

## Review

# Effect of Sodium Selenite and Vitamin E on the Renal Cortex in Rats: An Ultrastructure Study



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## ABSTRACT

This study examined the use of vitamin E to alleviate toxic effects of sodium selenite. Adult male albino rats ( $n = 50$ ) was divided into five groups. Group 1 was control, Groups 2 and 4 were treated with sodium selenite (2 mg/kg) for 2 and 4 weeks, respectively, Groups 3 and 5 were treated with sodium selenite (2 mg/kg) and vitamin E (100 mg/kg) for 2 and 4 weeks, respectively. Renal tissues were studied using anti-BCL2 and examined ultrastructurally. Positive Bax immunoreactivity was detected after 2 and more positive after 4 weeks and nearly all groups improved with co-administration of vitamin E. Ultrastructural study revealed lesions in Bowman's capsule and proximal convoluted tubules. The submicroscopic study revealed damage and necrosis of cortical structures after 2 and 4 weeks, respectively. After 4 weeks, cellular changes were seen, such as vacuolation and moderate degeneration of cells, widening of the urinary space scattered through the cortex with loss of cellular details, formation of apical buds, degeneration, and cellular rupture. Present findings disclosed an ameliorative effect of adding vitamin E to sodium selenite-induced changes in cortical tissues. Clinically, it is advised to add vitamin E to avoid selenium overdose hazards.

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## 1. Introduction

Selenium is a critical trace element for human health, potent antioxidant, and increasingly recognized for its clinical significance in various pathophysiologic conditions (Tos-Luty et al., 2003). Humans and domestic animals acquire selenium mainly from the soil–plant–water system in which plants are an immediate source of the mineral (Tian et al., 2005). As a chain breaking, soluble lipid, vitamin E is an efficient antioxidant that has a well-known protective role against a wide range of human diseases (Niki and Noguchi, 2004).

Vitamin E and selenium complement each other in their role as antioxidants. Vitamin E inhibits free radical production, whereas selenium, as a constituent of glutathione peroxidase, neutralizes the free radicals present in the biological matrix (Naziroglu et al., 2004). Thus, vitamin E and selenium have a sparing effect on each other (Tiwary et al., 2006). Vitamin E is thought to be involved

in an unlimited range of physiological and biochemical functions. The molecular mechanism of these functions could be mediated by either antioxidant activity or by its action as a membrane stabilizer, as its basic function is to protect all membrane lipids and unsaturated fatty acids against oxidative degradation (Wang and Quinn, 2000).

Daily required selenium for adults is 200 µg/day. The upper limit of the tolerable intake of selenium is 400 µg/day, as determined by the Food and Nutrition Board of the Institute of Medicine based on the avoidance of hair and nail brittleness and loss and initial signs of chronic selenium toxicity (Nuttall, 2006). The toxicity occurs when a human is exposed to excess consumption, overdose through selenium medications, or excessive and unregulated use of supplementation. The most common reported symptoms are gastrointestinal disturbances, garlic odor in the breath, lethargy, irritability, and nervous irregularities. However, extreme cases can lead to hepatic cirrhosis, edema of the lung, and death (Reid et al., 2004). Selenium can exert a pathogenic effect either in deficiency or in excess (i.e., excess of this chemical may lead to changes similar to those observed in its deficiency). These changes are caused by free radical degeneration, inhibition of synthesis of proteins, and intensification of lipid peroxidation (El-Demerdash, 2004). Inorganic selenium as sodium selenite ( $\text{Na}_2\text{SeO}_3$ ) is frequently used, along with vitamin E, for supplementation in animals identified

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with selenium deficiency or in animals living in selenium deficient areas (Hemingway, 2003).

The toxic outcomes of selenium in vitro, despite being non-specific, are avoided by the presence of vitamin E (Beyrouly and Chan, 2006). Supra-nutritional doses of vitamin E can reduce lipid peroxidation and mortality in selenite-treated animals (Saito et al., 2003). Therefore, the aim of this study is to determine if vitamin E should be taken daily alongside selenium to alleviate the toxicity of selenium overdose.

## 2. Material and methods

### 2.1. Chemicals

All chemicals, including vitamin E ( $\alpha$ -tocopherol) and sodium selenite (Na-selenite:  $\text{Na}_2\text{SeO}_3$ ) used in this study were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).

### 2.2. Animals

Adult male Wistar rats ( $n=50$ ), weighing 200–225 g, were obtained from the Experimental Animal Laboratory, King Abdulaziz University. They were housed under standard lightening and relative humidity ( $50 \pm 15\%$ ) conditions in a temperature-controlled room ( $25 \pm 2^\circ\text{C}$ ). The animals were allowed to acclimatize to the laboratory environment for 10 days, and then they were allowed free access to standard dry pellet diet and water ad libitum.

