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ORIGINAL STUDY

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Effect of vitamin E and selenium against aluminum--induced nephrotoxicity in pregnant rats

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Abstract: Kidney is one of the most affected organs by aluminium toxicity. This study aimed to investigate the effect of aluminium chloride on the kidney of pregnant rats and to assess the efficiency of vitamin E and selenium in ameliorating this effect. Forty virgin albino rats were randomly divided into two main groups. Control rats were further divided into negative control group (C1, n = 10) which received distilled water and positive control group (C2, n = 10) that received vitamin E (VE, 150 mg/kg/day) and selenium (NaSe 150 μg/kg/day) for 3 months through intra-gastric tube. The experimental group was divided into an E1 subgroup in which rats received aluminium chloride ($AlCl_3$, 150 mg/kg/day, n = 10) and E2 subgroup (n = 10) in which animals received the same dose of $AlCl_3$ plus VE and selenium at the same doses as C2 group for 3 months through intra-gastric tube. Conception of rats was allowed. $AlCl_3$, VE and NaSe were given through intragastric tube during the whole length of the pregnancy, at the same doses as before pregnancy. At the 20th day of gestation dams were sacrificed, kidneys were dissected and processed for routine histological and immunohistochemical staining for identification of T-lymphocytes and macrophages. Integrated optical density of both cell types was assessed. AlCl3 administration induced histopathological changes in the kidney of pregnant rats and increased the density of CD3 and CD68 immunoreactive cells, suggestive of the associated aluminium-induced inflammatory process. Vitamin E and selenium minimized these harmful effects. The results suggest that diets rich in vitamin E and selenium and their supplements are advised particularly during pregnancy to alleviate the effects of possible excessive aluminium exposure. *(Folia Histochemica et Cytobiologica 2013, Vol. 51, No. 4, 312–319)*

Key words: aluminium nephrotoxicity; pregnancy; rat; T cells; macrophages; vitamin E; selenium

Introduction

Aluminium (Al) is extensively used in building, canning, tanning, automobile, aviation, paint, paper, ceramic and glassware industries [1]. Since Al is ubiquitous, exposure to this element is in fact unavoidable. This means that pregnant women may be potentially exposed to Al in food, drinking water, soil ingestion, and some medications. During pregnancy, dyspepsia is a common complaint and antacids which contain Al are widely used to reduce the dyspeptic symptoms. However, the consumption of high amounts of Al compounds during pregnancy can mean a potential risk of Al accumulation and toxicity [2].

It was found that one of the main organs targeted by Al exposure is the kidney [3]. The nephrotoxic actions of aluminium arise from its accumulation in the kidneys, with the resultant degeneration of the renal tubular cells. It has been suggested that Al generates reactive oxygen species that cause the oxidative damage to cellular lipids, proteins, and DNA [4]. Few studies were focused on studying the effect of aluminium on the histological structure of kidney during pregnancy. One of them found that administration of aluminium lactate through gavage during the day 0–19 of gestation caused significantly higher levels of aluminum in plasma, liver, spleen and kidneys of pregnant rats when compared to non-pregnant female rats [5].

Vitamin E (VE) is an important component of human diet which protects the body's biological systems

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by counteracting lipid peroxidation [6, 7]. Because of the health risks induced by many environmental pollutants, several studies evaluated the relative antioxidant potency of VE [8, 9]. Selenium (Se) is also generally recognized to be a trace element of great importance for human health which protects the cells from the harmful effects of free radicals [10].

Although some studies examined the toxic effects of Al-containing substances in mature animal models [10–12], little attention was paid to the effect of Al on pregnant rats. Therefore, this study was carried out to investigate the efficiency of VE and Se in alleviating the Al-induced toxicity on kidney in pregnant rats.

