

The possible protective Effects of Alfa Lipoic Acid on Diethanolamine-Induced Renal Toxicity in Adult Male Albino Rats: A Histological and Immunohistochemical Study

Original
Article

Heba K. Mohamed¹ and Fatma Y. Meligy²

¹Department of Human Anatomy and Embryology, ²Histology and Cell Biology Department, Faculty of Medicine, Assiut University, Egypt.

ABSTRACT

Background: Diethanolamine (DEA) is an organic agent, used in cosmetic preparations and has been shown to cause degenerative changes in the kidney. Alfa lipoic acid (ALA) is a disulfide natural compound with established antioxidant effects.

Aim of the work: The aim of the current study was to evaluate the protective role of ALA on the renal histological structure of DEA-treated rats.

Materials and Methods: Thirty adult male albino rats were equally classified into three groups: a control group receiving distilled water, DEA-treated group receiving DEA (425mg/kg/b.w) orally six days/week for two weeks and DEA+ALA-treated group receiving ALA (100 mg/kg/b.w.) simultaneously with the same dose of DEA for the same duration. The specimens were obtained from the kidney and processed for histological and immunohistochemical examination. Morphometric studies and statistical analysis were performed.

Results: Light microscopic examination of DEA-treated rats showed disturbed renal architecture, increased glomerular size, widened Bowman's space and cell debris in the lumen of the tubules. Congested interstitial blood capillaries and loss of the brush border in some proximal convoluted epithelial cells were also noticed. Ultrastructural examination following DEA administration showed thickened glomerular basement membrane and electron-dense podocytes with fusion of their feet-processes. The tubular lining cells showed electron-dense mitochondria with distorted cristae and loss of cytoplasmic organelles and nuclear heterochromatin. Moreover, DEA administration induced intense cytoplasmic expression of caspase-3 and inducible nitric oxide synthase. The administration of ALA in DEA-treated rats significantly improved the histological and immunohistochemical changes in renal corpuscles and proximal and distal convoluted tubules, compared to DEA-treated rats.

Conclusion: It could be concluded that ALA protects the kidney against the damaging effects induced by DEA.

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Key Words: Alpha Lipoic Acid; diethanolamine; kidney; rats; ultrastructure.

Corresponding Author: Heba K. Mohamed, PhD, Department of Human Anatomy and Embryology, Faculty of Medicine, Assiut University, Egypt, **Tel.:** +20 1001016547, **Fax:** +20 88 2343703 **E-mail:** hebaelgamae73@yahoo.com

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INTRODUCTION

Cosmetic products contain different chemical substances, including possible carcinogens and endocrine disruptors^[1]. One of these chemicals is diethanolamine (DEA), which is a general ingredient of care products^[2]. Diethanolamine belongs to the family of ethanolamines, which consists of monoethanolamine (MEA), DEA, and triethanolamine (TEA). These chemicals have two functional groups (amino and hydroxyl), enabling them to act as intermediates of surfactants in soaps and pharmaceuticals applications^[3]. Diethanolamine is an organic compound that does not occur in nature, but results from ethylene oxide and ammonia reaction. Its amide salts are present in cosmetics, detergents, shampoos and hair

conditioners to make the cosmetic product more creamy or sudsy. Moreover, it has been used as a pH adjuster to neutralize other ingredients with high acidity^[4].

Exposure to DEA and its conjugates occurs via dermal contact with personal care products, detergents, and other surfactants^[5, 6]. Environmental release of DEA can occur from industrial sites, dispersing agents in agricultural chemicals or from the disposal of consumer products. Biodegradation results in an environmental half-life of days to weeks^[7, 8]. In rodents, oral absorption is essentially complete. Both male and female rats show significant elimination of DEA in urine, as well as bioaccumulation (especially in hepatic and renal tissues) with tissue/blood ratios up to 200. Oral DEA intake is more dangerous

than dermal administration because the liver receives the toxicant directly through the portal system^[9].

Diethanolamine was used as a carcinogen in lab animals (rats and mice), mainly to induce liver neoplasms^[3]. In addition, its toxic adverse effects include renal tubule hyperplasia, thyroid gland follicular cell hyperplasia and skin hyperkeratosis^[10]. Diethanolamine is incorporated into phospholipids with long residence times^[11]. The resulting alteration of biomembrane structure and function has been proposed as a mechanism of DEA toxicity^[12] that affects multiple organs in lab animals, such as the brain, liver, kidneys and testes^[13-16].

Alpha lipoic acid (ALA) [1,2-dithiolane-3- pentanoic acid or thioctic acid] is a naturally occurring disulphide compound, synthesized by an enzymatic pathway in plants and animals mitochondria from octanoic acid and cysteine as sulfur sources^[17]. It is an endogenously produced coenzyme, involved in the enzymatic reactions of pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase, required for cellular energy production^[18]. It is recognized as a potent antioxidant due to its ability to prevent lipid peroxidation and scavenge the reactive oxygen species (ROS), such as proxy nitrite, nitric oxide (NO), hydroxyl radicals, and superoxide anion^[19-23]. Moreover, it has anti-inflammatory properties and has been shown to inhibit the progression of created atherosclerotic plaques^[24]. In a recent study in male rabbits, ALA has been shown to protect against diabetic nephropathy by scavenging oxidative free radicals^[25].

In light of the above inferences from other evidence, we performed the present study to investigate the possible nephroprotective role of ALA against DEA -induced nephrotoxicity.

MATERIAL AND METHODS

Chemicals

- Diethanolamine was purchased from El-Gomhuria Company for Chemicals and Medical Trading (Assiut, Egypt) as a colorless liquid. Its chemical structure is $(\text{CH}_2(\text{OH})\text{CH}_2)_2\text{NH}$, while ALA (Thioctic acid) was obtained from Marcyrl Pharmaceutical Industries, Egypt as a white powder.
- Caspase-3 was purchased from Thermo scientific Company, USA.
- Inducible nitric oxide synthase (iNOS) was purchased from Thermo Scientific Company, USA. Other reagents were of analytical grade and were obtained from commercial sources.

Animals:

Thirty adult male albino rats, weighing 200-250 gm, were used in this study. Animals were kept in the animal house of Assiut University in cages containing bedding of fine wood, changed twice weekly under a 12-h light dark cycle and a temperature of 25 ± 5 C°. All rats were supplied with food and water ad libitum. This experiment was complied

with the standard guidelines of animal ethics committee (Assiut University) in accordance with the internationally accepted principles for the laboratory animal use and care. Rats were randomly divided into three equal groups (10 rats each): a control group (group I) receiving distilled water only, DEA-treated group (group II) receiving DEA (425mg/kg/b.w) orally^[26] and DEA+ALA-treated group (group III) receiving ALA (100 mg/kg/b.w.) dissolved in 3 ml of sterile distilled water simultaneously with DEA (425mg/kg/b.w)^[27]. All treatments were administered six days a week for two weeks.

