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Evaluation of the Possible Protective Effect of Vitamin E on Nicotine-Induced Nephrotoxicity in Rats

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Abstract

The goal of the current work was to investigate whether vitamin E had any protective effects against nicotine-induced nephrotoxicity in rats. Thirty mature male rats, weighing 200–250 g, were placed into 3 groups at random. Rats in the control group obtained saline, whereas Nicotine group: rats supplemented with nicotine (0.8 mg/kg/day; s.c.). Vitamin E+ nicotine group: rats were co-administered vitamin E (100 mg/kg/day; orally) followed by nicotine. The experiment duration was 30 days. Exposure of rats to nicotine resulted in significant rise in serum urea and creatinine levels, serum total oxidant status, tumor necrosis Factor-α and kidney tissue nitric oxide, malondialdehyde and caspase-3 levels. Otherwise, significant decrease in total antioxidant capacity. Moreover, the kidney histopathology investigation revealed significant damage. In addition to significant decrease in rats' weight and increase in relative kidney weight. On the other hand, the supplementation of Vitamin E altered the biochemical parameters and the histopathological findings. The results demonstrated that supplementation of Vitamin E protects against nicotine- induced kidney oxidative stress, inflammation and apoptosis.

Keywords: Nicotine; vitamin E; nephrotoxicity; anti-oxidant; tumor necrosis factor-α; caspase-3.

1. Introduction

There is mounting evidence that smoking use seriously harm renal health and is among the factors contributing to chronic renal disease (CRD). The most prevalent harmful alkaloid in cigarette smoke is nicotine, which is mostly accountable for renal impairment **(Arany et al., 2011)**. After intake, nicotine reaches the blood and briskly redistributes throughout the body to different organs. Almost all nicotine is metabolized in the liver with the production of cotinine which is the primary metabolite of nicotine and used as a sign of its intake. The kidney is likely in charge of eliminating the cotinine that the liver produces **(Sobkowiak and Lesicki, 2013)**.

Nicotine raises oxidative stress level and promotes the formation of oxygen free radicals and lipid peroxidation responsible for the harmful effect on the kidneys **(Arany et al., 2016)**. Moreover, nicotine triggers the production of pro-inflammatory proteins e.g., nuclear factor kappa (NF-κB), which causes an inflammatory response. In renal tubular epithelial cells, nicotine produced apoptosis via causing the production of ROS and activation of the NF-κB signaling pathways. Furthermore, nicotine can increase urine osmolality and decrease free water clearance by directly promoting the release of antidiuretic hormone (ADH). Also, persisting exposure to nicotine decreased the glomerular filtration rate (GFR). Additionally, nicotine has proangiogenic potential,

which causes various types of glomerular damage **(Sudheer et al., 2008; Kim et al., 2016)**.

Vitamin E (lipid soluble antioxidant) has the power to stop or postpone the onset of chronic diseases brought on by free radicals by preventing lipid peroxidation and oxidative DNA damage in the cells. **Iranloye and Oludare (2011)** investigated that vitamin E boosted the antioxidant levels in the liver and heart in nicotine-treated rats and lowered the high levels of biochemical oxidative biomarkers.

So, this experiment was done to investigate the underlying mechanisms of nicotine- induced nephrotoxicity and to assess the protective effect of vitamin E on nicotine- induced nephrotoxicity in rats.

2. Materials and Methods

2.1. Animals

Mature male rats (average weight 200 –250 gm) were utilized in the experiment. They were acquired from animal house Faculty of Medicine, Sohag University. Animals were kept at 22-24ºC room temperature, in the normal light/dark cycle and were fed a common animal nutriment with free water access. The Institutional Animal Care and Use Committee (IACUC) Sohag University, Egypt authorised the experimental protocol (Sohag-IACUC approval protocol No.5/5/2023/01).