### 2.3. Experimental design

The animals were randomly separated into five polypropylene cages of ten individuals each and were administered 0.5 ml of treatment solution via oral gavage. Group 1 (control) received a daily dose of corn oil for a period of 4 weeks. Groups 2 and 4 were given daily doses of sodium selenite at a dose of 2 mg/kg body weight diluted in 0.5 ml distilled water for periods of 2 and 4 weeks, respectively. Groups 3 and 5 were given daily doses of sodium selenite at a dose of 2 mg/kg body weight diluted in 0.5 ml distilled water and 0.5 ml vitamin E (100 mg/kg body weight) dissolved in corn oil orally for periods of 2 and 4 weeks, respectively.

Sodium selenite (2 mg/kg body weight) and vitamin E (100 mg/kg body weight) doses were chosen according to previously published data (Turan et al., 2003; Yilmaz, 2005) and the dose of vitamin E was previously shown to be effective in lowering toxicity (El-Demerdash, 2004). The chemicals were administered in the morning (between 8:00 AM and 9:00 AM) to non-fasted animals. At 2 and 4 weeks after treatment, the rats were sacrificed and the kidneys were removed, collected and weighed, and sampled for histopathological examination.

King Abdulaziz University's Faculty of Medicine Hospital Biomedical and Research Ethics Committee approved the study protocol. The general guidelines for the use and care of living animals in scientific investigations from the National Institutes of Health (NIH Publication No. 80-23) were followed. All experiments reported here complied with current laws and regulations of King Abdulaziz University, Saudi Arabia, on the care and handling of experimental animals.

### 2.4. Histological and morphometric evaluation

Renal samples were dissected longitudinally. Then they were fixed in 10% neutral buffered formalin, dehydrated in ascending grades of alcohol, and embedded in paraffin wax. Next, paraffin

sections (5  $\mu\text{m}$  thick) were stained with hematoxylin and eosin stain for routine histological inspection (Ibrahim et al., 2011). The sections were viewed and photographed with an Olympus light microscope (Olympus BX53, Tokyo, Japan) and an attached photograph machine (Olympus E-330, Olympus Optical Co. Ltd.). Images were analyzed using Image-Pro Plus v4.5 (Media Cybernetics, Shanghai, China).

### 2.5. Immunohistochemistry using an anti-Bax antibody

Overexpression of Bax protein accelerates apoptotic cell death (apoptosis inducers). Immunohistochemical detection of Bax and determination of the expression level were achieved using a mouse monoclonal anti-Bax antibody and avidin–biotin complex staining as described by the manufacturer.

Sections were cut into 4  $\mu\text{m}$  slices and then fixed in a 65  $^\circ\text{C}$  oven for 1 h. Trilogy (Cell Marque, CA, USA, Cat# 920p-06) is a product that combines the 3 pretreatment steps: deparaffinization, rehydration, and antigen unmasking. Using this product enhances standardization of the pretreatment procedure, thereby, producing more consistent and reliable results. The slides were placed in a Coplin jar filled with 200 ml of the trilogy working solution, and the jar was securely positioned in an autoclave (Morsy and El-Moselhy, 2013). The autoclave was adjusted so that the temperature reached 120  $^\circ\text{C}$  and maintained this temperature for 15 min, after which the pressure was released and the Coplin jar was removed to allow the slides to cool for 30 min. Sections were then washed and immersed in Tris-buffered saline (TBS) to adjust the pH; this step was repeated between each step of the immunohistochemistry procedure.

The endogenous peroxidase activity was determined by immersing the slides in 3% hydrogen peroxide for 10 min. The Power-Stain™ 1.0 Poly HRP (Horseradish Peroxidase) DAB (Diaminobenzidine) Kit Cat# 54-0017 (Genemed Biotechnologies, CA, USA) was used to visualize any antigen-antibody reaction in the tissues (for qualitative detection of antigen). The primary antibody used for Bax immunohistochemical staining (Bax Cat # orb4655, Biorbyt, Cambridge, United Kingdom) was diluted 1:500, and 2–3 drops were applied. The slides were then incubated in the humidity chamber overnight at 4  $^\circ\text{C}$ . Subsequently, polyclonal HRP-linked antibody conjugates were applied to each slide and incubated for 20 min. The DAB chromogen was prepared, and 2–3 drops were applied to each slide and incubated for 2 min. The DAB stain was rinsed off, counterstaining was performed with Mayer hematoxylin, and then a cover slip was attached before the slides were examined under a light microscope. The cells that displayed brown precipitation were considered positive for Bax expression (Albamonte et al., 2013).

### 2.6. Electron microscopy

Renal samples were cut into small pieces (1 mm<sup>3</sup>) and fixed in 2.5% glutaraldehyde (pH 7.4) in phosphate buffer for 2 h at room temperature. Post-fixation was performed in the same phosphate buffer containing 1% osmium tetroxide ( $\text{OsO}_4$ ). The tissues were dehydrated in graded ethanol solutions, transferred to propylene oxide, and finally embedded in Epon 812. Semithin sections (1  $\mu\text{m}$  thick) were cut using a glass knife and stained with toluidine blue. Ultrathin sections were obtained using LKB ultratome (Ultratome NOVA, LKB 2188, Bromma, Sweden) and spread on copper grids. The sections were stained with uranyl acetate followed by lead citrate and examined at 80 kV with a transmission electron microscope (JEM-2000EX; JEOL, Tokyo, Japan) (Hirose et al., 2012).