Histological study

At the end of the experimental period, the animals were sacrificed under general anesthesia by inhalation of chloroform. Four rats from each group were perfused intracardially with 10% formaldehyde solution. The specimens were immersed into 10% formaldehyde solution to continue fixation for two more days, processed and embedded in paraffin and serially cut to 5 μm -thick sections to be stained with Hematoxylin and Eosin (HandE)^[28].

Ultrastructural studies of the kidney in all groups were performed. Six rats from each group were perfused intracardially with 4% glutaraldehyde in cacodylate buffer (pH=7.4). The specimens were cut into thin slices (1 \times 1 mm) and immersed in 4% glutaraldehyde solution for 20 h, then washed with PBS and fixed in 1% osmium tetroxide. Semithin sections (1 μm) were prepared and stained with 1% toluidine blue and examined under a light microscope. Ultrathin sections (80-90 nm) were cut with an ultramicrotome, stained with uranyl acetate and lead citrate and carried on copper grids^[29] to be examined and photographed with transmission electron microscope (Jeol-JEM- 100 CXII; Jeol, Tokyo, Japan) in Assiut University Electron Microscopic Unit.

Immunohistochemical study

The expression of inducible nitric oxide synthase (iNOS) and caspase-3 was detected in formalin-fixed, paraffin-embedded sections^[30]. Sections (5 μm thick) were deparaffinized in xylene, rehydrated in alcohol and added to iNOS and caspase-3 rabbit polyclonal antibodies (1:100 dilution) for 20 minutes at room temperature. Sections were boiled in 10mM citrate buffer (pH 6.0) for 15 minutes followed by cooling at room temperature for 20 minutes. Later, sections were processed according to the manufacturer's instructions, using the universal kit. After completion of the reaction, counterstaining was done using Mayer's hematoxylin, dehydrated and cover slipped using DPX. Negative controls were processed according to the same protocol, except for the use of the primary antibody. A lung slide was used as the positive control for iNOS and a tonsil slide was used as the positive control for caspase-3.

Morphometric procedure and statistical study

A morphometric study was performed, using computer-assisted image analysis (Soft Imaging System,

AnalySIS-2004, Olympus Company, Tokyo, Japan). The cortical thickness, glomerular size and renal space measurements were done, using an objective lens (X40) in six non-overlapping fields in ten randomly chosen sections from three different animals from each group. The morphometric data of each animal group were statistically analyzed by SPSS software (version 22, Chicago, USA), using the ANOVA followed by post-hoc Tukey's test. The results were expressed as the mean \pm standard deviation (SD) and a *p* value < 0.05 was considered statistically significant.

RESULTS

Light microscopic results

HandE stained sections from control rats showed a normal renal architecture. The kidney is covered by a capsule and the outer cortex and inner medulla are noticed (Fig. 1A). The cortex contained the renal corpuscles, proximal (PCT) and distal convoluted (DCT) tubules. Each renal corpuscle was formed of a glomerulus, surrounded by a Bowman's capsule with its two layers (parietal and visceral) and renal space in between. The PCT had a narrow lumen, lined by a simple high cuboidal epithelium with intact brush border, vesicular round nuclei and acidophilic cytoplasm. The DCT showed wider lumen, lined by low cuboidal cells with ill-defined brush border (Fig. 1B).

The semithin sections, stained by toluidine blue, revealed that the renal corpuscle is formed of a glomerulus, surrounded by a Bowman's capsule, lined by simple squamous epithelium, which is the parietal layer and the inner visceral layer consisted of glomerular epithelium (the podocytes). The intra-glomerular mesangial cells could be seen. The PCT were lined by high cuboidal cells with prominent brush border and well-formed basal striations. The nuclei were rounded, vesicular and basal. The DCT had shorter cuboidal cells with basal striations and no brush border (Fig. 2A).

The sections from the kidney of DEA-treated rats, stained by HandE, showed deformed renal tissue architecture with increased cortical thickness and glomerular size (Histogram 1 and 2). Some glomeruli showed complete degeneration, while others showed lobulation with wide Bowman's spaces (Histogram 3). Vacuolations were observed in some tubular cells and cell debris from the desquamation of tubular epithelium was scattered in lumen of the tubules. Areas of hemorrhage in the interstitial tissue were seen (Figs 1C, D and E).

The semithin sections showed that renal corpuscles had widened spaces and dilated congested blood vessels. Some nuclei of the glomerular epithelium appeared dark, whereas others looked pale. Dilated and congested interstitial blood capillaries were noticed (Fig. 2B). Some of the epithelial lining of the PCT lost the brush border and the basal striations. The cytoplasm of some cells showed marked vacuolations. Some cells appeared pale with poorly stained rarified cytoplasm and other cells appeared darkly stained

with dense irregular nuclei. Some of the epithelial lining of DCT showed cytoplasmic vacuolations and loss of basal inholdings (Fig. 2C).

The HandE stained sections from the DEA+ALA-treated group showed preservation of the renal structure compared to DEA-treated rats, in the form of preserved cortical thickness with average glomerular size and no widening of the Bowman's space (Fig. 1F and Histograms 1, 2 and 3). Some renal convoluted tubules appeared intact; however, others still had slight vacuolations in the cytoplasm of tubular epithelial cells (Fig. 1G). The semithin sections of this group showed restoration of the brush border and basal striations of the PCT cells (Fig. 2D).

Immunohistochemical results

The immunohistochemical study of the control groups showed weak expression of iNOS and negative expression of caspase-3 in the renal tissue (Figs 3A and 4A, respectively). On the other hand, sections from DEA-treated rats showed intense iNOS and caspase-3 cytoplasmic immunoreactivity, mainly in the glomeruli and in the renal tubules (Figs 3B and 4B, respectively). Interestingly, the cytoplasmic expression of iNOS and caspase-3 was markedly reduced in DEA+ALA - treated group (Figs 3C and 4C, respectively).

Electron microscopic results

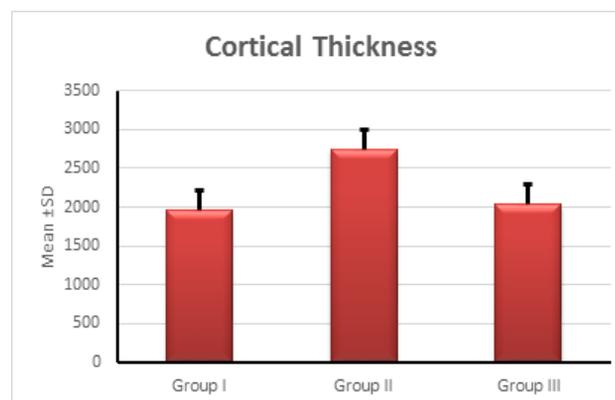
The ultrastructural examination of glomeruli from normal control rats showed the podocytes with large and indented nuclei and several primary cytoplasmic processes which arise from their body and give secondary processes or pedicles. These processes were separated by narrow slits, covered by slit membranes (Fig. 5A). The filtration barrier appeared in which the glomerular basement membrane (GBM) was outlined by the feet processes of podocytes from the outside and lined by fenestrated endothelium from the inside (Figs 5A and 5B). The cells of the PCT showed numerous apical microvilli, basal enfolding of plasma membrane, ovoid euchromatic nuclei with prominent nucleoli and several basally-located mitochondria. The cells rested on a well-developed basement membrane (Fig. 5C). The DCT lining cells had few apical microvilli and their nuclei were oval, more apically located with less basal infoldings of the plasma membrane and elongated mitochondria. The cells rested on a well-developed, less electron-dense basement membrane (Fig. 5D).