2.2. Drugs and chemicals

Vitamin E and Nicotine were utilized in the present study. They were acquired from Pharma Co. for pharmaceuticals and chemical industries, Egypt and Sigma Aldrich Company, England, respectively. Kits for measurement of kidney function tests gained from Egyptian Company for Biotechnology, Cairo, Egypt. Kits for measurement of total oxidant status (TOS), total antioxidant capacity (TAC), nitric oxide (NO) and malondialdehyde (MDA) levels were obtained from Biodiagnostic Company Pharmaceutical Industries, Egypt. Moreover, tumor necrosis Factor-alpha (TNF-α) and caspase-3 kits were obtained from Wuhan EIAab Science Co. Ltd (China) and Elabscience Co, Egypt, respectively.

2.3. Experimental design

Experimental animals from all groups were left to acclimatize for one week, and then were distributed into three groups of ten animals each. The experiment duration was 30 days.

Control group: rats received 0.5ml saline subcutaneously (s.c)

Nicotine group: rats received nicotine; 0.8 mg/kg/day dissolved in saline s.c. (Azab et al., 2022).

Vitamin E+nicotine group: rats received vitamin E; 100 mg/kg/day orally **(Oyeyemi et al., 2015)** followed by nicotine; 0.8 mg/kg/day dissolved in saline s.c.

All the animals were weighed after the completion of the experiment. The serum from blood samples was separated by centrifugation and was utilized for the measurement of kidney function tests, TOS, TAC and TNF-α. Each rat's two kidneys were removed and weighed; the right kidney was employed to measure the NO, MDA and caspase-3 levels. While the left kidney was used for histological examination.

2.4. Determination of relative kidney weight

Relative kidney weight (RKW) % = (Rt kidney weight+Lf kidney weight)/(Final body weight) × 100 **(Imafidon et al., 2016)**.

2.5. Assay of kidney function tests

Spectrophotometric measurements of serum urea and creatinine levels were performed using commercially available kits bought from the Egyptian Company for Biotechnology in Cairo, Egypt.

2.6. Assay of total oxidant status & total antioxidant capacity

Serum level of TOS and TAC were determined by a colorimetric method according to **Fossati et al. (1980)** and **Koracevic et al. (2001)**, respectively. Their absorbance was measured at 510 NM. TOS and TAC were expressed as mM/L.

2.7. Oxidative stress index calculation

As stated by **Harman (1956)**, oxidative stress index (OSI) was calculated using the following formula: (TOS/TAC) ×100.

2.8. Assay of tumor necrosis Factor-alpha

Enzyme-linked immunosorbent assay (ELISA) rat-specific kit was used for measurement of serum TNF-α, and it was expressed as pg/ ml.

2.9. Assay of nitric oxide and malondialdehyde

Levels of NO and MDA in kidney tissue were measured by a colorimetric method in kidney tissue homogenate according to **Montgomery and Dymock (1961)** and **Ohkawa et al. (1979)**. respectively. The sample's nitrite level was expressed as μmol/ g tissue. While MDA level was expressed as nmol/g tissue.

2.10. Assay of caspase 3

Kidney tissue caspase-3 was measured using ELISA kit. Caspase 3 level was expressed as ng/ g tissue.

2.11. Histopathological examination

Following fixation in 10% formal saline, parts of the right kidney tissues collected and dehydrated in ascending grades of alcohol concentrations, and then embedded in Paraffin wax. After that, light microscope examination of 5 m thick sections stained with hematoxylin and eosin (H&E) was performed **(Bancroft and Gamble, 2002)**.

2.12. Statistical analysis of data

Mean ± SE was used to express the values. Tukey post hoc analysis was carried out after the one-way analysis of variance test (ANOVA) to determine whether there were any differences between the groups. At $p<0.05$, differences were considered significant. SPSS program, software package (version 26) was used in the analysis.

