

Original Research Article

Effect of vitamin E on biochemical and ultrastructural changes in acrylamide-induced renal toxicity in rats

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Received: 07 April 2017

Revised: 20 April 2017

Accepted: 26 April 2017

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ABSTRACT

Background: Acrylamide (ACR) is a widely used chemical in industry and it accounts for major health problems as it has been detected in highly consumed food items, carbohydrate-rich food items cooked at high temperature. Accordingly the population is highly exposed to ACR. The aim of the study was to assess the effect of vitamin E on biochemical and ultrastructure changes in acrylamide-induced renal toxicity in wistar albino rats.

Methods: Three groups of adult albino wistar rats weighing about (200-250 gm) were used in this study to investigate the effect of vitamin E on acrylamide induced renal toxicity; 10 rats in each group; Group I: Control group, Group II: Acrylamide treated and Group III: Acrylamide-Vit E treated group. Blood samples were collected for estimation of serum creatinine, blood urea nitrogen (BUN), lactic dehydrogenase (LDH) and albumin. Kidneys specimens were processed for light and electron microscopic studies. Kidney sections were stained with H&E, MT, PAS and immuno-histochemical stains for detection of NF- κ p65 and Bcl-2. Morphometric study was done followed by biochemical and morphometric statistical analysis.

Results: Acrylamide treated rats showed degeneration of cells lining PCT and DCT, atrophy of glomeruli and fibrosis. Ultrastructurally; tubules lining cells showed loss of microvilli, basal membrane in foldings and mitochondrial changes. Podocytic changes include: Vacuolation, irregularity and disorientation of their processes and thinning of glomerular basement membrane. Significant increase in the mean number of NF- κ p65 positive cells and decrease in the mean area% of Bcl-2 immuno-expression, increase in the mean area % of collagen fibers and urinary space diameter and decrease in thickness of epithelial cells of PCT and DCT were also observed. Serum creatinine, BUN and LDH were increased whereas serum albumin was decreased. Vitamin E co-administration with ACR improve all these histological, ultrastructural and biochemical changes.

Conclusions: Acrylamide induced renal toxicity could be ameliorated by vitamin E co-administration.

Keywords: Acrylamide, Kidney, Vitamin E, NF- κ p65, Bcl-2, Ultrastructure

INTRODUCTION

Acrylamide (ACR) is an odorless, white crystalline, reactive and highly water-soluble monomer. These properties facilitate its rapid absorption and distribution through the body.¹ It is solid at room temperature with a molecular formula of C₃ H₅ NO.² It is present in plants

like potatoes, carrots, radish, lettuce, Chinese cabbage, parsley, onions, spinach and rice also in sugar and olives.^{3,4} ACR represents a chemical widely used in industry and manufacturing of polyacrylamides which are common in personal care products as lotions, deodorants and cosmetics, also in paper industry.⁵ It was also highly detected in tobacco and cigarette smoke.⁶

ACR monomer may form in foods cooked at very high temperatures.⁷ It is present at high concentrations in carbohydrate rich food as potato and grain-base foods prepared at high temperature such as French fries and potato chips. Since ACR is formed from natural chemicals in food while cooking, its levels in organic foods are similar to inorganic ones.⁸ It has become a major public health concern as it is detected in highly consumed food.⁹

ACR toxicity has been reported to various body organs; neurotoxicity is the main effect, reproductive system toxicity.^{10,11} It has a significant binding capacity to liver, kidney, erythrocyte and brain.¹² The US EPA and IARC have classified acrylamide as B2, a carcinogen and as 2B a human carcinogen, respectively.¹³ The toxicity of ACR is due to its biotransformation to a more potent molecule that initiates cellular toxicity. Therefore, the significant pathogenic pathway is the oxidative biotransformation of ACR by cytochrome P450 2E1 (CYP2E1).¹⁴ The resulting metabolite is an epoxide glycidamide, which is more reactive towards DNA and proteins than ACR.^{15,16} Once absorbed, ACR may be conjugated by glutathione-S-transferase (GST) to N-acetyl-S-(3-amino-3-oxopropyl) cysteine or it reacts with cytochrome P450 (CYP450) to produce glycidamide. Several metabolic studies have been conducted that focused on the interaction of ACR with CYP450 and GST in rats and mice. The results of these studies indicated that liver, brain, kidney and erythrocyte GST have high binding capacity with ACR.^{17,18}

ACR induced oxidative stress, resulting in generation of reactive oxygen species ROS which plays a critical role in the initiation and progression of fibrotic diseases. Two important markers for ROS Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and advanced oxidation protein products (AOPPs). NADPH oxidase is recognized as a key mediator of cell proliferation and matrix accumulation in renal disease. AOPPs are not only markers of oxidative stress but also cause renal injury. Inhibition of NADPH oxidase and/or reducing AOPPs production might be a corner stone for the therapeutic intervention of variety of fibrotic kidney disorders.¹⁹ AOPPs induce vascular endothelial and smooth muscle cell dysfunction, promote podocyte depletion and apoptosis, upregulate the expression of fibronectin and collagen IV in mesangial cells.²⁰⁻²²