In DEA-treated rats, the ultra-sections showed podocytes with electron-dense cytoplasm and loss of the heterochromatin from some parts of the nucleus. The GBM appeared thickened with disruption of its layers. The podocytes' feet processes were irregular in some areas and disappeared in others. Fusion and broadening of some feet processes were also noticed (Fig. 6A). The PCT epithelial cells showed loss of the microvillar brush border of the apical cell membrane in some areas and apparent thickening of the basement membrane in others (Fig. 6B). The cytoplasm revealed areas of lysis and

vacuolation, extensive loss of cytoplasmic organelles with pushing of the nucleus towards the cell base and loss of its heterochromatic appearance. Few damaged and distorted mitochondria with disrupted cristae were seen around the nucleus and in the basal cytoplasm. The DCT epithelial cell had large areas of vacuolar degeneration in the cytoplasm, loss of nuclear heterochromatic appearance and massive lysis of its organelles. Some of DCT cellular mitochondria were giants, others were electron dense, and some had distorted cristae. Many lysosomes were seen. A dilated, fragmented rough endoplasmic reticulum could be observed (Fig. 6C).

In group III, the ultra-sections showed more or less normal thickness of the GBM and absence of pedicle fusion. The fenestrated endothelium appeared well-arranged (Fig. 7A). The PCT epithelial cells showed restoration of the microvillar brush border in some areas. The nucleus appeared nearly normal with heterochromatic appearance. Some areas of vacuolations were present in the cytoplasm (Figs 7B and C). The DCT epithelial cells showed preserved heterochromatic character of the nucleus and abundant

mitochondria, few of which had distorted cristae. Some areas of minute vacuolations were present in the cytoplasm (Fig. 7D).



Histogram 1: The mean of the cortical thickness in the studied groups

GI Vs II $p < 0.001$ GI Vs III $P < 0.10$ G II Vs III $P < 0.001$

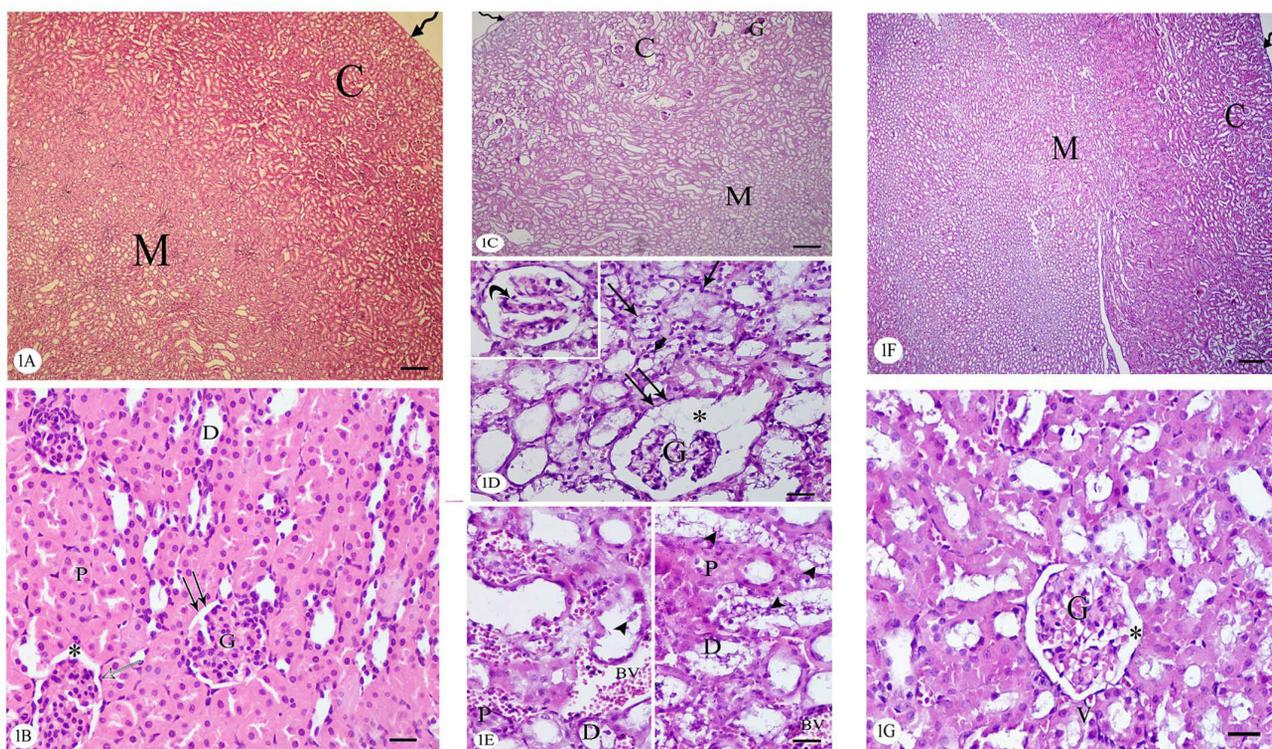


Fig. 1 Photomicrographs of sections in the kidney cortex: group I (A and B), group II (C-E), and group III (F and G). Scale bar of (A, C and F) is 500 μ m, (B, D, E and G) is 50 μ m. A. The cortex (C) and the medulla (M). The capsule is noticed (wavy arrow). B. Renal corpuscle (double arrow) with the parietal layer of Bowman's capsule (open arrow), glomerulus (G) and the renal space (star) are preserved. Note the proximal convoluted tubules (P) with high cuboidal acidophilic cells and narrow lumen and distal convoluted tubules (D) with low cuboidal cells and wide lumen. C. Apparent increase in the thickness of the cortex (C) with degenerative changes in some glomeruli (G) and medulla (M). Note the capsule which appears to be irregular and more thickened (wavy arrow). D. The renal corpuscles are dilated (double arrow), glomeruli appear shrunken (G) and the Bowman's space is widened (star). Some renal convoluted tubules (arrows) are damaged with vacuolation of cytoplasm of tubular epithelial cells. Deeply stained pyknotic nuclei are seen (thick arrow). Inset: A markedly lobulated glomerulus (curved arrow) is noticed. E. Degenerated some proximal (P) and distal tubules (D) with loss of normal architecture. Some of the lining epithelial cells are exfoliated into the lumen (arrow head). There are dilated congested blood vessels in the interstitium (BV). F. The cortex with average thickness (C) and the medulla (M). Note the capsule (wavy arrow). G. Renal corpuscle (double arrow) with average size. Glomeruli (G) appear well-organized with nearly normal Bowman's space (*). Some renal tubules (rt) are still damaged with slight vacuolations in the cytoplasm of their tubular epithelial cells (V). The majority of the tubules appear intact.