3. Results

3.1. Effect of vitamin E on body weight and relative kidney weight

Nicotine administration produced significant ($p<0.05$) decrease in rats' body weight compared to the control group. Moreover, vitamin E administration produced significant ($p<0.05$) increase in rats' body weight compared to nicotine group **(Table 1)**. As regard RKW nicotine group displayed a significant ($p<0.05$) elevation in comparison to the control group. However, vitamin E + nicotine group showed significant ($p<0.05$) decrease compared to nicotine group **(Table 1)**.

3.2. Effect of vitamin E on kidney function

As shown in **Table 2**, serum urea and creatinine showed significant ($p<0.05$) rise in nicotine group in comparison to the control group. Whereas, vitamin E + nicotine group showed significant ($p<0.05$) diminution in serum urea and creatinine compared to nicotine group.

3.3. Effect of vitamin E on total oxidant status & total antioxidant capacity

Total oxidant status in nicotine group raised significantly ($p<0.05$) in comparison to the control group. However, vitamin E + nicotine group produced significant dwindling ($p<0.05$) in serum TOS in comparison to the nicotine group **(Table 3)**.

Table 1. Body weight, kidney weight and RKW of different studied groups

Parameter	Control	Nicotine	Vitamin E + Nicotine
Body weight (g)	232 ± 5.33	186* ± 4.52	228• ± 11.23
Kidney weight (g)	1.01 ± 0.04	1.62* ± 0.09	1.12• ± 0.07
RKW	0.44 ± 0.02	0.87* ± 0.05	0.50• ± 0.04

Mean ± SE of 10 observations. RKW= Relative kidney weight. * $p< 0.05$ vs the control group. • $p< 0.05$ vs the nicotine group.

Table 2. Kidney function tests of different studied groups

Parameter	Control	Nicotine	Vitamin E + Nicotine
Urea (mg/dl)	42.9 ± 1.89	55.9* ± 2.09	44.7• ± 1.21
Creatinine (mg/dl)	0.90 ± 0.04	2.08* ± 0.07	0.98• ± 0.06

Mean ± SE of 10 observations. * $p< 0.05$ vs the control group. • $p< 0.05$ vs the nicotine group.

Table 3. Serum TOS, TAC and OSI of different studied groups

Parameter	Control	Nicotine	Vitamin E + Nicotine
TOS (mM/L)	0.335 ± 0.029	0.736* ± 4.52	0.382• ± 0.024
TAC (mM/L)	2.62 ± 0.195	0.9* ± 0.043	2.03• ± 0.105
OSI	13.77 ± 1.89	84.12* ± 6.53	19.03• ± 1.29

Mean ± SE of 10 observations. TOS= Total oxidant status, TAC= Total antioxidant capacity, OSI= Oxidative stress index. * p< 0.05 vs the control group. • p< 0.05 vs the nicotine group.

Total antioxidant capacity in nicotine group was significantly (p<0.05) decreases compared to the control group. Whereas, in vitamin E+nicotine group it exhibited a significant (p<0.05) elevation compared to nicotine group **(Table 3)**.

3.4. Effect of vitamin E on oxidative stress index

Table 3 showed significant (p<0.05) increase in OSI in nicotine group in comparison to the control group. Treatment of the rats with vitamin E + nicotine produced significant (p<0.05) decrease in OSI compared to in nicotine group.

3.5. Effect of vitamin E on tumor necrosis Factor-α

Tumor necrosis Factor-α level exhibited a significant (p<0.05) elevation in nicotine group in comparison to the control group. On the other hand, vitamin E + nicotine group exhibited a significant (p<0.05) decrease in TNF-α compared to in nicotine group **(Fig. 1)**.