Vitamin E, as an important antioxidant, plays a role in inhibition of mutagen formation and repair of membranes and DNA.²³ So vitamin E is a non-enzymatic antioxidant which can act to reduce oxidative stress. Studies have reported that vitamin E can reduce lipid peroxidation caused by toxic substances.^{24,25} Vitamin E supplementation in cancer patients showed that it has an important cytoprotective effect.^{26,27}

Since ACR is present in many commonly consumed foods and industries, the general population is highly

exposed to it. The aim of this study is to investigate renal toxicity of acrylamide ACR and the ameliorative effect of vitamin E on the toxicity.

METHODS

Animals and housing

The experiment was conducted on thirty adult albino wistar rats, weighing about 200-250 gm. The animals were acclimated for about one week and housed in plastic cages at room temperature prior to experiment in Mansoura Medical Research Center (MRC). Throughout the experiment, the animals were housed under standard laboratory conditions. Care and treatment of animals were approved and practices were performed according to approval of ethics regulation at Mansoura University.

Chemicals

Acrylamide (99.50%) and vitamin E pure were purchased from Hi Media Laboratories Pvt. Limited, Mumbai. All other required chemicals were used of highest quality available.

Experimental design

Experiment was designed to study toxicity of acrylamide on kidney for the duration of 4 weeks. The rats were assigned to one of the three groups, with ten rats each as follows:

- Group I (Control group): given distilled water for 4 weeks by oral gavage tube.
- Group II (Acrylamide treated group): treated orally with acrylamide dissolved in distilled water at a dose of 20 mg/kg body weight once daily for 4 weeks.²⁸
- Group III (Acrylamide –Vitamin E treated group): Was treated orally with acrylamide dissolved in distilled water at a dose of 20 mg/kg body weight daily with vitamin E at a dose of 50 IU/kg body weight once daily for 4 weeks.²⁸

Blood samples and kidney tissue-specimens collection

At the end of the experiment after 4 weeks, rats of all groups were anesthetized by intraperitoneal injection of sodium thiopental (40 mg/kg of body weight) (Sigma Chemical Co., ST Louis, MO, USA). Blood samples and renal specimens were collected.

Biochemical study

Blood samples were collected in non-heparinized tubes for biochemical analysis of various kidney function to detect levels of creatinine, albumin, LDH, blood urea nitrogen (BUN). The enzyme activity was measured by a kinetic method using commercial kit (Egyptian company for biotechnology).

Light microscopic (LM) study

kidneys were excised and fixed in 10% neutral buffered formalin; then routinely processed to obtain paraffin blocks which were cut into sections of 5µm thickness, mounted on glass slides, deparaffinized in xylene and stained by: hematoxylin and eosin stain (H&E), Masson trichrome (MT), periodic acid schiff (PAS) reagent stains, immuno-histochemical stains for detection of NF-kβ and Bcl2 using avidin-biotin-peroxidase complex (ABC) techniques (The primary anti- Bcl-2 and anti- NF-kβp65 antibodies were rat anti-mouse Bcl-2 monoclonal antibody (Genemed Biotechnologies Inc., South San Francisco, CA 94080, USA) and rabbit polyclonal NF-kβp65 antibody (dilution 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) respectively.²⁹⁻³¹ Sections were then examined and observed under light microscope.

Electron microscopic (EM) study

Small kidney specimens (1 mm³) of kidneys were immediately fixed in glutaraldehyde (2.5%), and then post fixed in osmium tetroxide (1.0%), dehydrated, and embedded in epoxy resin. Semithin sections (1 µm thickness) were prepared and stained with Toluidine blue. Ultrathin sections were cut at thickness of 80nm mounted on copper grids and stained with Uranyl acetate 5% for 15 min followed by lead citrate for 8 min.³² Sections were examined by transmission electron microscope (JEOL2100 electron microscope, Tokyo, Japan) at Electron Microscopy Unit, Faculty of Agriculture, Mansoura -University, Egypt.

Morphometric study

From H&E stained sections (x400); the epithelial height of PCT and DCT and the width of urinary space (the distances between the parietal and visceral layers) of renal corpuscles were measured). From MT stained sections (x400); area% of collagen fibers deposition in the kidney tissues were measured. From immune-stained sections (x400); number of NF-kβp65 positive cells (nuclear reaction) and the area % of Bcl-2 immun-expression were measured. For each mentioned stain measured parameter, five non-overlapping fields/rat sections were examined (The total was 50 measurements /group) and photographed using color video camera (digital camera CH- 9435 DFC 290). The photographs were analyzed using Leica Qwin 500 (Imaging System, Cambridge, UK) within a frame area of 293.4288 µm². Morphometry was carried out at the Image Analysis Unit, Anatomy Department, Faculty of Medicine, Taibah University, Al Madinah Al Monawarrh, KSA.