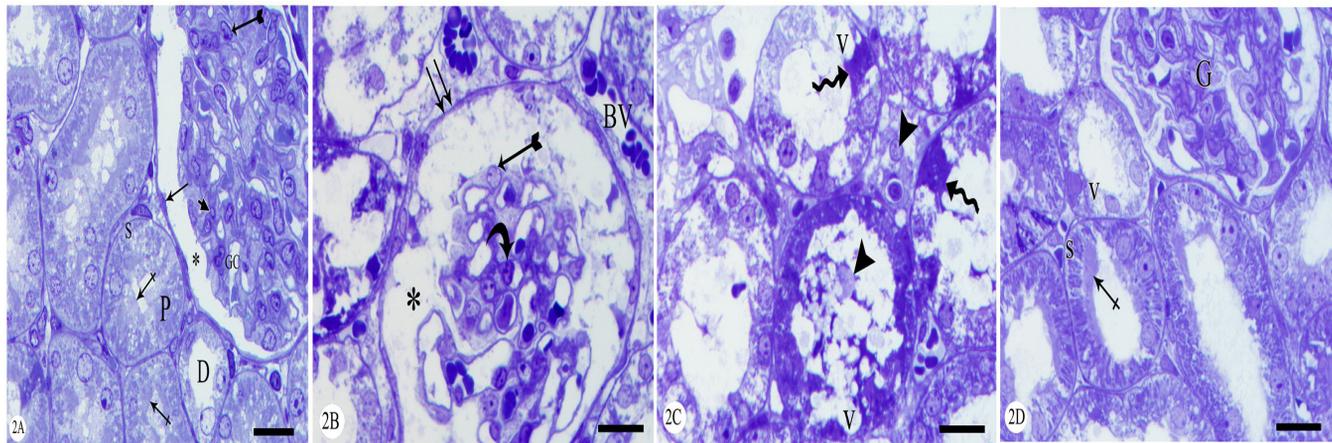


Fig. 2 Photomicrographs of semithin sections in the kidney cortex: group I (A), group II (BandC), and group III (D). Scale bar=20 μ m A. Part of a renal corpuscle with simple squamous epithelium lining the parietal layer of Bowman's capsule (arrow). The podocytes (tailed arrow) which line the visceral layer, the glomerular capillaries (GC) and the intra-glomerular mesangial cells (short arrow) can be noticed. The renal space (star) is preserved. A proximal convoluted tubule (p) lined by high cuboidal cells with brush border (crossed arrow) and well-formed basal striations (S) is observed. Note a distal convoluted tubule (D) with shorter cells, numerous basal striations, wide lumen and no brush border. B The renal corpuscle (double arrow) with widened renal space (star) and dilated congested blood vessel (bv) are observed. Some of the nuclei of podocytes appear dark (curved arrow), whereas other nuclei appear pale (tailed arrow). Note the dilated congested interstitial blood capillaries (BV). C. Extensive vacuolation of the lining epithelium of the renal tubules (V) with loss of the brush border, loss of the basal striations and poorly stained nuclei. There are changes in the staining density in cells: some cells appear darkly stained with dense irregular nucleus (wavy arrow) and others are pale. Note the cell debris and exfoliated cells inside the lumen (arrow heads). D. Preservation of the renal structure in the form of nearly normal glomerulus (G), preservation of the brush border of the proximal tubule (crossed arrow) and basal striations (S), but there are some areas of vacuolation in the cells (V).

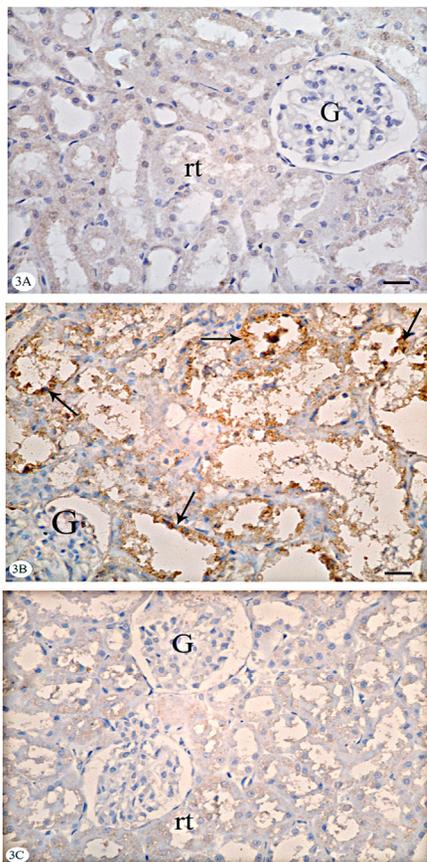


Fig.3 Photomicrographs of iNOS immune-stained paraffin sections:group I (A), group II (B), and group III (C).Scale bar=50 μ m. A. Negative iNOS immunoreactivity in the glomeruli (G) and renal tubules (rt). B. Intense iNOS- immunoreactivity in the glomeruli (G) and renal tubule (arrows). C. Weak iNOS- immunoreactivity in the glomeruli (G) and renal tubules (rt).

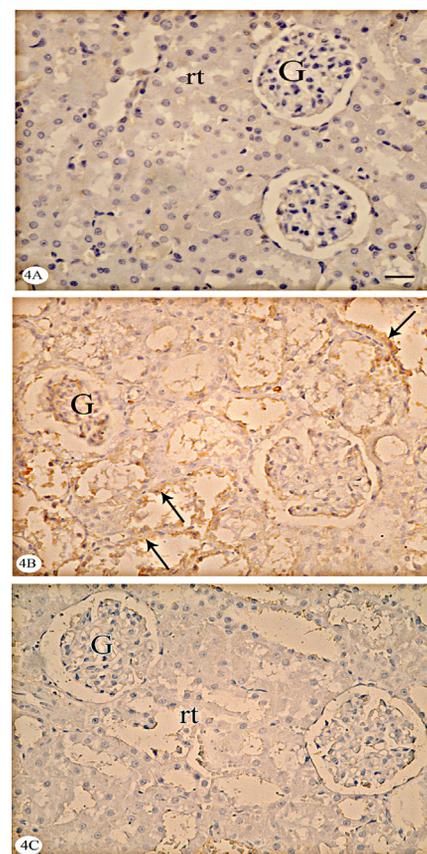


Fig.4 Photomicrographs of caspase-3 immunostained paraffin sections: group I (A), group II (B), and group III (C). Scale bar=50 μ m. A. Negative caspase-3immunoreactivity in the glomeruli (G) and renal tubules (rt). B. Strong caspase-3immunoreactivity in the glomeruli (G) and renal tubule (arrows) C. Faint caspase-3immunoreactivity in the glomeruli (G) and renal tubules (rt).