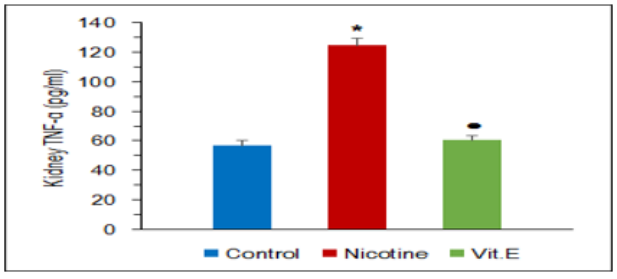

Figure 1. Serum tumor necrosis factor-α of different studied groups. Mean± SE of 10 observations. TNF-α = tumor necrosis factor-α.* p< 0.05 vs the control group. • p< 0.05 vs the nicotine group.

3.6. Effect of vitamin E on NO and MDA

Nicotine group revealed a significant (P<0.05) rise in kidney NO level in comparison to the control group. Whilst, vitamin E + nicotine group revealed a significant (p<0.05) reduction in kidney NO level in comparison to nicotine group **(Fig. 2).**

Malondialdehyde significantly (p<0.05) increased in nicotine group compared to the control group. While, it significantly (p<0.05) decreased in vitamin E + nicotine group compared to nicotine group **(Fig. 3)**.

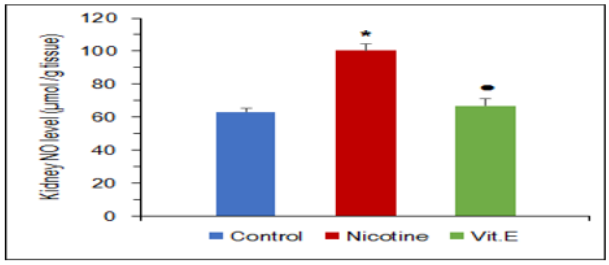

Figure 2. Kidney NO level of different studied groups. Mean± SE of 10 observations. NO = nitric oxide. * p< 0.05 vs the control group. • p< 0.05 vs the nicotine group.

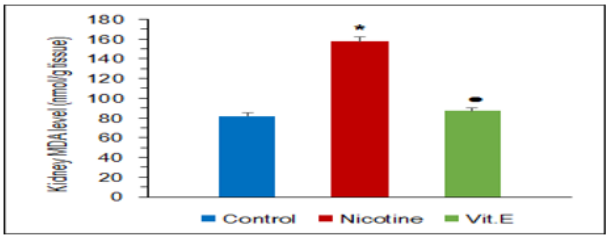

Figure 3. Kidney MDA level of different studied groups. Mean± SE of 10 observations. MDA = malondialdehyde. * p< 0.05 vs the control group. • p< 0.05 vs the nicotine group.

3.7. Effect of vitamin E on caspase-3

As regard caspase-3 level, nicotine group expressed a significant (p<0.05) elevation in kidney caspase-3 level in comparison to the control group. Nevertheless, compared to the nicotine group, the vitamin E + nicotine group significantly (p<0.05) decreased kidney caspase-3 level **(Fig. 4)**.

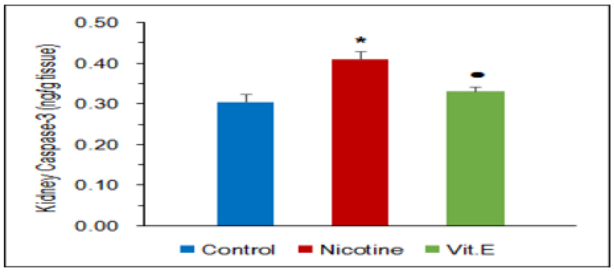

Figure 4. Kidney caspase-3 level of different studied groups. Mean± SE of 10 observations. * p< 0.05 vs the control group. • p< 0.05 vs the nicotine group.