Material and methods

Animals and design of the study. Forty virgin albino Sprague Dawley rats weighing between 150 and 180 g were purchased from animal house in King Fahd Medical Research Center, Jeddah, Saudi Arabia. The animals were housed in stainless steel cages and maintained on a 12-hour light-dark cycle and room temperature of 27°C ± 1°C under hygienic conditions. Water was offered *ad libitum*. Rats were randomly divided into two main groups: Control (n = 20) and experimental (n = 20) groups. Control group was further divided into negative control group (C1) which received distilled water, and positive control group (C2) that received VE (150 mg/kg/day) [13] and Se in the form of sodium selenite (Na_2SeO_3) (150 μg/kg/day) for three months through intragastric tube [14]. This study was approved by the biomedical research ethics committee, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.

The experimental group was further divided into two subgroups: the first group (E1) received orally aluminium chloride ($AlCl_3$) (150 mg/kg BW/day) [15] for three months through intragastric tube and the second one (E2) received the same dose of $AlCl_3$ plus VE and Se at the same dose as positive control (C1 group) for three months. $AlCl_3$, VE and Se used in this study were purchased from Aldrich Chemical Company (Milwaukee, MN, USA).

One fertile male albino rat was introduced into each cage and left overnight. Pregnancy was determined by the presence of spermatozoa in the vaginal smear next morning and this was considered the first day of gestation (GD1) [16]. $AlCl_3$,VE and Se were given to pregnant rats through intragastric tube during the whole length of the pregnancy at the same doses as before pregnancy. At the same time negative control group was given saline through intragastric tube during the whole length of the pregnancy.

At the time of GD20 dams were sacrificed. The abdomen was opened to dissect the kidney which was fixed in 10% formalin and proceeded for paraffin blocks. Paraffin sections 6 μm thick were stained with hematoxyline and eosin (H&E) for routine histological examination, and Masson trichrome method for visualization of connective tissue [17].

Immunocytochemical staining and image analysis. For immunohistochemical (IHC) staining, sequential serial paraffin sections 4 μm thick were prepared using an avidin-biotin-complex (ABC) technique and stained with mouse monoclonal anti-CD3 (in concentration of 1:200, Novocastra Laboratories, Newcastle, UK) and anti-CD68 (1:50–1:100, Dako A/S, Glostrup, Denmark) primary antibodies for the identification of T lymphocytes and macrophages, respectively. Shortly, the sections were deparaffinized, hydrated and treated with 0.5% hydrogen peroxide in methanol for 10 min to block activity of endogenous peroxidases, and washed in tap water. Sections were then incubated in 10 mM citrate buffer, pH 6.0, and heated in a microwave oven to 95°C during two cycles of approximately 5 min each for antigen recovery of the CD3, and CD68. After cooling, sections were washed in phosphate-buffered saline (PBS, pH 7.6) for 5 min, then placed in saline (0.9% NaCl). Sections were incubated with the primary antibodies for 1 h at 25°C and treated thereafter with the Dako EnVision + System for 30 min, followed by incubation with 1 mg/ml DAB solution (3,3' tetrahydrochloride diaminobenzidine; SigmaAldrich, St. Louis, MO, USA) in PBS pH 7.4, plus 1% hydrogen peroxide (Merck, Darmstad, Germany) for 5 min. The slides were counterstained with Harris hematoxylin and mounted in Permount resin. They were washed with PBS between each reaction step [18, 19].

Positive labeling in CD3 was identified by a brown color of the cell membrane. Pattern of CD68 expression was defined as cytoplasm staining. Slides were photographed using an Olympus Microscope BX-51 (Olympus, Tokyo, Japan) with a digital camera connected to a computer. Image analyses were performed with a 40× objective lens and a 10x ocular lens (Olympus) using Image-Pro Premier Software version 6.0. Integrated optical density (IOD) of CD3- and CD68-stained cells were measured in 30 view fields of each kidney.

Statistical analysis. Data were analyzed using non-parametric Kruskal-Wallis test followed by a post-hoc analysis (based on Dunn's procedure) was used to analyze each pair of groups and thereby avoid multiple-comparison effect. The IOD was expressed in mean values ± standard error. P value less than 0.05 was considered to be significant.