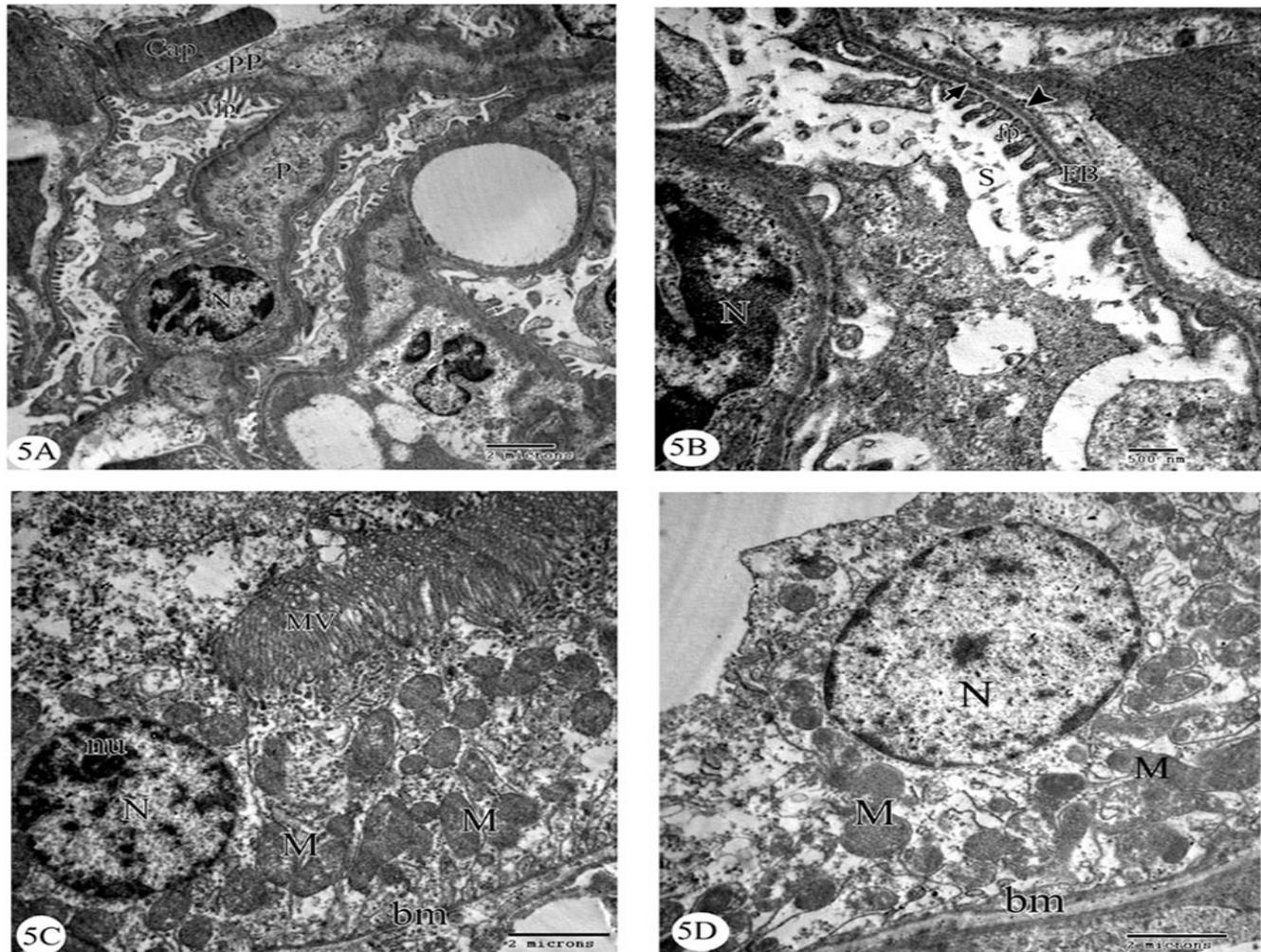


Fig. 5: Electron micrographs of ultrathin sections from the kidney cortex of group I: A. Part of the glomerulus which reveals the podocyte (p) with highly indented nucleus (n) and electron dense cytoplasm. From the podocytes arise the primary processes (pp), from which emerge the feet processes (fp). Note the blood capillary (cap). B. A higher magnification of the same previous section showing the filtration barrier (FB) which reveals the glomerular basement membrane (short arrow) attached by feet processes (fp) of podocytes from outside, and lined by fenestrated endothelium (arrow head) from inside. Note the nucleus of the podocyte (N). C. The epithelial cell of a proximal convoluted tubule with the apical cytoplasm containing numerous long apical microvilli (MV). The cytoplasm is electron dense has a large euchromatic nucleus (N) with a distinct peripheral nucleolus (nu) and peripherally arranged heterochromatin. Well-developed basal infoldings made by elongated radially arranged mitochondria (M) can be seen. The cell rests on a well-developed, less electron dense basement membrane (bm). D. The epithelial cell of a distal convoluted tubule. The cytoplasm is electron dense and contains a large oval apical nucleus (N). Few blunt apical microvilli (arrow) are seen. Elongated, basally-arranged mitochondria (M) are observed. The cells rest on a well-developed, less electron-dense basement membrane (bm).