3.8. Histopathological changes

Kidney section of control group showed the renal cortex contains the renal corpuscle and the renal tubules. The renal corpuscle is formed of renal glomeruli surrounded by the Bowman's capsule which is formed of parietal and visceral layers. The proximal tubules (P.T) are lined with cuboidal cells with deeply acidophilic cytoplasm and rounded vesicular nuclei. The apical cell membranes have brush borders. The distal tubules (D.T) exhibit wide lumen and are lined with low cuboidal cells with rounded vesicular nuclei with no brush borders **(Fig 5A)**. Otherwise, kidney of nicotine group showed that, the renal corpuscle had dilated urinary space, congestion of the glomerular capillaries and glomerular separation. Most of the proximal tubules showed dilated lumen and degenerated brush borders. Some proximal and distal tubular cells had deeply stained pyknotic nuclei. Interstitial tissue showed inflammatory cell infiltration **(Fig 5B)**. Examination of kidney sections of vitamin E+nicotine group showed an apparent decrease in the urinary space with less congested glomerular capillaries and glomerular separation compared to nicotine group. Some proximal and distal tubules restored their normal shape. Some proximal and distal tubular cells still had pyknotic nuclei **(Fig 5C)**.

4. Discussion

Smoking is a significant risk factor that has the unique ability to make renal impairment worsened **(Orth and Hallan, 2008)**. Both males and females who smoke cigarettes are at an increased risk of developing chronic kidney disease or passing away from end-stage renal disease **(Ishani et al., 2006)**. Smoking greatly accelerates the progression of renal disease by causing microvascular injury and hastening glomerulosclerosis. Smoking also seriously affects kidney structure and function **(Ramalingam et al., 2019)**. Over 4000 different substances are found in typical cigarette smoke, including carbon monoxide, reactive oxygen species, ketones, and stable reactive aldehydes **(Huang et al., 2005)**. Nicotine is one of the biologically stable and active substances found in tobacco that is easily absorbed through both active and passive smoking, and is responsible for the addictive effect of smoking. Moreover, it plays a significant role in the etiology of many diseases, including kidney disease. Nicotine exposure through chronic cigarette smoking appears to be the reason that hasten the microvascular complications. Furthermore, nicotine downregulates eNOS, which is a first step in the pathophysiology of kidney injury, and causes vascular endothelial dysfunction in rats. Additionally, smoking has been shown to exacerbate nephropathy by triggering hyperlipidemia and high-grade oxidative stress **(Balakumar et al., 2009; Chakkarwar, 2011; Ben Saad et al., 2019; Zhang et al., 2019)**.

Accordingly, the current research was prepared to assess the underlying mechanisms of nicotine-induced nephrotoxicity. Moreover, to assess the protective effect of vitamin E on nicotine- induced nephrotoxicity in rats. It was observed in the present study that giving rats nicotine reduced their appetite, which most likely caused them to weigh less than controls. Contrary significant increase in RKW in nicotine group compared to the control group can be attributed to the reduction of body weight and increased kidneys weight which may results from edema of the kidneys due to nicotine-induced tubular necrosis. Moreover, nicotine administration produced significant rise in serum urea and creatinine levels and pathological changes in glomeruli, suggesting that renal impairment was brought on by nicotine exposure. Our results are in agreement with the results of **Ibrahim et al. (2016)** and **Azab et al. (2022)**. According to **Ahmed et al. (2015)**, smokers' urea and creatinine levels were considerably higher than those of the control group that might be due to increase renovascular resistance as a results of cigarette smoking. Increased renovascular resistance significantly decrease GFR, filtration fraction, and renal plasma blood. Moreover, the decrease in GFR will cause the distal tubular flow rate to drop, which will raise