**Table 1**  
Body weights of rats treated with sodium selenite.

Treatment duration	Body weight (g)			Kidney weight (g)		
	Control group (n = 10)	Sodium selenite group (n = 10)	Sodium selenite and vitamin E group (n = 10)	Control group (n = 10)	Sodium selenite group (n = 10)	Sodium selenite and vitamin E group (n = 10)
1st day	196.10 ± 1.101	196.10 ± 1.101	196.10 ± 1.101	–	–	–
2 weeks	199.40 ± 0.699	194.50 ± 0.527 <i>P</i> < 0.0001	199.00 ± 0.816 <i>P</i> < 0.207	0.628 ± 0.002	0.619 ± 0.001 <i>P</i> < 0.0001	0.626 ± 0.003 <i>P</i> < 0.128
4 weeks	209.50 ± 0.527	204.10 ± 0.876 <i>P</i> < 0.0001	208.60 ± 0.516 <i>P</i> < 0.005	0.648 ± 0.002	0.639 ± 0.001 <i>P</i> < 0.0001	0.646 ± 0.003 <i>P</i> < 0.128

Values are expressed as a mean ± standard deviation. The analysis was made using one-way ANOVA test (LSD).

**Table 2**  
Comparison of the mean values of kidney histological parameters (µm) of the experimental groups with the control group.

Groups	Renal corpuscle	Urinary space	PCT
Control group	82.96 ± 7.83	9.54 ± 2.26	39.28 ± 3.24
Group receiving sodium selenite for 2 weeks	65.59 ± 8.04 ** <i>P</i> < 0.01	7.11 ± 2.28	35.11 ± 2.89
Group receiving sodium selenite for 4 weeks	54.66 ± 9.33 *** <i>P</i> < 0.001	7.4 ± 2.35	18.63 ± 1.66 *** <i>P</i> < 0.001
Group receiving sodium selenite + vitamin E for 2 weeks	70.52 ± 12.70 * <i>P</i> < 0.05	6.34 ± 1.47 ** <i>P</i> < 0.01	36.3 ± 3.49
Group receiving sodium selenite + vitamin E for 4 weeks	60.81 ± 9.34 * <i>P</i> < 0.05	5.64 ± 1.54 *** <i>P</i> < 0.001	21.85 ± 2.78 *** <i>P</i> < 0.001

Values are expressed as a mean ± standard deviation. \**P* < 0.05 differences significant. \*\**P* < 0.01 differences considerably significant. \*\*\**P* < 0.001 difference very significant. For statistical significance, experimental groups have been compared to their respective control group.