Statistical analysis

All data were expressed as mean ± SEM. Statistical analysis was performed using IBM SPSS software version 21.00 (Chicago, Illinois, USA). One-way analysis

of variance (ANOVA) (data were normally distributed and variances of populations were equal), post-hoc and least significant difference (LSD) were performed for inter-group comparison. P >0.05, P ≤0.05 and P ≤0.001 were considered non-significant, significant and highly significant, respectively.³³

RESULTS

Light microscopic results

Acryl group showed disturbed architecture of renal cortex with corpuscle shrinkage up to partial or complete loss with congestion of the glomerulus and an apparent widening of the urinary space. Some PCT and DCT showed disturbance of their shape with degeneration, and vacuolation of their lining cells. Other tubules show marked dilatation and their cells appear small with pyknotic nuclei and an apparent increase in the interstitial tissue in between is observed (Figure 1B).

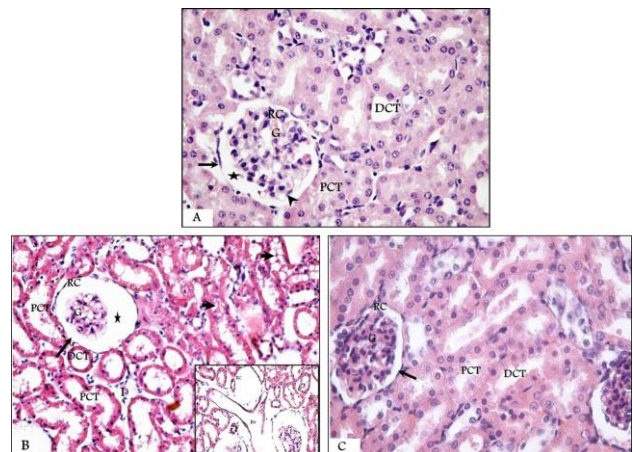


Figure 1: Photomicrographs of H&E stained sections in the renal cortex of A): Control group, showing normal renal cortex with glomeruli, parietal and visceral (arrow head) layers of Bowman's capsule, urinary space, PCT and DCT. B): Acryl group showing RC shrinkage, congestion of the glomerulus up to its complete loss (Inset), partial loss of parietal layer and widening of the urinary space. PCT and DCT show degeneration and vacuolation (arrow head) of lining cells with pyknotic nuclei. C): Acryl-Vit E group, with preserved normal renal architecture. (H&E stain; A and CX400; B and Inset BX200).

RC: renal corpuscle, G: glomeruli, Arrow: parietal layer of Bowman's capsule, Star: urinary space, PCT: proximal convoluted tubules, DCT: distal convoluted tubules, I: interstitial tissue, Red arrows: pyknotic nuclei.

Acryl-Vitamin E group, showed nearly preserved renal cortex architecture which showed apparently normal shaped renal corpuscle with decrease glomeruli congestion, intact parietal layer of Bowman's capsule and minimal widening of urinary space. Preservation of normal shape and lining of PCT and DCT are also detected (Figure 1C).

Masson trichrome stained sections of Acryl group, increasing amount of collagen fibers deposition among glomerular capillaries, however the renal tubules (RT) were still surrounded with minimal amount of collagen. Moreover, areas of loss of RC or RT and their replacement with fibrous tissue were also detected (Figure 2B and C). Acryl-Vitamin E group showed an apparent decrease in the deposition of collagen fibers around the wall of RC and among glomerular capillaries. RT and blood vessels were also surrounded with minimal amount of collagen fibers when compared with Acryl group (Figure 2D).

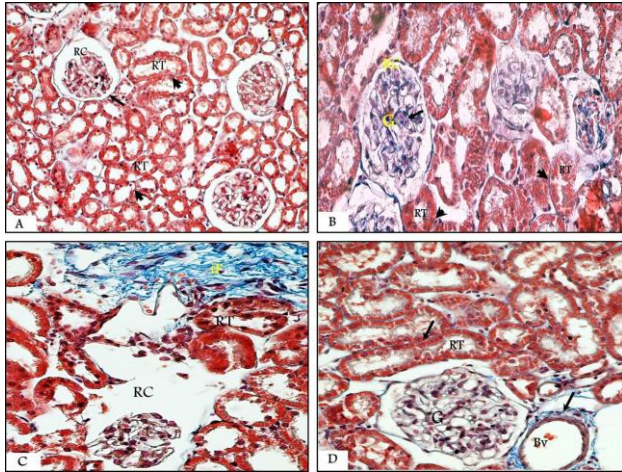


Figure 2: Photomicrographs of Masson trichrome stained sections in the renal cortex of A): Control group: minimal collagen fibers (arrow heads). B): Acryl group: increasing amount of collagen fibers deposition among glomeruli with renal tubules (RT) surrounded with minimal collagen around renal tubules. C): Acryl group, show area of loss of RC or RT and their replacement by fibrous tissue (F). D): Acryl-Vit E group shows decreased fibrous tissue (arrow). (Masson trichrome stain; AX200; B, C and DX400).