Results

This study showed that there were no changes in the renal cortex structure observed in the control (C2) group in which rats received VE and Se compared to the (C1) group (Figure 1A, B). Kidneys of rats which were treated with $AlCl_3$ (E1 group) showed dilated Bowman's capsule and congested glomerular capillaries in almost all the corpuscles of renal cortex. Large number of cells lining the proximal convoluted tubules (PCT) appeared degenerated. The peritubular

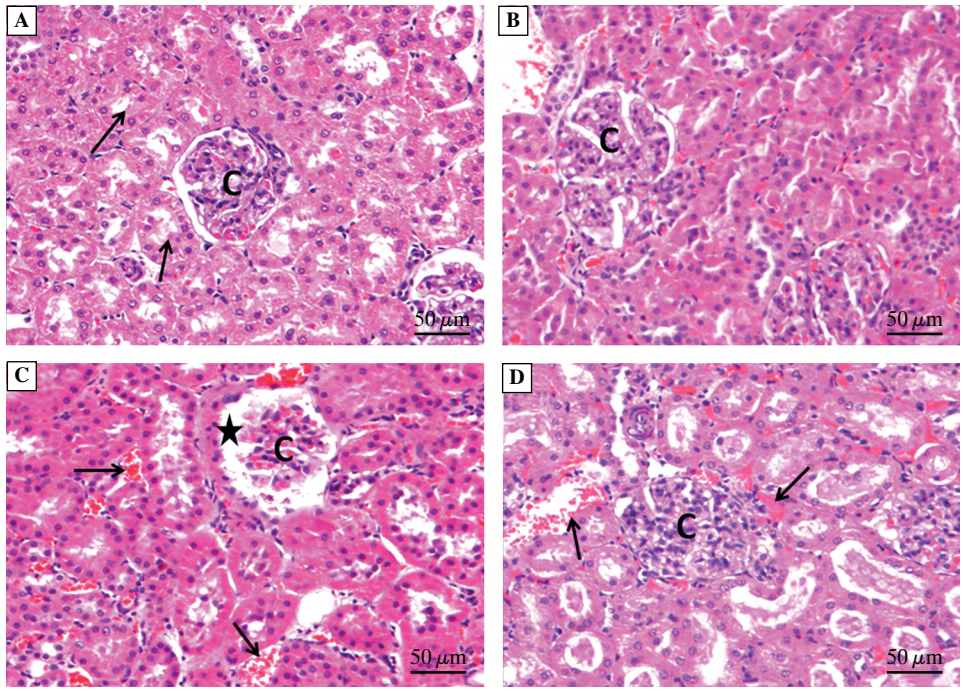


Figure 1. Histological structure of the renal cortex of pregnant rats as revealed by H&E staining, scale bar = 50 μm. **A.** Group C1: renal corpuscle (C) is surrounded by many proximal convoluted tubules (PCTs) (arrows) lined with high cubical cells with acidophilic cytoplasm. **B.** positive control (C2) group which received VE and Se shows normal kidney structure. **C.** In $AlCl_3$-treated group (E1) renal corpuscle (C) dilated Bowman's capsule (star), congestion of glomerular capillaries and multiple areas of hemorrhage between the PCTs (arrows) are present. **D.** Kidney of rat of E2 group (E2) ($AlCl_3$ plus VE and Se) shows hypercellular renal corpuscle (C) with less hemorrhage areas than in group E1 (arrows). Most of the PCT lining intact cells have acidophil cytoplasm and vesicular nuclei

capillaries were dilated and congested (Figure 1C). Although administration of $AlCl_3$ plus VE and Se (group E2) did not improve the structure of dilated peritubular capillaries, it resulted in an increase of cellularity of renal corpuscules and a decrease in glomerular capillary congestion. Most of the proximal convoluted tubules (PCT) were lined by intact cells (Figure 1D).

In order to assess the extent of the $AlCl_3$-induced fibrosis, Masson trichrome staining was used. In the kidneys of rats which received only $AlCl_3$ (group E1) and $AlCl_3$ with VE and Se supplementation (group E2) the amount of collagen fibers around renal tubules clearly increased in comparison to both control groups (C1 and C2) (Figure 2A–D).