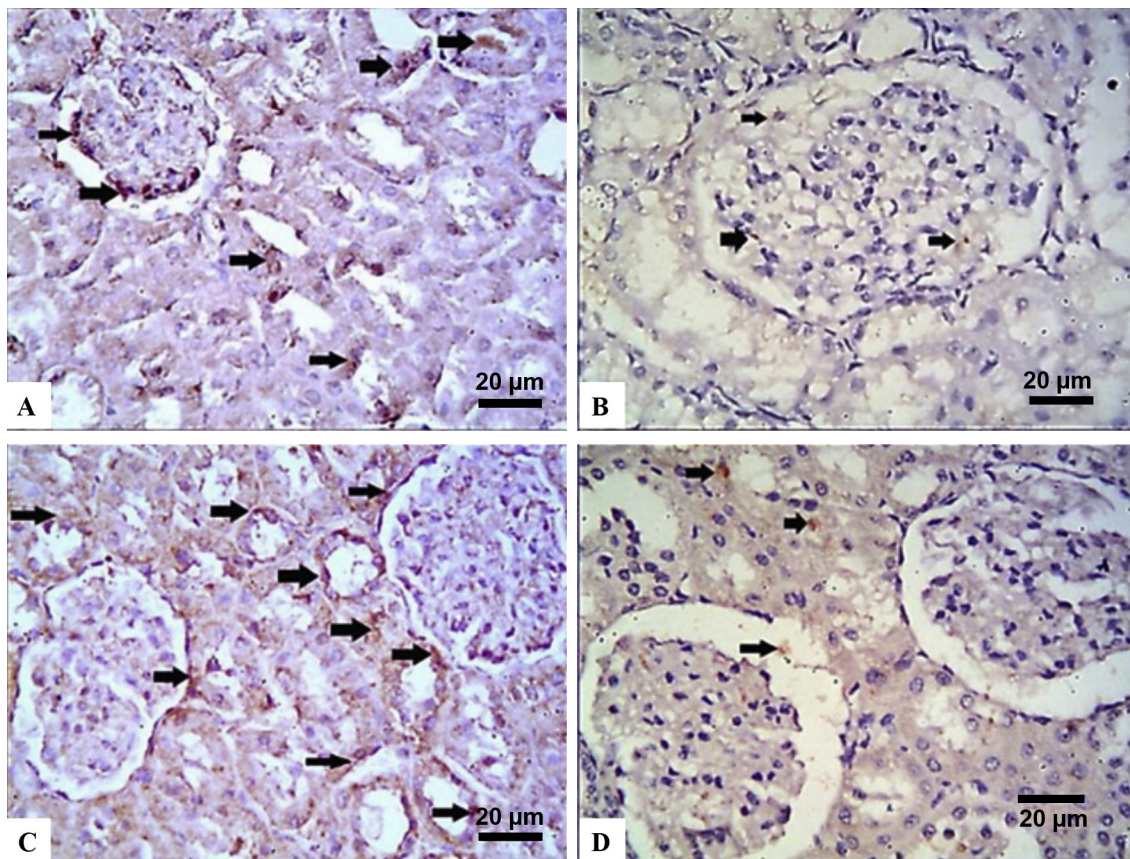
## 2.7. Statistical analysis

Data are expressed as a mean ± standard deviation. Statistical analysis of all data was performed using SPSS for Windows (Version 22; SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used, followed by post hoc analysis using LSD test. Values were considered statistically significant when the *p*-value was less than 0.05.

## 3. Results

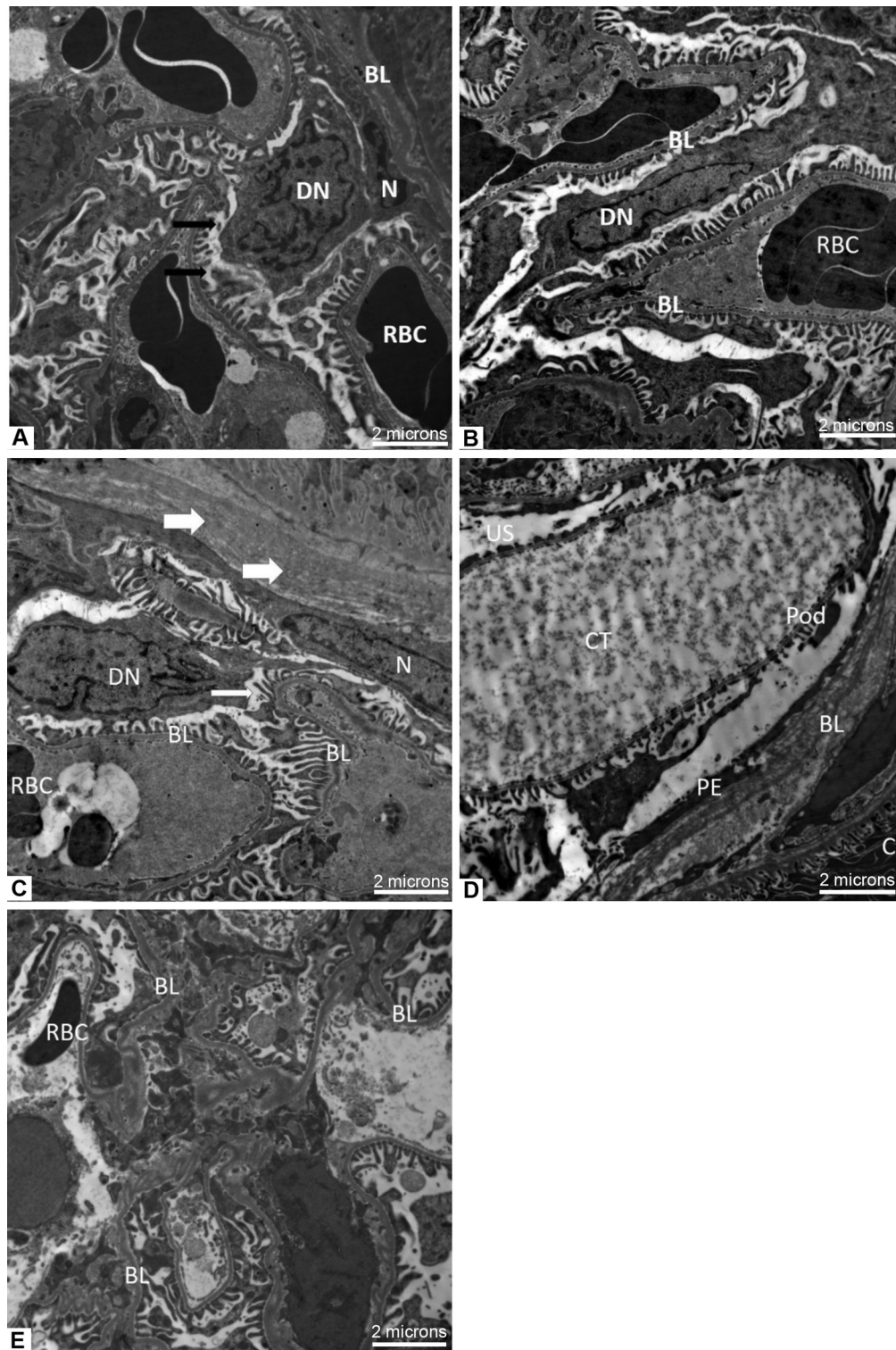
### 3.1. Effect of sodium selenite on rat general health

In sodium selenite-treated groups, few clinical signs, such as reduced activity, cumulative weakness, petty diarrhea, and hair loss, were observed. Death was not observed in any of the experimental groups during the treatment period (4 weeks).