G: glomeruli, RT: renal tubules, RC: renal corpuscle, F: fibrous tissue, Arrow heads: collagen fibers, Bv: blood vessels.

PAS stained sections of Acryl group, the RC, PCT and DCT showed partially lost and ill-defined weak PAS positive basement membranes and PCT showed partial to complete loss of their apical brush borders (Figure 3B). Acryl-Vitamin E group appeared with PAS positive and intact basement membranes of RC, PCT and DCT and the brush borders of cells of PCT (Figure 3C).

Anti-NF-k β p65 immunostained sections of control kidney showed NF-k β negative immunoreaction in RC components and renal tubules epithelium (Figure 4A). Acryl group exhibited strong positive NF-k β immunoreaction in the epithelial cells of RT and cells of glomerulus (Figure 4B), whereas in Acryl- Vitamin E group, weak positive NF-k β immunoreaction was detected in few numbers of cells of glomerulus and renal tubules (Figure 4C).

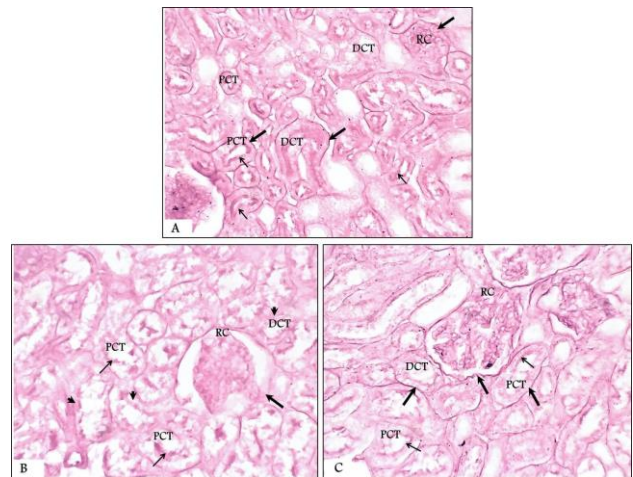


Figure 3: A photomicrograph of PAS stained sections in the renal cortex of A): Control group shows renal corpuscle (RC), proximal (PCT) and distal convoluted tubules (DCT) with PAS positive well defined basement membranes (thick arrows). PCT epithelial cells have prominent PAS positive apical thick brush borders (thin arrows). B): Acryl group shows weak PAS positive basement membranes (arrow heads) with partial to complete loss of apical brush borders of PCT (arrows). C): Acryl-Vit E group appears with PAS positive (arrows) areas. (PAS stain; A, B and C 400 X).

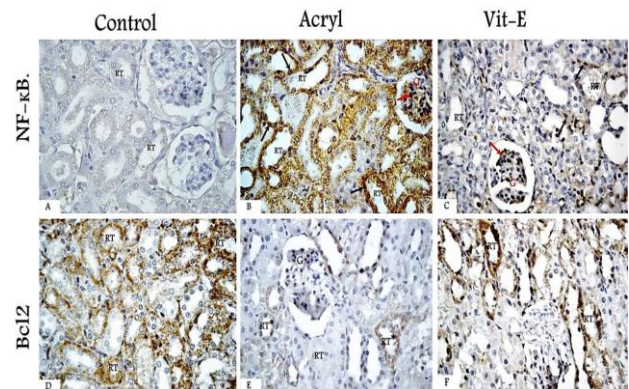


Figure 4: A photomicrograph of Anti-NF-k β p65 immune-stained sections of renal cortex (A, B and C) and Anti-BCL2 immuno-stained sections of renal cortex (D, E and F).

A) and D): Control kidney showed NF-k β p65 negative immunoreaction (A). It exhibit strong Bcl2 positive cytoplasmic reaction in cells of most tubules (D). B) and E): Acryl group exhibited strong positive NF-k β p65 immunoreaction in the epithelial cells of RT (black arrows) and cells of glomerulus (red arrows) (B). It showed very weak Bcl2 immunoreaction in few cells of renal tubules (E) C) and F): Acryl- Vit E group show weak NF-k β p65 immunoreaction in few cells (red arrow) of glomerulus and few cells (black arrow) of renal tubules (C). It showed strong Bcl2 positive reaction in cells of most tubules. G: glomerulus (F). (Anti-NF-k β p65 immune-stain; A, B and Cx400 and Anti-BCL2 immuno-stain; D, E and Fx400). RT: renal tubules G: glomerulus, RT: renal tubules.