The IHC staining revealed lack or the presence of only few CD3 positive T lymphocytes in the kidney of both control groups (Figure 3A, B). On the contrary, kidney of rats treated with $AlCl_3$ (group E1) displayed many CD3 positive cells in the peritubular cellular infiltrates (Figure 3C, D) that were less frequently observed in kidney of rats which received $AlCl_3$ plus VE and Se (Figure 3E). There was a significant increase in the Integrated Optical Density of CD3 positive cells in kidney of the E1 group ($AlCl_3$ — treated rats) compared to all other groups. There was no significant difference between control groups in comparison to rats which received $AlCl_3$ plus VE and Se (E2 group). The IOD of CD3 positive cells in kidney of rats which were given $AlCl_3$ plus VE and Se significantly decreased in comparison to rats treated with AlCl3 alone (Figure 4).

No CD68 immunoreactive cells were found in kidney sections of both control groups (Figure 5A, B). However, kidney of $AlCl_3$-treated rats (group E1) showed many CD68 positive cells in the peritubular cellular infiltrates in both renal cortex and medulla (Figure 5C, D). These cells were less frequently observed in the E2 group which received $AlCl_3$ plus VE and Se (Figure 5E). The IOD of CD68 positive cells in the kidney of the E1 and E2 groups were significantly increased compared to both control groups. However, The IOD of CD68 immunoreactive cells was significantly decreased in the E2 group which received $AlCl_3$ plus VE and Se compared to the E1 group which received only $AlCl_3$ (Figure 6).

Discussion

Aluminium-induced damage to body organs has been reported in several studies and accumulation in the kidney has been related to worsening renal func-

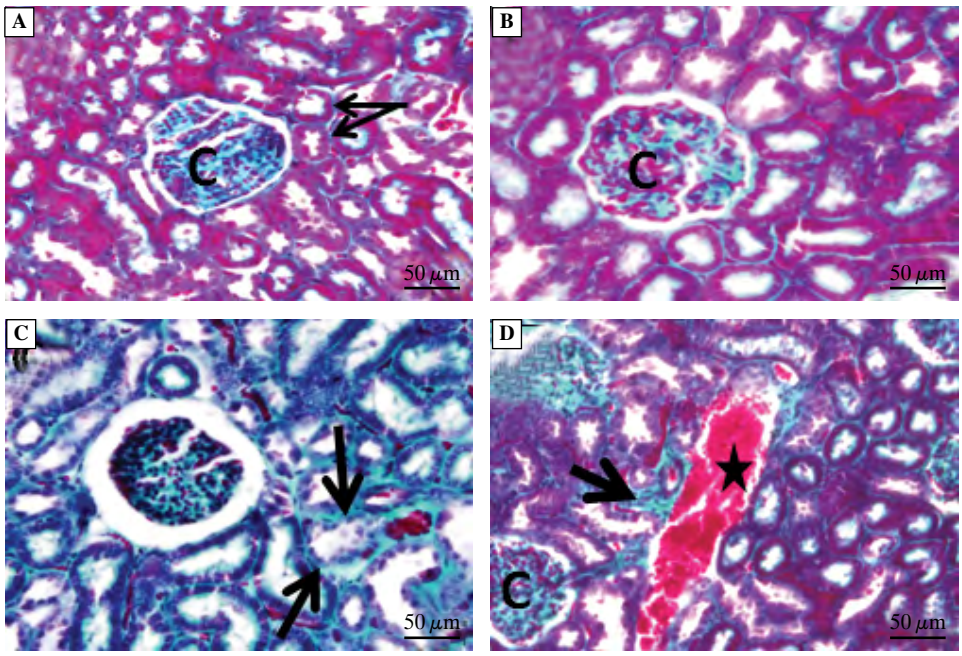


Figure 2. Histological structure of the renal cortex of pregnant rats as revealed by Masson trichrome (**A–D**) staining; scale bar = 50 μm. **A.** Group C1: renal corpuscle (C) is surrounded by tubules separated by few blue-stained collagen fibers (thin arrow). **B.** Group C2 has similar kidney structure as the control. **C.** Group E1: increased amount of collagen fibers (thick arrow) around the dilated and atrophied renal tubules. **D.** Group E2: increased amount of collagen fibers around tubules (thick arrow). Areas of hemorrhages (star) between the tubules

tion [20, 21]. Indeed the kidney may be exposed to high concentrations of aluminium during the normal process of renal excretion making kidney vulnerable to aluminium-mediated toxicity [22].