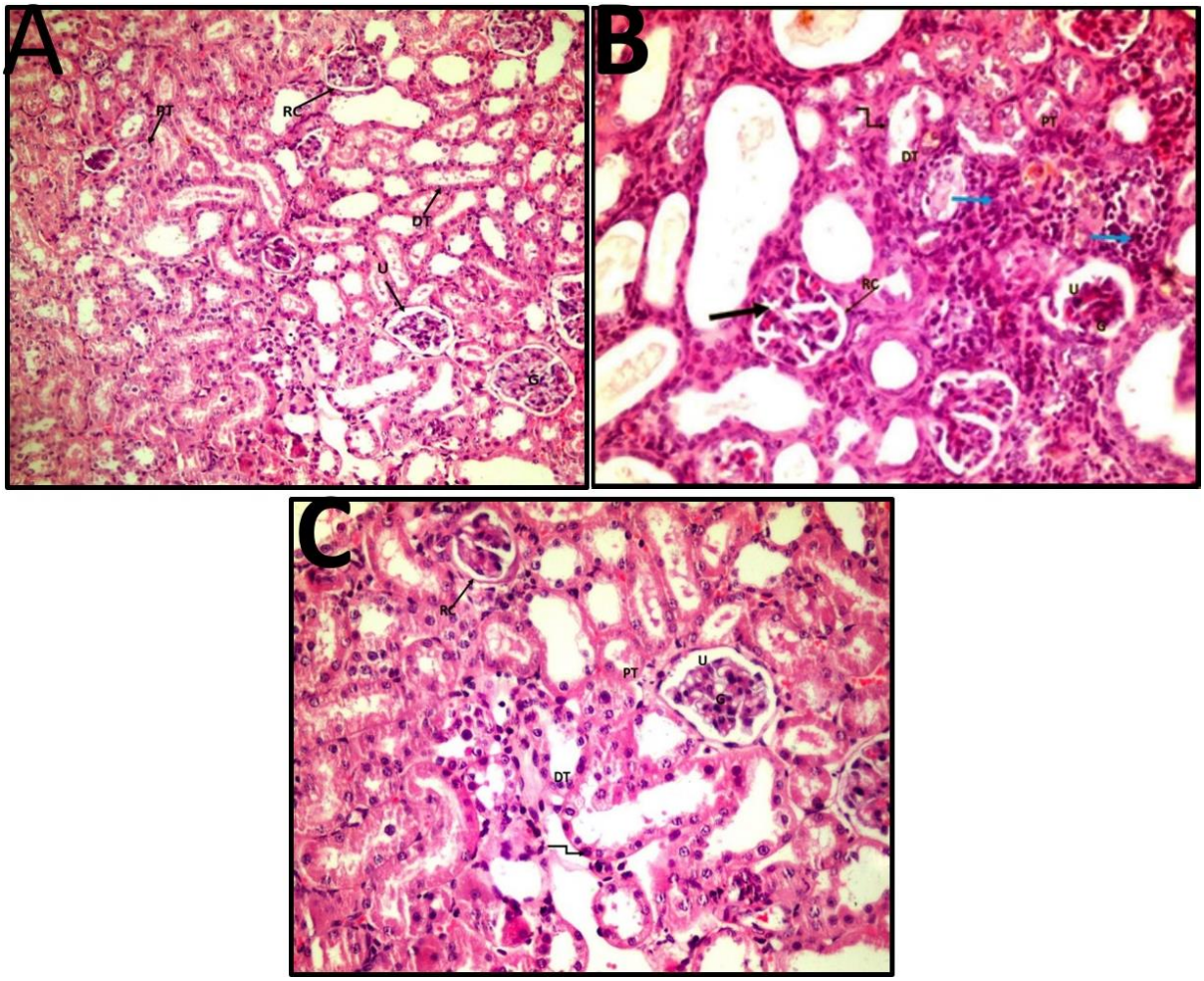

Figure 3. Kidney histopathological changes. (A) Photomicrograph of the control rats showing; the renal corpuscle (RC), glomerular capillaries(G), urinary space (U), proximal tubule (P.T), and distal tubule (D.T), **(B)** the rats treated with nicotine showing renal corpuscle (RC), congestion of glomerular capillaries (G) with glomerular separation (thick arrow) and widening of urinary space (U). Some P.T and D.T showed pyknotic nuclei (curved arrow) with loss of brush border of P.T. Note inflammatory cell infiltration in the interstitial tissue (blue arrow), **(C)** the rats treated with nicotine and vitamin E showing renal corpuscle (RC) with less glomerular (G) separation and congestion with decrease of urinary space (U) compared to group II. Some proximal tubules (P.T) and distal tubules (D.T) still showed pyknotic nuclei (curved arrow). Interstitial tissue showed less inflammatory cell infiltration compared to group II. (H&E, **A**: x200, **B** and **C**:x400)