Anti-BCL2 immuno-stained sections of Control renal cortex exhibited strong Bcl2 positive cytoplasmic immune reaction which was detected in cells of nearly all renal tubules (Figure 4D). Acryl group showed very weak Bcl2 immunoreaction in few numbers of cells of renal tubules and glomerulus (Figure 4E). On the other hand, Acryl- Vitamin E group showed strong Bcl2 positive reaction in the epithelial cells of the large number of renal tubules (Figure 4F).

Electron microscopic results

In Acryl group, both DCT and PCT showed signs of degeneration of its cells in the form of PCT cells loss of brush borders, degeneration and loss of normal orientation of mitochondria and separation of cells with loss of lateral border interdigitations in cells of DCT. Glomerulus showed abnormal shape and orientation and marked irregularity of secondary feet processes of podocytes with extensive vacuolations of their cytoplasm. Lumen of glomerular capillary contained eosinophil cell with its characteristic cytoplasmic ellipsoid granules. Apparent thinning of glomerular basement membrane was evident (Figure 5E-H).

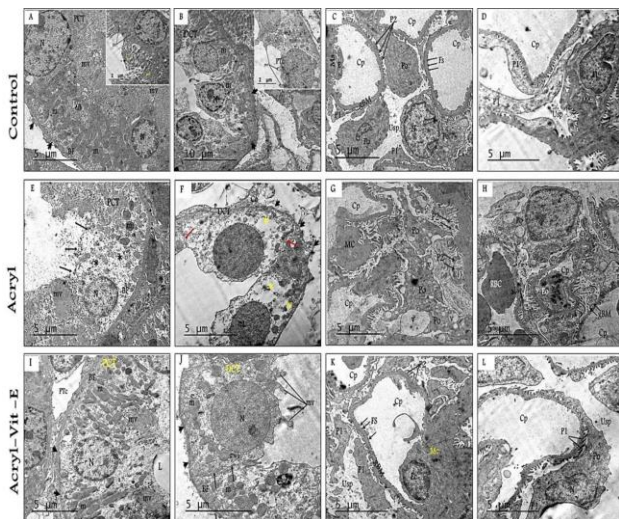


Figure 5 (A-D): Electron micrographs of control kidney.

A): PCT lining cell surrounded with intact basement membrane (arrow head) with show basal infolding (BF) in between them longitudinally arranged mitochondria (m). The cells contain basal rounded nuclei (N), apical endocytic vacuoles (Ev) with microvilli (mv) forming the characteristic brush border of PCT (Inset). B): DCT lining cell surrounded with intact basement membrane (arrow head) which show basal infolding (BF) with longitudinally arranged mitochondria (m) in between. The lining cells contain rounded nuclei (N) with apical microvilli (mv) (Inset). C): glomerular capillary loops (Cp) invested with 2nd feet (P2) which extend from 2nd processes (P1) of podocytes (Po). Capillaries lined by endothelial cells (E) which show fenestrae (Fs) and rest on glomerular basement membranes (gBM). D): Capillary loop (Cp), 2nd processes (P1) of podocytes, mesangial cell (Mc) and urinary space (Usp). Acryl group (E,F,G and H); E): PCT degenerated of cells with loss of

brush borders (arrows), focal loss of cytoplasm (asterisks) and degeneration of mitochondria(m). F): Degenerated DCT with thin and irregular basement membrane (BM), nuclear lysis (N) and loss of lateral interdigitations (red arrows). G): Irregular of capillary loops (Cp), irregular 2nd feet (P2) of vacuolated (V) podocytes. H): Glomerular capillary (Cp) with an eosinophil (Eo) with ellipsoid granules. Thin glomerular basement membrane (gBM). Acryl -Vit E group (I, J, K and L); I) preserved PCT structure. J): preserved DCT cells with basement membrane (arrow heads), lateral cell borders interdigitations (arrows). K and L): Glomerular capillaries with normal 1st (P2) and 2nd feet (P1) processes of podocytes. (Uranyl acetate and Lead citrate X 2000, Print Mag. X 11700).

In Acryl -Vitamin E group, the PCT and DCT appeared of nearly normal shape with their lining cells showed preservation of apical microvilli of PCT, basement membrane with basal infoldings and longitudinally oriented mitochondria, lateral interdigitations and apical border stubby microvilli of DCT. Glomerular capillaries appeared of normal shape and showed normal shape and orientation of primary and secondary feet processes of podocytes which invested the glomerular basement membranes (Figure 5I-L).