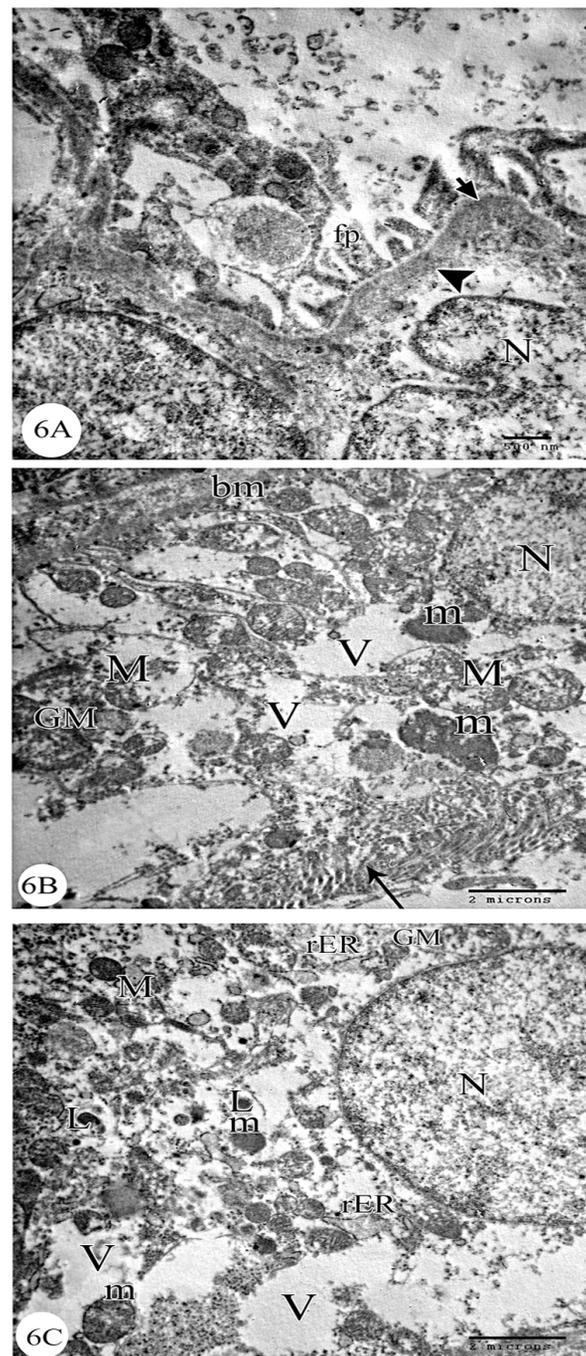


Fig. 6: Electronmicrographs of sections from the kidney cortex of group II: A. portion of the glomerular capillary basement membrane which appears thickened (short arrow) with disruption of its layers. Fusion and broadening of some of the podocytes feet processes (fp) and disappearance of fenestrated endothelium in some places (arrow head) are noticed. A part of the indented nucleus of the podocyte is seen showing loss of heterochromatin in certain parts (N). B. The proximal tubular epithelial cell in which, the apical cell membrane loses microvillar brush border in some areas with short apical microvilli in other places (arrow). The cytoplasm reveals many areas of lysis and vacuolations (V). Extensive loss of most of cytoplasmic organelles with pushing of the nucleus (N) towards the cell base and loss of its heterochromatic appearance were observed. Damaged and distorted mitochondria with disrupted cristae (M) are seen around the nucleus and in the basal cytoplasm. Some giant mitochondria (GM) and electron dense mitochondria (m) are observed. Note apparently thickened basement membrane (bm). C. The distal tubular epithelial cell which reveals loss of their normal architecture in the form of areas of vacuolar degeneration in the cytoplasm (v) with massive loss of most of its organelles. The cytoplasm contains scattered mitochondria with destroyed cristae (M). Some of mitochondria are giants (GM) and others are electron dense (m). Many lysosomes (L) are seen. Dilated fragmented rough endoplasmic reticulum (rER) is observed. The nucleus reveals loss of its heterochromatic appearance (N).

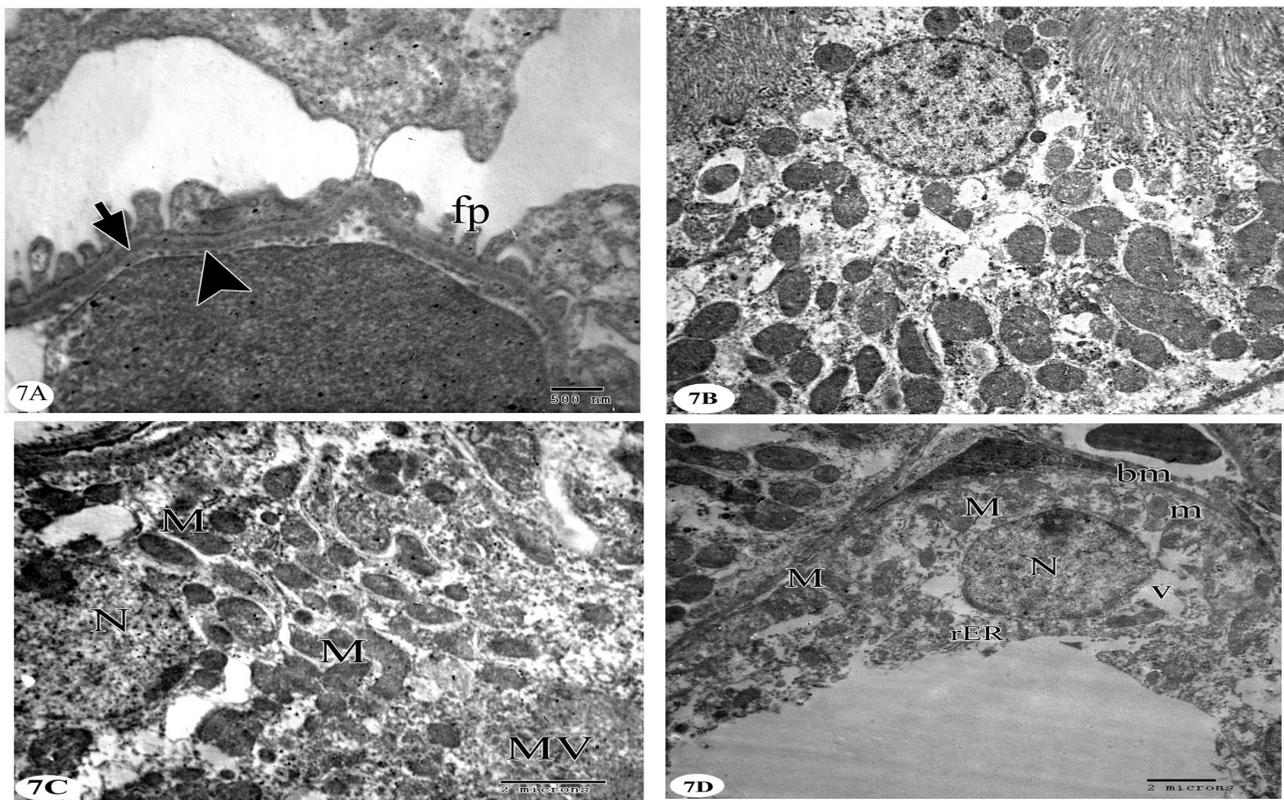
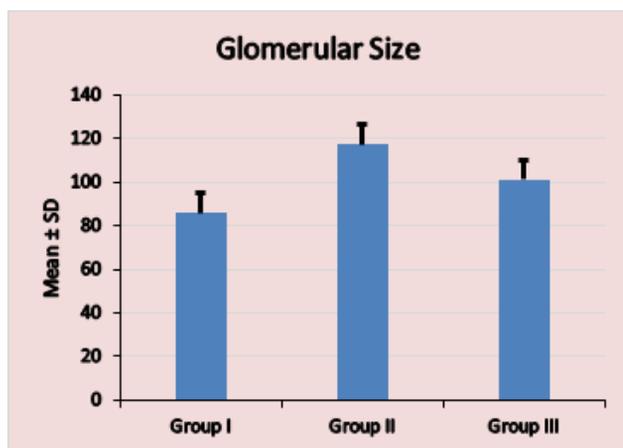
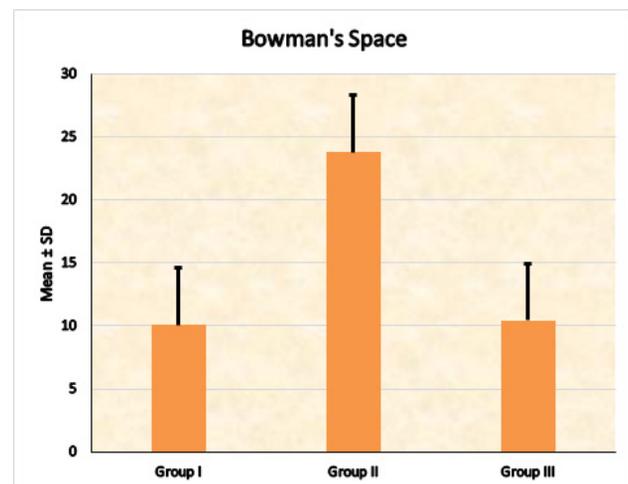