In this study, rats received $AlCl_3$ for three months through intragastric tube before pregnancy to mimic chronic toxicity of aluminium as may occur in humans. The high dose of orally administered $AlCl_3$ was chosen because the intestine plays a role of a protective barrier against aluminum toxicity since only a small fraction (0.1 to 0.5%) of ingested aluminum is absorbed [23].

In the present study, kidney of pregnant rats in negative and positive control groups had normal structure and showed no histopathological changes. This finding extends our observations made in a previous study that pregnancy did not affect the kidney histological structure in rat [24]. As the maternal control rats showed normal kidney architecture with very minimal capsular spaces and rounded glomeruli intimately surrounded by the Bowman's capsule [24].

Our study is the first one which investigated the effects of prolonged aluminium administration on the kidney structure of pregnant female rats. In male rats treated orally with aluminium for 6 months the kidneys showed abnormal proximal tubules with irregular brush border [25] and dilated, focal areas of interstitial fibrosis, partial sclerosis of glomeruli and hypercellularity in mesangium [26]. We were able to extend these observations to the other gender to describe in detail histopathological changes in the kidneys of pregnant rats.

Aluminium might accumulate in the kidneys of pregnant rats exposed to aluminium compounds following exposures to very high doses [27, 28]. In an earlier study, aluminum levels of plasma, liver, spleen, and kidneys were significantly higher in treated pregnant rats than non-pregnant female rats after oral ingestion of aluminum lactate (400 mg Al/kg/d.) [5]. The primary effects of $AlCl_3$ on brain [29] and kidney [30] are thought to be mediated via damage to cell membranes. Aluminium induces generation of reactive oxygen species that cause membrane lipid peroxidation resulting in loss of membrane integrity, decrease of its fluidity, disruption of membrane bound receptors and ion channels which all lead finally to cell death [10].

The involvement of innate (macrophages) and adaptive (T lymphocytes) immunity seems to be crucial in the onset and persistence of renal inflammation, tubular damage and fibrosis [31, 32]. These cells have been involved in the clearance of infective pathogens and the scarring as well as in the healing of tubulointerstitial lesions irrespective of the nature of the original renal insult [33–35].