Biochemical and morphometric statistical results

A highly significant increase in the mean concentration of serum Creatinine, BUN and LDH, and a highly significant decrease in the mean concentration of serum albumin was detected in group II when it was compared to Control group. On the other hand, A highly significant decrease in the mean concentration of serum Creatinine, BUN and LDH and a highly significant increase in the mean concentration of serum albumin was observed in group III when it was compared to group II (Table 1).

A highly significant decrease in the mean epithelial thickness of PCT and DCT and a highly significant increase in the mean diameter of urinary space in Group II when it was compared to control group. Group III showed a highly significant increase in the mean epithelial thickness of PCT and DCT and a highly significant decrease in the mean diameter of urinary space when it was compared to group II (Figure 6).

Regarding the main area percentage of collagen fibers deposition in the renal tissue, it showed a highly significant increase in group II when it was compared to control and a highly significant decrease in group III when it was compared to group II (Figure 7A).

A highly significant increase in the mean number of NF- κ p65 and a decrease in the main area % of Bcl-2 immuno-expression were observed in the group II when it was compared to control ones. A highly significant decrease in the mean number of NF- κ p65 and increase in the main area % of Bcl-2 immuno-expression was observed in the group III when it was compared to group II (Figure 7B and C).

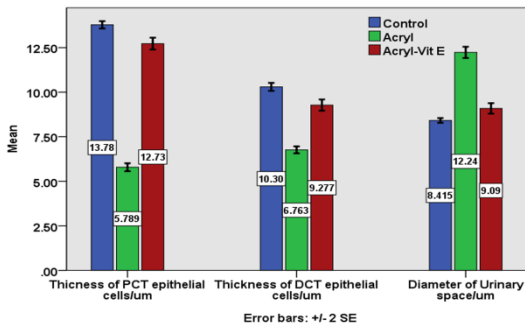


Figure 6: Clustered bars show a highly significant decrease in the mean epithelial thickness of PCT&DCT and a highly significant increase in the diameter of urinary space in Acryl groups when it is compared to control group. Acryl-Vit E group show a highly significant increase in the mean epithelial thickness of PCT and DCT and a highly significant decrease in the diameter of urinary space in Acryl groups when compared to Acryl group. Values are expressed as mean ± standard error of mean (SE). P1< 0.001, P3< 0.001. (P1; Acryl vs Control. P3; Acryl vs Acryl-VitE).

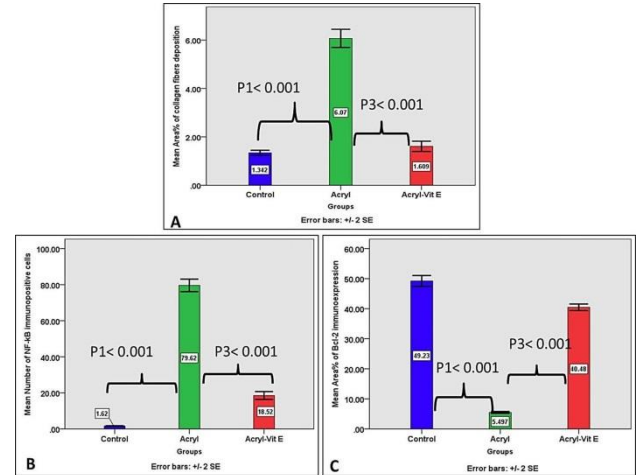


Figure 7: Bars morphometry of A) Mean area % of collagen fibers deposition, B) Mean number of NF-kβ immune-positive cells C); Mean area% of Bcl-2 immuno-expression in the renal tissue of different groups. Values are expressed as mean ± standard error of mean (SE). P1; Acryl vs Control, P3; Acryl vs Acryl-VitE.

Table 1: Mean concentration of creatinine, BUN, LDH and Albumin in the serum (mg/dL).

	Creatinine (mg/dL)	BUN (mmol/L)	LDH (U/L)	Albumin (g/dL)
Group I (Control)				
Mean± SE	0.66± 0.32	37.28 ± 1.24	462.48± 9.76	4.25 ± 0.056
Group II (Acryl group)				
Mean ± SE	1.48± 0.11	112.47 ± 3.96	1397.25 ± 30.50	3.15 ± 0.061
P value 1	< 0.001***	< 0.001***	< 0.001***	< 0.001***
Group III (Acryl-Vit E Group)				
Mean± SE	0.86 ± 0.04	48.54 ± 1.80	570.87 ±15.63	3.85 ± 0.109
P value 2	0.063 (non-significant)	0.005**	0.001***	0.001***
P value 3	< 0.001***	< 0.001***	< 0.001***	< 0.001***

Data are expressed as Mean ± SE: (SE :Standard error of mean), P value 1; Acryl group compared to control group, P value 2; Acryl-Vit E , compared to control group, P value 3; Acryl-Vit E group compared to Acryl group, Non-significant, P value >0.05, ** Significant P value ≤0.01, ***highly significant, P value ≤ 0.001.