Fig. 7 Electronmicrographs of sections from the kidney cortex of group III: A. More or less normal glomerular capillary basement membrane (short arrow). No fusion in the pedicles is detected (fp). The fenestrated endothelium appears well arranged (arrow head). Band C. preservation of normal structure in the proximal tubular epithelial cell in the form of long microvillar brush border (MV). The nucleus appears more or less normal (N) and numerous mitochondria with the characteristic basal infoldings are seen and most of them appear healthy (M). Some areas of vacuolations are present in the cytoplasm (V). Note the basement membrane still thickened (bm). D. Remarkable preservation of normal structure in the distal tubular epithelial cell in form of heterochromatic character of the nucleus (N) and abundant mitochondria (M) but some appeared with distorted cristae (m). Note rough endoplasmic reticulum (rER). Minute areas of vacuolations are present (V). Note the basement membrane (bm).



Histogram 2: The mean of the glomerular size in the studied groups

GI Vs II $p < 0.001$ GI Vs III $P < 0.001$ G II Vs III $P < 0.001$



Histogram 3: The mean of the Bowman's space in the studied groups

GI Vs II $p < 0.001$ GI Vs III $P < 0.10$ G II Vs III $P < 0.001$

DISCUSSION

The published literature on DEA is insufficient due to the lack of adequate data on human exposure, reproductive and developmental toxicity, chronic inhalation, and health effects. The present study revealed that DEA administration in rats resulted in renal degenerative changes in the renal corpuscle and tubules. These results were compatible with those of Anon^[31] who reported that DEA, if not neutralized, may cause systemic toxicity mainly to the liver, kidneys, red blood cells and the nervous system following oral and/or dermal exposure in laboratory animals.

Pharmacokinetic studies showed that following absorption of orally-administered, radiolabeled DEA, about 70% remained in the hepatic and renal tissues, whereas the residual substance was excreted mainly in urine and faeces^[32, 33]. Moreover, several of DEA metabolites, such as N-methyl-DEA and dimethyl-DEA, were found in urine in significant amounts. It was reported that the changes were most prominent in the glomeruli, proximal and distal tubules^[10, 34]. The present findings were in agreement with other studies showing that prolonged DEA exposure induces nephropathy and tubular epithelial necrosis^[14-16]. Investigators from the National Toxicology Program postulated that the cellular changes in DEA-treated animals might be due to alterations in membrane phospholipid structure^[13]. In harmony, El-Mehallawi *et al.*^[26] reported that DEA might interfere with the synthesis of phospholipids, essential for normal membrane structure and function.

Interestingly, intense caspase-3 and iNOS cytoplasmic immunoreactivity were detected in this study, mainly in the glomeruli and renal tubules following DEA exposure. Noiri *et al.*^[35] reported that iNOS activation can lead to excessive production of NO, which can cause cytotoxic injury and renal failure exaggeration. Marked decreases in the expression of iNOS and caspase-3 were noticed in the current study after ALA administration. The present results were supported by El-Sayed *et al.*^[36] who noticed that ALA administration reduced the renal contents of NO and caspase-3 in doxorubicin (DOX)-treated rats. Forstermann^[37] postulated that the biological action of NO is mediated by activation of cytosolic guanylyl cyclase and secondary generation of cGMP. The stimulation of the NO/cGMP system can be reversed by ALA, which may explain its nephroprotective effect, as shown before in diabetic rats^[38].

In our work, increased cortical thickness and diameter of the renal corpuscle was noticed in DEA-treated rats, which was confirmed by morphometric analysis. Mosberg *et al.*^[39, 40] recorded significant increases in liver and kidney weights after a two-week inhalation exposure to TEA in rats and mice and regarded these increases as an adaptive response rather than a frank adverse effect. In ultrastructural examination, histological changes were observed in the filtration barrier in the form of local thickening and corrugations of the GBM, as well as disorganization of podocyte foot processes covering the

surface of the GBM. This membrane acts as molecular sieves, filtering molecules on the basis of their charge, size and shape^[41]; therefore, glomerular diseases are associated with pathological GBM alterations^[42]. Similar changes had been noted in the kidneys of experimental animals upon exposure to other trace metals, such as lead^[43] and aluminum chloride^[44].

In the current study, cellular vacuolations were observed in the proximal and distal tubules. Some investigators explained this finding as a cellular defense mechanism to segregate injurious toxic substances and prevent their interference with cellular metabolism^[45]. Of note, the cellular lining of the PCT appeared to be the affected by DEA injury, probably because they are the first to encounter the toxic substances after glomerular filtration^[46]. Moreover, loss of microvilli and basal inclusions in some regions of the PCT were detected in this study, probably because nephrotoxic substances as DEA directly damage the integrity of the PCT cell membrane^[47].

The degenerative changes that were observed in this study could be attributed to the structural similarity of DEA to the naturally occurring MEA, which is a structural component of membrane phospholipids. Diethanolamine can interact with the phospholipid metabolism and alter the membrane structure and enzyme activities^[10, 13, 48]. The resulting diethylamino-phospholipids possess a longer half-life and therefore can accumulate in body tissues^[12, 33]. Barbee and Hartung^[11] found that repeated treatment of rats with DEA significantly inhibited the formation of phosphatidyl-choline and phosphatidyl-ethanolamine in the liver, compared to control rats. Kamendulis and Klauning^[49] postulated that reduced cellular choline levels was due to DEA inhibition of choline uptake, which leads to alteration of DNA methylation.

The ultrastructural findings in this study revealed degenerative changes in the mitochondria which are compatible with the finding of Barbee and Hartung^[50] who noted changes in liver mitochondrial activity in rats following exposure to DEA in drinking water for up to 5 weeks. Mitochondria depend on phospholipids for proper function; therefore, any alteration in phospholipid metabolism, such as that induced by DEA, could result in loss of mitochondrial structural integrity and function^[11]. Moreover, altering the membrane permeability by DEA enhances the Mg²⁺-dependent ATPase activity leading to increased K⁺ influx. This is followed by H⁺ ejection in order to maintain ion balance and mitochondrial swelling due to Pi entry^[50]. It is possible that small increases in the mitochondrial membrane permeability below certain thresholds will produce swelling and elevations in oxygen consumption without affecting the ability to synthesize ATP^[51]. This may explain the swollen appearance of some mitochondria in tubular cells in our examination.