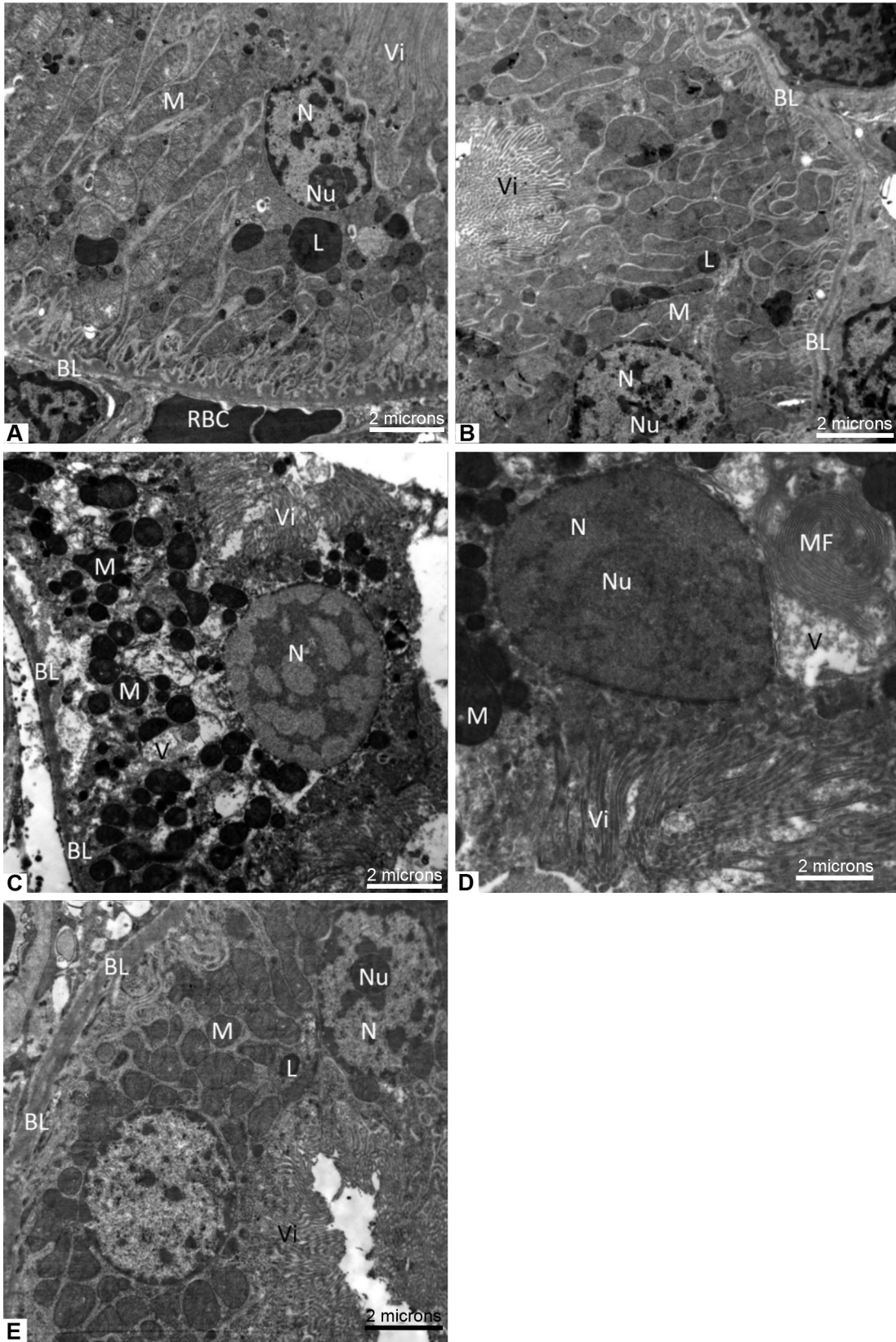


**Fig. 1.** Immunohistochemical Bax staining. (A) Positive Bax immunoreactivity in the kidney of the groups treated with sodium selenite 2 mg/2 week. (B) Faint Bax immunoreactivity in the kidney groups treated with sodium selenite 2 mg/2 week and vitamin E. (C) Strong positive Bax immunoreactivity in the kidney of the groups treated with sodium selenite 2 mg/4 week. (D) Weak Bax immunoreactivity in kidney groups treated with sodium selenite 2 mg/4 week and vitamin E. (Bax immunostaining 200×).