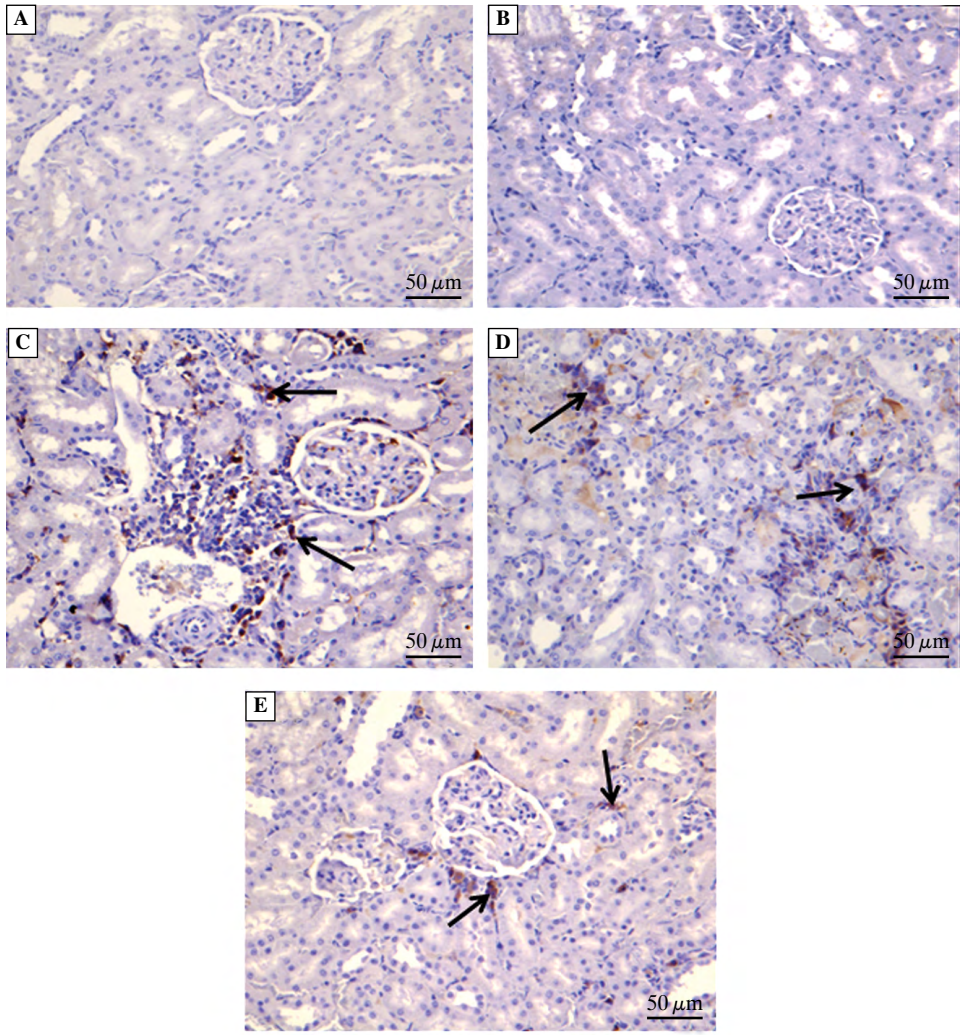
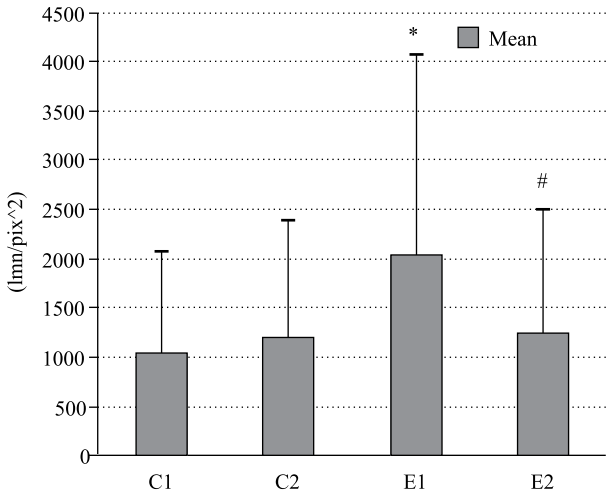


Figure 3. The presence of T lymphocytes in the renal tissue of pregnant rat as revealed by IHC staining. **A.** Group C1: lack of CD3 immunoreactive cells. **B.** Group C2: lack of CD3 positive cells. **C.** Group E1: renal cortex: many CD3 positive cells (arrow) in the peritubular cellular infiltrates. **D.** Group E1, renal medulla: many CD3 positive cells (arrow) in the peritubular cellular infiltrates. **E.** Group E2: renal cortex with lower number of CD3 positive cells (thin arrow) compared to the group E1. IHC detection of CD3 cells was performed as described in Methods, scale bar = 50 μm



Groups	Mean ± SD
Negative control (C1)	1044.2 ± 1534.3
Positive control (C2)	1201.3 ± 4885.3
$A1C1_3$ (E1)	2031.6 ± 2776.6*
[$A1C1_3$ + VE + Se] (E2)	1245.4 ± 2922.7#

*p < 0.05 compared to all groups; #p < 0.05 compared to A1C13 group

Figure 4. Integrated Optical Density (IOD) of CD3 positively stained cells in the kidney. *group E1 significantly differs from other groups (P < 0.05), no significant difference between C1 and C2 groups in comparison to the group E2. #group E2 significantly different from group E1 (P < 0.05). IOD was determined as described in Methods