DISCUSSION

In this research, our aim was to study the effects of ACR and vitamin E on the kidney. In group II (ACR-treated group) showed shrunked renal corpuscle with loss of parietal layer of Bowman’s capsules and wide urinary space. The cells of renal tubules showed vacuolations and pyknotic nuclei. Other research used several doses of ACR (0.5, 5, 50, 100 mg/kg ACR per day for 11 days intraperitoneally) to investigate the nephrotoxic effect of ACR. The H&E technique of the low doses (0.5 and 5 mg/kg) showed normal renal cortex while, the high doses of (50 and 100 mg/kg) showed proliferative glomerulonephritis. It had been concluded that, the ACR induced acute nephrotoxicity.³⁴ These findings were in harmony with another research that was studied the

effects of several doses of ACR on the kidney which showed degeneration of the glomerular tuft with lymphocytic infiltration. The cells lining renal tubules showed cytoplasmic vacuolations and loss of brush borders.^{35,36}

Our results from ACR-treated group revealed that the renal corpuscle appeared with significant increase in the amount of collagen fibers deposition among its glomerular capillaries. This is can explain by, ACR induced oxidative stress, resulting in generation of reactive oxygen species ROS which plays a critical role in the initiation and progression of fibrotic diseases. Renal fibrosis is a common pathway of progressive renal diseases leading to end-stage renal disease regardless of the etiology. The progression of fibrosis involves

interstitial hypercellularity, matrix accumulation, and atrophy of epithelial structures, resulting in loss of normal function and ultimately organ failure.³⁷

The PAS results of this research were similar to another research that was done to study the effect of cisplatin (CP) on the kidney, revealed, weak positive PAS reaction in the brush border and basement membranes of the healthy PCT and DCT in CP-treated rats.³⁸

All the above mentioned apoptotic figures were confirmed by strong positive cytoplasmic and nuclear NF- κ B immunoreaction in the epithelial cells of the renal cortex compared with very weak Bcl2 immunoreaction. These results were approved statistically and by another study which concluded that the ROS activates NF- κ B, which then ultimately leads to activation of ERK1/ERK2 pathway that lead to fibronectin expression.^{37,39} In a previous study, there was downregulation of Bcl2 in gentamicin induced nephrotoxicity. The NF- κ B controlled through interaction with an inhibitor protein called I κ B. In normal cells, NF- κ B is inactive in cytoplasm through its binding with its inhibitors, p105 and I κ B α -like proteins. The increased ROS in rat kidney causes the degradation of its inhibitor I κ B-alpha or Proteolytic cleavage of p105, and free NF- κ B dimers translocate to nucleus and activate the target anti-inflammatory genes.⁴⁰

The histopathological results of the effect of ACR on the kidney (Group II) were approved by electron microscopic study of the effect of ACR on the RC, PCT and DCT. These findings agreed with ultrastructure findings of the effect of cisplatin (CP) on the kidney revealed that the renal corpuscles showed wide capsular space, fused foot processes of podocytes, dilated congested capillary loops and irregular capillary basement membrane. The PCT of the same study revealed irregular thick basement membrane with loss of its basal infoldings. However, the DCT of CP-treated rats showed apoptotic nuclear changes with fragmented nuclear envelop, thick basement membrane with few basal infoldings, small sized degenerated mitochondria, many lysosomes and cytoplasmic vacuoles.³⁸

The harmful effects of ACR were also approved by the biochemical results which showed high significant increase in the mean concentration of serum creatinine, BUN and LDH, and a high significant decrease in the mean concentration of serum albumin.

In this study ACR was used in a dose of 20 mg/kg body weight once daily for 28 days.²⁸ Other researchers used ACR only in three doses (2, 10 and 30 mg/Kg/day for eight weeks). They found no significant differences in s. creatinine level between the low dose ACR, but it showed a significant decrease in both mild and high dose groups associated with significant decrease in albumin showed in all treated groups.³⁵

On the other hand, some authors reported that the BUN and creatinine levels significantly increased in the ACR treated group (40 mg/kg/day intraperitoneally). They explained these results as the higher dosage of ACR with a different route of administration could have increased the absorption of the toxin and in turn increased the damage.⁴⁰

Other researchers used different doses of ACR (10, 30, 60 and 90 mg/kg/day for 6 weeks) followed by Recovery period was adopted thereafter for 4 weeks. They found that the albumin in rats fed on 10 mg/kg dietary ACR was significantly decreased, but there was non-significant change in albumin level in rats fed on 90 mg/kg dietary ACR. While in the recovery period, there were no significant differences in the concentrations of the albumin between the groups of their experiment. The hypoproteinaemia in rats fed on different concentrations of ACR might have resulted from hepatocellular dysfunction.¹⁴