**Fig. 2.** (A) An electron micrograph of a section of the group treated with sodium selenite 2 mg/2 week showing the Bowman's capsule of the glomeruli formed by hypertrophied basal lamina (BL) with narrowing of the urinary space and flattened, elongated parietal epithelium (N). The urinary space is narrow or nearly absent (thin black arrows), and the capillary tuft is moderately congested Red Blood Corpuscles (RBC). Note: The visceral epithelium shows dentate nucleus (DN). (B) An electron micrograph of a section of the kidney of the group treated with sodium selenite 2 mg/kg and vitamin E showing the glomerular tuft with apparent congestion of the capillaries (RBC) and the Bowman's capsule showing thickening of the basal lamina (BL) with improved urinary space narrowing and hypertrophy of the podocyte. Note: The visceral epithelium shows dentate nucleus (DN). (C) An electron micrograph of a section of the group treated with sodium selenite 2 mg/4 week showing the Bowman's capsule greatly thickened basal lamina (thick white arrows) and parietal epithelium flat and elongated (N). The urinary space is greatly reduced or nearly absent (thin white arrows) and the capillary tuft (RBC) is congested with obvious moderate thickening of the basal lamina (BL) and hypertrophy of the podocyte. Note: The visceral epithelium shows dentate nucleus (DN). (D) An electron micrograph of a section of the group treated with sodium selenite 2 mg/4 week showing dilation of the capillary tuft (CT) and filled with plasma and the shrinkage and atrophy of the podocyte (Pod). Shrinkage and condensation of the cytoplasm of the parietal epithelium (PE) includes thickening of the Bowman's capsule basal lamina (BL), narrowing of the urinary space (US), and numerous invaginated corrugated membrane profiles (C) at the base. (E) An electron micrograph of a section of the group treated with sodium selenite 2 mg/4 week and vitamin E showing the Bowman's capsule with apparently moderate thickening of the basal lamina (BL) with seemingly improvement of the urinary space narrowing and less congestion of the capillary tuft (RBC). The size of the mesangial cell and matrix are reduced.





### 3.2. Evaluation of body and kidney weights

After 2 and 4 weeks, the body weights in the sodium selenite-treated groups were significantly lower than in the control and sodium selenite+vitamin E-treated groups ( $P < 0.0001$  for all). While insignificant differences in body weight were seen between the control and sodium selenite+vitamin E-treated groups ( $P < 0.207$ ) after 2 weeks, significant differences were seen after 4 weeks ( $P < 0.005$ ). After 2 and 4 weeks, the kidney weights in the sodium selenite-treated group were significantly lower than in the control and sodium selenite+vitamin E-treated groups ( $P < 0.0001$  for all); meanwhile, insignificant differences were seen between the control and sodium selenite+vitamin E-treated groups ( $P < 0.128$  for both) (Table 1).

### 3.3. Morphometric results

The kidneys from the control group showed normal structure of the cortex. While, the experimental groups' kidneys revealed histological alterations that were mainly cortical. Renal corpuscle and proximal convoluted tubules are markedly affected while the distal convoluted tubules showed minimal or no detectable histological alterations. In addition, the medulla showed minimal or no changes in these groups.

Renal corpuscle diameter showed highly significant reduction in size in those treated with sodium selenite for 2 ( $P < 0.001$ ) and 4 weeks ( $P < 0.0001$ ) compared to the control. Moreover, those treated with sodium selenite and vitamin E showed significant diminution in size compared to the control while the urinary space showed highly significant improvement with those treated with sodium selenite and vitamin E for 2 weeks ( $P < 0.001$ ) and 4 weeks ( $P < 0.0001$ ). In addition, proximal convoluted tubular diameter revealed diminution in diameter after sodium selenite alone for 4 weeks ( $P < 0.0001$ ) with apparent significant improvements after treatment with sodium selenite and vitamin E ( $P < 0.0001$ ) (Table 2).

### 3.4. Immunohistochemical results

To determine the mechanism underlying the anti-apoptotic effects of vitamin E, Bax (a marker protein of apoptosis) was detected using immunohistochemistry. The results revealed that Bax was mainly expressed in the cytoplasm of renal cells, and the intensity of Bax expression in the sodium selenite-treated group was higher than that in the control group. Sodium selenite augmented renal expression of Bax, which was probably accompanied by renal apoptosis. Moreover, co-administration of vitamin E

reduced renal expression of Bax, which was likely associated with its anti-apoptotic effects. Bax-stained cells were not observed in the control group, while sodium selenite-treated rats for 2 weeks had dense immune-positive cells (Fig. 1A) and those treated with sodium selenite for 4 weeks showed more dense immune-positive cells (Fig. 1C). The groups exposed to sodium selenite along with vitamin E for 2 or 4 weeks had minimal immune-positive cells (Fig. 1B, D).

### 3.5. Ultrastructure results

The renal cortex, being a major portion of the kidney, was studied with particular regard to the cortical ultrastructural appearances. Ultrastructural submicroscopic observations of the renal tissues of the control rats disclosed a healthy picture of the Bowman's capsule, tubular system, and endothelial cells in the glomerular capillaries with a highly rich and fenestrated endothelium with large pores. The epithelium of the proximal renal tubular was distinguished by a dense brush border, rounded vesicular nucleus near the base or the central portion, endocytic vesicles in the apical portion, rare lysosomes, and elongated, rounded mitochondria with preserved cristae and rough endoplasmic reticulum cisternae placed between the mitochondria.