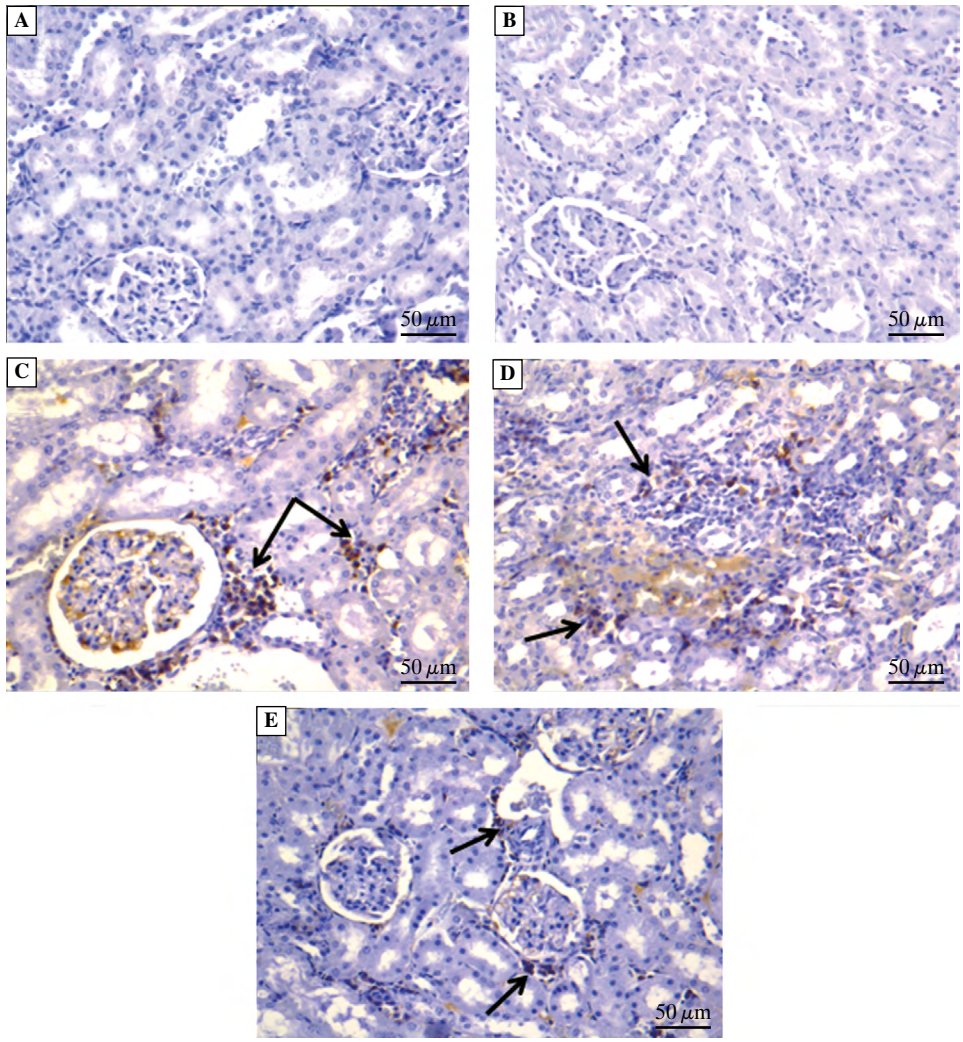
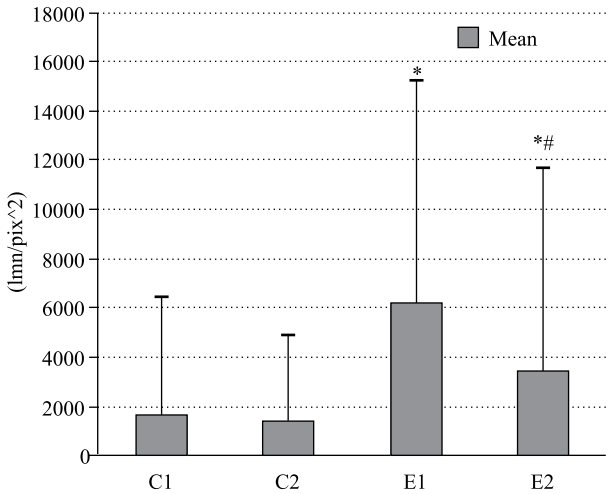