In the current study, concomitant administration of ALA and DEA resulted in preserved histological and immunohistochemical picture, compared to DEA-treated rats. Unlike other antioxidants, ALA can perform its

antioxidant action against oxidative stress in the cytoplasm, plasma membranes, and serum^[52]. The present results were in agreement with Sumathi *et al.*^[53] who confirmed the therapeutic potential of ALA in neurodegenerative disorders, oxidative tissue injury and heavy metal toxicity. Moreover, Ahmed^[25] noticed preserved normal appearance of glomeruli and other renal tubules after using ALA in diabetic rabbits.

Lipoic acid is a thiol-containing nucleophile that can react with endogenous electrophiles including free radicals, reactive drug metabolites and heavy metals^[54]. After cellular entry, it is rapidly reduced to dihydrolipoate by lipoamide dehydrogenase, thioredoxin reductase, and glutathione reductase in the presence of nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH). Dihydrolipoate is then released extracellularly, reduces cystine to cysteine, and thus promotes glutathione synthesis^[55]. Alpha-lipoic acid and its reduced form "dihydrolipoate" contribute to the mitochondrial energy metabolism, regenerate physiological antioxidants, scavenge ROS and chelate metal ions^[54, 56, 57]. These actions would protect against xenobiotics-renal damage by inhibiting lipid peroxidation and enhancing the renal antioxidant defense^[58].

In accordance with our results, several studies^[36, 59, 60] showed that ALA treatment can improve the renal function and histopathological changes through alleviating oxidative stress and enhancing antioxidant enzymes' activities. Intriguingly, Moini *et al.*^[61] have reported that ALA may also exert pro-oxidant properties in-vitro. It was shown to promote the mitochondrial permeability transition in isolated rat liver and permeabilized hepatocytes and to stimulate superoxide anion production in rat liver mitochondria, as well as sub-mitochondrial particles^[62]. This may be attributed to the differences in dosages and testing circumstances.

Moreover, Kang *et al.*^[63] stated that ALA has anti-inflammatory effects due to suppressing the nuclear factor- κ B (NF- κ B) signaling pathway, which inhibits the expression of adhesion molecules in human endothelial cells and monocytes. They added that ALA ameliorates cisplatin-induced renal injury by suppression of MCP-1 expression. Shanmugarajan *et al.*^[64] suggested that ALA nephroprotection is mediated by inhibiting neutrophil infiltration, regulating the generation of inflammatory mediators and balancing the oxidant-anti-oxidant status. Other authors^[65] reported that ALA prevent angiotensin II-induced leukocytic infiltration.

CONCLUSION

ALA protects the kidney against the histological and immunohistochemical changes, induced by DEA, probably through its anti-inflammatory and anti-apoptotic effects.

ABBREVIATIONS:

ALA: Alpha-lipoic acid, DCT: Distal convoluted

tubules, DEA: Diethanolamine, GBM: Glomerular basement membrane, iNOS: inducible nitric oxide synthase, MEA: Monoethanolamine, PCT: Proximal convoluted tubules, ROS: Reactive oxygen species, TEA: Triethanolamine

CONFLICT OF INTEREST:

All authors declare no related conflicts of interest.

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الملخص العربي

التأثيرات الوقائية لحمض ألفا ليبويك على التسمم الكلوي المحدث بثنائي إيثانولامين في ذكر الجرذان البيض البالغه : دراسة هستولوجية و هستوكيميائية مناعية

هبة كمال محمد و فاطمة ياسين مليجي

قسم التشريخ الأدمي و علم الأجنة وقسم الهستولوجيا وبيولوجيا الخلية - كلية الطب- جامعة أسيوط

المقدمة: يعتبر ثنائي إيثانولامين عامل عضوي يستخدم في مستحضرات التجميل و اتضح أنه يسبب تغيرات تآكلية. وقد وجد أن حمض ألفا ليبويك يعتبر مركب ثاني كبريتيد طبيعي معروف بتأثيراته المضادة للأكسدة.

الهدف من البحث: الهدف من هذه الدراسة هو تقييم الدور الوقائي لحمض ألفا ليبويك على التركيب الهستولوجي للكلية في الفئران المعالجة بثنائي إيثانولامين.

المواد والطرق المستخدمة: تم تقسيم إجمالي عدد 30 من الفئران بالتساوي إلى ثلاث مجموعات. المجموعة الضابطة وأعطيت هذه المجموعة مياه مقطرة. والمجموعة المعالجة بثنائي إيثانولامين وقد أعطيت جرعة تعادل 425مجم/كجم من وزن الجسم عن طريق الفم 6 مرات في الأسبوع لمدة أسبوعين والمجموعة المعالجة بثنائي إيثانولامين وحمض ألفا ليبويك وقد أعطيت جرعة من حمض ألفا ليبويك تعادل 100مجم/كجم من وزن الجسم بالتزامن مع نفس جرعة ثنائي إيثانولامين ولنفس المدة. تم أخذ العينات من الكلى وأعدت للدراسة الهستولوجية و الهستوكيميائية المناعية. وقد أجريت دراسة مورفومترية وإحصائية.

نتائج البحث: قد اظهر الفحص بالميكروسكوب الضوئي أن الفئران المعالجة بثنائي إيثانولامين بها خلل في تركيب النسيج الكلوي، زيادة في حجم الكبيبات، اتساع في الفراغ الكلوي وبقايا خلايا في تجايف القنيات. كما وجد أيضاً احتقان في الاوعية الدموية وفقدان للحافة الفرشائية المكونة للأنايبب الملتفة الدانية. كما اوضح الميكروسكوب الالكتروني زيادة في سمك غشاء الكبيبات القاعدى وظهور خلايا قديمة داكنة مع حدوث التحام في الزوائد القديمة. وقد أظهرت الخلايا المبطنة للقنيات وجود ميتوكوندريا داكنة ذات حواجز مشوهة مع فقد في عضيات السيتوبلازم. كما أظهرت الدراسة الهستوكيميائية المناعية في المجموعة المعالجة بثنائي إيثانولامين ظهور تفاعل إيجابي ملحوظ في السيتوبلازم للكاسباس-3- والإنزيم المستحث المخلوق لأوكسيد النيتريك (iNOS). المجموعة المعالجة بثنائي إيثانولامين وحمض ألفا ليبويك أظهرت تحسن ملحوظ في الخلل الناشيء في التركيب الهستولوجي و الهستوكيميائي المناعي للكبيبات الكلوية و القنيات الملتفة القريبة و البعيدة مقارنة بالمجموعة المعالجة بثنائي إيثانولامين.

الخلاصة: نستخلص من أن هذه الدراسة ان حمض ألفا ليبويك له تأثير وقائي على الكلية من التأثيرات الضارة التي يسببها التعرض لثنائي إيثانولامين.