After 2 weeks, sodium selenite treated group exposed Bowman's capsule with hypertrophy of the basal lamina, narrowing of the urinary space with congested capillaries. While the epithelium of proximal convoluted tubule revealed pleomorphic mitochondria, abundant microvilli and thin basal lamina (Figs. 2A and 3A).

Moreover, after 2 weeks, sodium selenite and vitamin E treated group exposed Bowman's capsule with thickening of the basal lamina, with improvement of the urinary space narrowing and less congested capillaries. While the epithelium of proximal convoluted tubule revealed apparently healthy mitochondria with preserved cristae abundant microvilli and thin basal lamina (Figs. 2B and 3B).

After 4 weeks, sodium selenite treated group exposed Bowman's capsule with greatly thickened basal lamina, markedly reduced urinary space with congested capillaries and shrinkage and atrophy of podocytes. While the epithelium of proximal convoluted tubule revealed degenerated ballooned mitochondria with lost cristae, clumped microvilli and thick basal lamina (Figs. 2C, D and 3C, D).

After 4 weeks, sodium selenite and vitamin E treated group exposed Bowman's capsule with apparently moderate thickening of the basal lamina, seemingly improvement of the urinary space narrowing and less congested capillaries. While the epithelium of proximal convoluted tubule revealed noticeably healthy unaffected mitochondria, plentiful microvilli and sparingly thickened basal lamina (Figs. 2E and 3E).

**Fig. 3.** (A) An electron micrograph of a section of the group treated with sodium selenite 2 mg/2 week revealing the tubular epithelium of proximal convoluted tubule. The basal lamina (BL) is thin with numerous invaginated membranes in the cell cytoplasm. The cell contains abundant pleomorphic elongated mitochondria (M) and numerous electron dense rounded bodies varying in size that seem to be lipid components (L). The nucleus (N) is dentated, vesicular, and contain a large nucleolus (Nu). The cell surface having abundant, closely packed microvilli (Vi) and congestion of the peritubular capillary (RBC). (B) An electron micrograph of a section of the group treated with sodium selenite 2 mg/2 week and vitamin E revealing the tubular epithelium of proximal convoluted tubule. The cell contains abundant pleomorphic elongated mitochondria (M) with preserved cristae and an overall decrease in electron dense rounded bodies that vary in size and seem to be of lipid components (L). The nucleus (N) is rounded, vesicular, contains large nucleolus (Nu) nuclei, and regains the characteristic appearance of chromatin. The cell surface has abundant, closely packed microvilli (Vi). The basal lamina (BL) is thin with numerous invaginated membranes in the cell cytoplasm. (C) An electron micrograph of a section of the group treated with sodium selenite 2 mg/4 week revealing the tubular epithelium of proximal convoluted tubule with thickening of the basal lamina (BL) of the tubule. The nucleus (N) of the tubular epithelial cell has lost its characteristic electron density of the nuclear chromatin, the mitochondria (M) is obviously less abundant, vacuolated, lost cristae and more electron dense, vacuolation (V) is all over the cytoplasm, and the surface microvilli (Vi) is clumped and not straight. (D) Higher magnification of a section of the group treated with sodium selenite 2 mg/4 week revealing the tubular epithelium of proximal convoluted tubule. The nucleus (N) of the tubular epithelium has lost its electron density of the nuclear chromatin and an apparent nucleolus (Nu), the myelin like figure (MF) and the surface microvilli (Vi) are long, clumped, and not straight as usual, and there are more electron dense pleomorphic mitochondria (M) and apparent vacuoles (V). (E) An electron micrograph of a section of the group treated with sodium selenite 2 mg/4 week and vitamin E revealing the tubular epithelium of proximal convoluted tubule. The cell contains abundant pleomorphic elongated noticeably healthy unaffected mitochondria (M) with preserved cristae, few electron dense rounded bodies varying in size that seem to be of lipid components (L). The nucleus (N) is rounded and vesicular with an apparent nucleolus (Nu). The cell surface has abundant, closely packed long microvilli (Vi). The basal lamina (BL) is sparingly thickened with numerous invaginated corrugated membrane profiles at the base of the cell.

#### 4. Discussion

Amongst the necessary human micronutrients, selenium is peculiar due to its valuable physiological activity and toxicity. It may have anti-carcinogenic properties at low concentrations, while at concentrations higher than those needed for nutrition, it can be carcinogenic and genotoxic (Valdiglesias et al., 2010).

The current study suggests significant correlation between body weight and selenium intake. Sakr and El-Abd (2009) previously reported these findings in which they found a proportional relationship between selenium intake and body weight reduction with diminished food intake. The inhibitory effects of sodium selenite on the secretion of growth hormone and somatomedin C (insulin-like growth factor 1-binding proteins) may explain growth retardation (Wang et al., 2007). In addition, a significant decrease in kidney weight of the selenium-treated animals was seen and this was found to be related to the duration of treatment. There was relative improvement with the co-administration of vitamin E after 2 weeks and more amelioration was seen after 4 weeks and this agrees with the study of Tos-Luty who studied the effect of sodium selenite (Tos-Luty et al., 2003).