urea reabsorption **(JoAnn and Robert, 2011)**. The kidneys easily filter creatinine, a non-protein waste product, and because the serum creatinine level depends on the GFR, it is regarded as a sign of renal dysfunction **(Zhang et al., 2019)**.

Oxidative stress almost always related to organ toxicity, in the present study administration of nicotine produced significant increase in serum TOS and TNF-α levels, kidney tissue MDA, NO and caspase-3 levels with significant decrease in serum TAC compared to the control group. Moreover, it increases OSI compared to the control group. Numerous investigations have demonstrated that nicotine has an oxidative stress potential and mitochondrial ROS release damages organs and tissues **(Arany et al., 2011; Mohamed et al., 2022)**. Furthermore, **Mohamed et al. (2022)** reported that nicotine-induced oxidative damage together with its antioxidant suppression have been found to exacerbate tubular necrosis in rats' renal tissue and might be the cause of increased caspase-3 level in kidney tissue. The increased serum level of TNF-α by nicotine administration may be due to induction of proinflammatory cytokines and the overexpression of their genes. These cytokines can stimulate leukocytes to generate additional

chemokines and pro-inflammatory cytokines, which will aggravate the inflammation and is the cause of inflammatory kidney state. TNF-α also activates the death receptor pathway, which leads to caspase-mediated apoptosis **(Sun et al., 2013)**. The present biochemical abnormalities are confirmed by histopathological abnormalities in the renal tissue in the form of dilated urinary space in renal corpuscle, congestion of the glomerular capillaries and glomerular separation. Most of the proximal tubules showed dilated lumen and degenerated brush borders. Some proximal and distal tubular cells had deeply stained pyknotic nuclei. And inflammatory cell infiltration in the interstitial tissue. These results are in accordance with the results of **(Menshawy et al., 2019; Azab et al., 2022; Mohamed et al., 2022)**.

The present study revealed that concurrent treatment with vitamin E protected from nicotine-induced nephrotoxicity by improvement of body weight and RKW. In addition to significant decrease in serum urea and creatinine levels. Also, there were effective restoration of serum TOS, TAC and TNF-α levels. Moreover, kidney MDA, NO and caspase-3 levels were significantly decreased. The restoration of the renal architecture under a light microscope provides proof for this conclusion. The renal protective impacts of vitamin E might be allocated to its antioxidant and free radicals scavenging properties. These findings are in accordance with **(Nematbakhsh et al., 2012; Abo-Elmaaty et al., 2020; Erdemli et al., 2020)**. Moreover, the anti-apoptotic activity of vitamin E was verified by a significant reduction of kidney tissue caspase-3 level. This is might be because vitamin E effects on the prevention of mitochondrial oxidative stress and the prevention of mitochondrial release of apoptotic signaling molecules into the cytoplasm **(Weber et al., 2003)**. Furthermore, the radical scavenging ability of vitamin E may be the cause of its anti-inflammatory effects. Likewise, the anti-inflammatory effects of vitamin E could be explained by suppression of NF-κB protein expression during the inflammatory process **(Sarir et al., 2015)**.

5. Conclusion

The current study confirms nicotine-induced nephrotoxicity in rats based on the current biochemical findings, which are substantiated by histological data. This toxic effect is due to induction of oxidative stress, inflammatory mediators and apoptotic pathways. Treatment of the rats with vitamin E protects against this nephrotoxic effects.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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