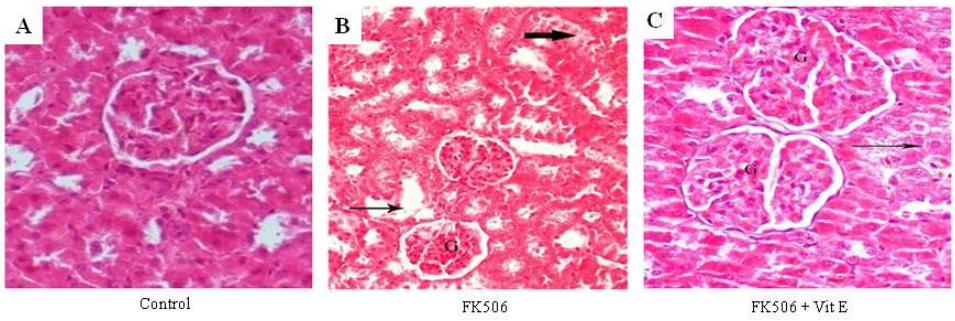

Fig. 4. A. A photomicrograph of a section of the kidney of a control rat showing the normal structure of the tissue (H&E 200). B. A photomicrograph of a section of the kidney of FK506 alone-treated rats showing sever damage of tubules, where some tubules show only dilatation of lumen (thin arrow), others show necrotic material in their lumen with degeneration of lining cells (thick arrow). The glomeruli show segmentation of their tuft of capillaries (G) (H&E 200). C. A photomicrograph of a section of the kidney of rats received FK506 + Vit E showing many tubules with vacuolated epithelium lining (thin arrow). The glomeruli show segmentation of their tuft of capillaries (G). In comparison to FK506 alone-treated group the necrotic tubules show improvement (H&E 200)

3.5 Effects of FK506 and/or Vit E on Serum Level of IL-2

To test whether the immunosuppressive effect of FK506 would also be affected in the presence of Vit E, serum IL-2 level was measured as a marker of the immunosuppressive efficiency of FK506 [13]. As shown in Fig. 5, treatment of animals with FK506 produced a significant reduction in the serum IL-2 level as expected. On the other hand, no significant changes were observed in rats treated with FK506 in combination with Vit E as compared to FK506 alone treated animals.

4. DISCUSSION

FK506 is one of the most efficient immunosuppressive agents that are commonly used in organ transplantation. However, the clinical use of FK506 is strongly limited by acute and chronic nephrotoxicity which remains a major clinical problem. Recently, it has been reported that over production of ROS and consequently oxidative stress plays a major role in FK506 (parent drug)-induced renal toxicity [4,5,14]. In 2012, Akool et al. [15] demonstrated that mitochondrial enzymes may represent the main sources of ROS induced by FK506 in the kidney. In addition, it was found that the antioxidant melatonin has the ability to protect against FK506-induced renal oxidative stress in rats [5]. In the present work, it was found that treatment of rats with FK506 resulted in a decline in renal function as indicated by significant increase in BUN and serum creatinine levels as well as histopathological changes. These data are in agreement with previous findings by several researchers who reported significant alteration in BUN and serum creatinine levels as well as histopathological changes following FK506 administration [16,17]. Interestingly, concomitant administration of Vit E along with FK506 improved the renal function as indicated by significant reduction in BUN and serum creatinine levels compared with FK506 alone-treated rats. Furthermore, administration of Vit E along with FK506 improved the histopathological changes to a great extent as indicated by improvement in the tubular damage. Previously, it has been demonstrated that FK506 generates ROS in renal cells [4,14]. Furthermore, alterations in renal function and structural damage have been shown to be associated with lipid peroxidation induced by FK506 [16,17]. In the present study, Vit E was found to attenuate lipid peroxidation induced by FK506 indicating that Vit E has the ability to exert cells protection against oxidative damage induced by FK506. The highly reactive peroxynitrite ($ONOO^-$) that is usually produced by the reaction between nitric oxide (NO) and superoxide (O_2^-) has been demonstrated to be involved in renal cellular damage [18]. Moreover, it has been reported that renal injury induced by FK506 is associated with the production of NO [5]. In the present work, it was observed that Vit E has the ability to inhibit the expression of iNOS (that catalyze the production of NO) induced by FK506 in renal tissues indicating that Vit E has the ability to exert cells protection against oxidative damage induced by FK506 via inhibition of iNOS expression and consequently NO level.

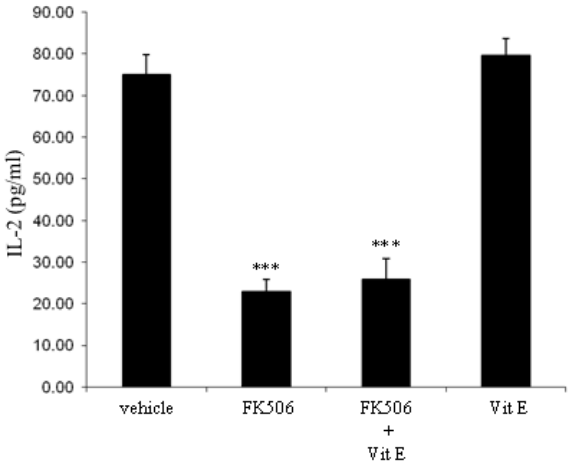

Fig. 5. Effects of FK506 and/or Vit E on serum level of IL-2 in male Wistar albino rats
*Data represent means ± S.D. (n=6), *** p < 0.001 versus control*

Furthermore, it was found that Vit E has the ability to inhibit FK506-induced NF-κB expression that plays an important role in the transcription of the inflammatory enzyme iNOS and other inflammatory genes in response to oxidative stress [19-21]. An efficient endogenous antioxidant defense system operates to scavenge the reactive oxygen species. The most important endogenous antioxidants are GSH, SOD and CAT [22-25]. In agreement with previous study [17], it was found that treatment of rats with FK506 produced a significant reduction in the endogenous antioxidants GSH, SOD and CAT. This reduction in oxygen radical scavenger system due to the toxic effects of FK506 leads to oxidative stress and renal injury. Interestingly, concomitant administration of Vit E along with FK506 significantly increased the renal level of GSH as well as SOD and CAT activities which play a major role in cells protection against oxidative damage. Moreover, the present work demonstrates that the immunosuppressive effect of FK506 was not affected in the presence of Vit E. In the present work, Vit E was found to protect against oxidative damage induced by FK506 as indicated by improvement not only in histopathological changes but also in renal function. These positive effects of Vit E were associated with an increase in oxygen radical scavenger system (endogenously produced) and decrease in the oxidative damage markers.

5. CONCLUSION

The findings of present work demonstrate that Vit E has the ability to protect against FK506-induced nephrotoxicity due to its ability to restore the balance between oxygen radical formation and the endogenous oxygen radical scavenger system which was disturbed by FK506 in renal tissue. These findings suggest that concomitant use of Vit E might be useful in reducing renal toxicity induced by FK506 via inhibition of ROS, iNOS, and NF-κB expression.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All procedures performed in this study were approved by the Institutional Animal Ethics Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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