Moreover, the present work denotes that the morphological effects of sodium selenite on the renal tissues are mainly cortical. These effects include a decrease in the diameter of the Bowman's capsule, widening of the urinary space, and decrease in the diameters of proximal convoluted tubules in comparison to the control. These results appeared after 2 weeks and were dramatic after 4 weeks. These latter findings agree with that of Khattab (2007), but with the co-administration of vitamin E, these parameters were relatively improved. On the contrary, the effects on the distal convoluted tubules were minimal, while the renal medulla showed faint or no effects, disagreeing with the findings of Khattab (2007).

In our findings, the expression of Bax in the sodium selenite-treated group was increased compared to the control group. However, co-administration of vitamin E decreased the expression of Bax compared to the sodium selenite-treated group. These results indicate that vitamin E might influence the renal expression of Bax.

Previous findings concluded that the presence of vitamin E inhibited the formation of reactive oxygen species (ROS), decreased the level of lipid peroxide, elevated the level of glutathione (GSH) and activities of superoxide dismutase and catalase, and blocked the reduction in the mitochondrial membrane potential and the activation of caspase-3 (Meng et al., 2011). Some investigations have indicated that vitamin E protects tissues by causing apoptosis of injured cells (Reddy Avula and Fernandes, 2000). In our study, vitamin E prevented the up-regulation of Bax expression induced by sodium selenite treatment. This may protect cells from injuries induced by sodium selenite through the down-regulation of oxidative stress and the prevention of mitochondrial-mediated apoptosis.

On the ultrastructure observation and with the co-addition of vitamin E, the Bowman's capsule showed amelioration thickening of the basal lamina with improvement of the narrowing of the urinary space after 2 weeks. Moreover, the Bowman's capsule showed more amelioration of the thickening of the basal lamina with seemingly improvement of the urinary space narrowing after 4 weeks.

The cellular and submicroscopic ultrastructure changes observed in the second and fourth week agree with the work of Khattab (2007) that described slight histopathological changes in the form of cloudy swelling of the tubular cells and smudgy appearance of the renal tubular cells. In addition, vacuolar degeneration and narrowing of the cellular lamina and alterations in the nuclear appearance was seen. The epithelial cells of the tubules were partially detached with necrotic changes and

damage of the brush borders with darkly stained pyknotic nuclei. Swollen microvilli with many vesicles and swollen, condensed mitochondria with dark matrices and indistinguishable cristae were observed (Khattab, 2007). Other authors also observed dilatation of the endoplasmic reticulum and changes in the appearance of the mitochondria, as well as the liposomes (Tos-Luty et al., 2003). These findings were determined to be due to the protein lipid membranes of those cellular organelles or the presence of glutathione peroxidase, which can be a site for the sodium selenite activity (Naziroglu et al., 2004; Tos-Luty et al., 2003).

The later findings agree with Khattab who studied the effect of sodium selenite at low and high doses. The proximal convoluted tubule cells showed coagulative necrosis, electron opaque bodies, and obviously demolished organelles, except for some heavily condensed lysosomes. The cytoplasm of these cells contained many degenerated mitochondria, some swollen and disrupted and others hypertrophied with condensed matrices and electron-dense inclusion bodies. The nuclei of some affected cells showing shrinkage contained many vacuoles with chromatin clumped at the nuclear membrane (Khattab, 2007).

Some authors ascribed focal areas of tubular dilatation, atrophy, and interstitial fibrosis as due to selenium deficient diets (Fujieda et al., 2007). Other authors reported sloughing or internalization of the brush border as an effect of toxic materials (Khattab, 2007). Similar results have been studied on the histology of the rat kidney cortex after treatment with a selenium deficient diet for 1–12 weeks and found that selenium deficiency induces protein urea and glucosuria with renal calcification, which may be mostly induced by injury to the proximal tubule via oxidative stress (Fujieda et al., 2007). In addition, the inhalation of selenium derivatives, such as dimethyl selenide, has been associated with tubular injury of the kidney, resulting in swelling and vacuolation of the proximal tubular cells and showing focal areas of tubular dilation, atrophy, interstitial fibrosis, and sloughing or internalization of the brush border of the proximal tubules (Cherdwongcharoensuk et al., 2005; Fujieda et al., 2007).

Our study indicates a duration-dependent influence of sodium selenite. These results agree with other authors' findings (Tos-Luty et al., 2003). In addition, more statistics and morphometric measures are recommended to evaluate the concept and more advanced techniques are recommended as stereological techniques to support our findings.

#### 5. Conclusion

The histopathological and ultrastructural changes of the renal cortex caused by the oral administration of sodium selenite are proportionate to the duration of its intake. The coadministration of vitamin E showed amelioration of these changes. The results of present work afford an improved awareness on the activity of sodium selenite and aid in elucidating the causes underlying the duality in action of it as antioxidant or toxic compound so to regulate its exact use in nutrition and medicine.

#### Authors' contributions

All authors participated in the design of this work and performed equally. All authors read and approved the final manuscript.

#### Conflict of interest statement

The authors declare that they have no conflict of interest.

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