Glycidamide has a close relationship to the toxic mechanism of ACR. In order to explore the toxic mechanism of ACR, some authors discussed the effects of intragastric and intraperitoneal administration of glycidamide-induced toxicity by determining the LDH, BUN and creatinine. They found that the same dose of glycidamide had more toxic effects and damage effects to the mice compared to the ACR. It could markedly increase the level of LDH and BUN.⁴¹

On the other hand, vitamin E is the main endogenous antioxidant which reacting with oxygen radical prevents the chain reaction of free radicals, protecting thus the membranes. However, the endogenous antioxidants reserves, such as vitamin E, gradually decrease in reactions with free radicals.⁴²

In group III (ACR-Vitamin E treated group), showed nearly preserved renal cortex with significant decrease in both the width of urinary space. In two different studies used Vitamin E. to minimize the Nickle and Colistin Methane Sulfonate induced nephrotoxicity, the histopathological studies showed remarkable reduction in the nickel-induced PCT degeneration and tubular necrosis, multiple foci of hemorrhage and inflammation with the presence of lymphocytes in interstitial tissue by the administration of vitamin E. There was a relatively normal appearing urinary space area of the RC.⁴³⁻⁴⁵ The results from the same (group III) showed also collagen fibers deposition in the renal tissue. These tubules were also surrounded with minimal amount of collagen fibers with absence of fibrosis. In another research used vitamin E against aflatoxin induced nephrotoxicity, the Masson's trichrome stain sectioned revealed few collagen fibers were be detected nearly similar to the control ones.⁴⁵

ACR-Vitamin E treated group (Group III) appeared with nearly preservation of PAS positivity and intactness of

basement membranes of renal corpuscle RC, PCT and DCT and the brush borders of cells of PCT. In PAS-stained sections to investigate the antioxidant effect of aged garlic extract against cisplatin induced nephrotoxicity, strong positive PAS reaction was observed in the brush border of proximal convoluted tubules (PCT), basement membrane of both PCT and distal convoluted tubules (DCT), and parietal layer of Bowman's capsule.³⁸

There was also weak positive cytoplasmic and nuclear NF- κ B immunoreaction with strong Bcl2 positive reaction. Several agents antagonize NF- κ B, such as drugs in clinical use for treatment of renal disease, e.g. steroids, statins, and vitamin D receptor activators. During recovery from renal injury, there was downregulation of NF- κ B and upregulation of anti-inflammatory genes. There is experimental evidence supporting the critical role of NF- κ B activation in the pathogenesis of renal inflammation.⁴⁶

The ultrastructure results from group III (ACR-Vitamin E treated group) revealed glomerular capillaries normal shape and showed normal shape and orientation of primary and secondary feet processes of podocytes. The ultrastructure study of PCT and DCT appeared of nearly normal shape with their lining cells showed preservation of apical microvilli of PCT, nuclei with prominent nucleolus. The basement membrane showed normal basal infolding and longitudinally oriented mitochondria, lateral cell borders interdigitations and apical border stubby microvilli of DCT. In another research to study the ultrastructure antioxidant effect of vitamin -E against Aflatoxin induced nephrotoxic effect, it showed nuclei of the podocytes and mesangial cells appeared vesicular nearly similar to those of control. It also showed, PCT were lined by cells having relatively normal appearing mitochondria, extensive microvilli at their apical border and euchromatin inside nucleus. The DCT were also lined by mildly affected cells containing many normal shaped mitochondria mostly infranuclear similar to those in the control and mildly affected basal in foldings.⁴⁵

The protective effects of Vitamin E on the ACR-treated rats were also confirmed in our study by the significant decrease in the mean concentration of serum creatinine, BUN and LDH and a highly significant increase in the mean concentration of serum albumin.

CONCLUSION

Apoptosis plays a central role in inflammation as well as in many renal diseases and drug induced nephrotoxicity.⁴⁶ Bcl-2 acts as an anti-apoptotic protein. Bcl-2 binding to the mitochondrial outer membrane inhibits cytochrome c activation.⁴⁷ The ACR-treated rats displayed the upregulation of NF- κ B and down regulated Bcl-2 expression. These results further suggested that ACR administration induces apoptosis as well as inflammation, resulting in tubular necrosis in kidney tissues. Vitamin E

supplementation with ACR significantly protected and prevented injuries associated with renal tubular apoptosis and minimized the renal protein expression of NF- κ B, thereby inhibiting renal tubular apoptosis associated with ACR-induced nephrotoxicity alone. The results from this study corroborate previous findings [20, 29, 30 &31]. Our results indicate that Vitamin E may be an appropriate target molecule for renal protection from ACR-induced nephrotoxicity.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee

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Cite this article as: Atef H, Attia GM, Rezk HM, El-Shafey M. Effect of vitamin E on biochemical and ultrastructural changes in acrylamide-induced renal toxicity in rats. *Int J Sci Rep* 2017;3(5):134-43.