Figure 5. The presence of macrophages in the renal tissue of pregnant rat as revealed by IHC staining. **A.** and **B.** Group C1 and Group C2, respectively: lack of CD68 positive cells in the renal cortex. **C** and **D.** Group E1, renal cortex and renal outer medulla, respectivelly: many CD68 positive cells (arrow) in the peritubular cellular infiltrates and in blood vessels. **E.** Group E2, renal cortex: much lower number of CD68 positive cells (arrow) in the interstium and blood vessels as compared to the group E1. IHC staining of CD68 cells was performed as described in Methods, scale bar = 50 μm



Groups	Mean ± SD
Negative control (C1)	1667.3 ± 4885.3
Positive control (C2)	1369.1 ± 3440.73
$A1C1_3$ (E1)	6120.2 ± 9255.6*
[$A1C1_3$ + VE + Se] (E2)	3561.7 ± 8263.6*#

*p < 0.05 compared to negative and positive control groups;
#p < 0.05 compared to $A1C1_3$ group

Figure 6. Integrated Optical Density (IOD) of CD68 positively stained cells in the kidney. *group E1 significantly differs from groups C1 and C2 (P < 0.05), no significant difference between C1 and C2 groups in comparison to the group E2. #group E2 significantly different from group E1 (P < 0.05). IOD was determined as described in Methods

Therefore, in this study, CD3 positive T-lymphocytes and CD68 positive macrophages were chosen to assess the effect of aluminium on the kidney structure of pregnant rats.

We found that prolonged oral $AlCl_3$ treatment increased the density of CD3 and CD68 immunoreactive cells in the kidney of pregnant and that concomitant vitamin E and selenium administration did not change this process. Our findings confirm and expand the results of other studies which showed that small number of macrophages and T lymphocytes present in the normal kidney increased in renal interstitium in human glomerular disease [32]. Moreover, studies in animal models indicated that macrophage is the dominant infiltrating cell in the initiation and progression of injury in chronic renal disease [36].

Our observations could be explained in the light of the previous studies which demonstrated that vitamin E has a high antioxidant capacity and plays an important role in body homeostasis. It was shown that vitamin E may protect cells and tissues from oxidative damage and prevent the formation of toxic oxidation products such as those formed from unsaturated fatty acids [4, 37]. Vitamin E was effective in scavenging lipid radicals with the particular function of preventing lipid peroxidation in membranes and lipoproteins [38–40].

Regarding antioxidant effects of selenium, it is believed that this trace element protects cells from the harmful effects of free radicals by inhibiting metal-mediated DNA damage. Its action is mediated through the glutathione peroxidases that inactivate hydroperoxides and hydrogen peroxide, potent lipid damaging factors [41]. Previous study demonstrated that combination of VE and Se significantly decreased level of free radicals in rat kidney which resulted from oral administration of $AlCl_3$ for 30 days [10]. This finding could explain the beneficial effect exerted by both vitamin E and selenium in ameliorating toxic effects of $AlCl_3$ on the kidney structure of female rats.

In conclusion, prolonged oral administration of $AlCl_3$ induced histopathological changes in the kidney of pregnant rats reflected by dilatation of Bowman's capsule, congestion of peritubular and glomerular capillaries, increased collagen fibers deposition, and increased density of CD3 and CD68 immunoreactive cells. Co-administration of vitamin E and selenium with aluminium partially alleviated the harmful effects of Al on the kidney structure of pregnant rats. Supplementation of a diet enriched in vitamin E and selenium should be advised particularly during pregnancy to alleviate the effects of possible excessive exposition to aluminium.

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