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Antioxidant effect of vitamin E on diphenylamine-induced hepato-renal oxidative stress and structural changes in rat fetuses

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Abstract

To date, studies on the effects of prenatal exposure to diphenylamine on developing fetuses are sparse. Therefore, further investigation is required to determine the potential prenatal hazard of this compound and to introduce possible treatment for these hazards. This study aimed to assess the biochemical, histopathological, and ultrastructural changes induced by diphenylamine in the developing liver and kidney of rat fetuses and the role of vitamin E in alleviating these changes. Fifty pregnant rats were divided equally into five groups, the group I was administrated distilled water, group II was administrated corn oil, group III was administrated 100 mg/kg/b.wt. vitamin E, group IV was administrated approximately 400 mg/kg/b.wt diphenylamine and group V was administrated diphenylamine + vitamin E at the above-mentioned doses from the 6^{th} to 15^{th} day of pregnancy. Diphenylamine induced undesirable histopathological and ultrastructural changes in the fetal liver and kidney. These changes were in the form of vacuolation, congestions of central veins, hemorrhage, leucocytic infiltration, degenerated cytoplasm, pyknotic nuclei, and swollen mitochondria and rER of hepatocytes. While the degenerative changes in the kidney were represented by degenerated brush border, lumen dilation, tubular hyalinization, vacuolation, degenerated nuclei, and mitochondria. Also, there was a significant decrease in the antioxidant enzymes i.e., superoxide dismutase and catalase, and a significant increase in reactive oxygen radicals and malondialdehyde. Treatment with vitamins E after diphenylamine restored all biochemical, histopathological, and ultrastructural damage cited above. In conclusion, vitamin E has antioxidant effects which could be able to antagonize diphenylamine prenatal toxicity.

Keywords: Diphenylamine, Vitamin E, Oxidative stress, Organogenesis, Histopathological.

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1. Introduction

Diphenylamine (DPA) is a parent compound of many derivatives, which are used for the production of dyes, pharmaceuticals, photography chemicals, and further small-scale applications. DPA and derivatives are most commonly used as stabilizers in nitrocellulose-containing explosives and propellants, perfumery, and as antioxidants in the rubber and elastomer industry. DPA is also a growth regulator and is widely used to prevent post-harvest deterioration of apple and pear crops (Drzyzga, 2003) so, people may be exposed to residues of DPA through the diet.

A few studies demonstrated the potential hazard of various DPA to the aquatic environment, bacteria, and animals (Drzyzga, 2003). Also, DPA has mutagenicity, genotoxicity, clastogenecity, cytotoxicity, acute toxicity via oral and intraperitoneal exposure, and metabolism/toxicokinetics (Probst et al., 1981; Babish et al., 1983; Dolara et al., 1993; Litton Bionetics, 1994). Moreover, Lenz and Carlton, (1990) indicated that acute exposures to DPA were toxic to the kidney of hamsters, rats, and gerbils.

Oxidative stress is an imbalance between the generation of the reactive oxygen species (ROS) and antioxidant defense system (superoxide dismutase, SOD, catalase, CAT, glutathione, GSH; glutathione peroxidase, GPx) which can lead to lipid peroxidation (LPO) and can cause cell damage, mitochondrial dysfunction and DNA damage (Davies, 2000; Scandalios, 2005; Slotkin and Seidler, 2010). It is well known that tissues and organs are more susceptible to toxic factors in the prenatal than mature age (Yamashita et al., 2011).

Vitamin E (Vit. E) is an important antioxidant that might have an important role in scavenging free oxygen radicals. It is considered one of the most effective lipid-soluble antioxidants which protect body tissues by preventing LPO, thus maintaining its permeability. Also, it may affect oxidative changes which occur in other cell organelles (Evstigneeva et al., 1998; Alsaggaf et al., 2016) and up-regulate antioxidant enzymes through regulation of antioxidant enzymes activity or gene expression (Hajiani et al., 2008). Moreover, Vit. E is effective against various diseases, such as cardiovascular, Alzheimer's, and nephropathy (Liu et al., 2015).

Little attention to the prenatal effect of DPA on rat fetuses has been taken. So, this study was aimed to investigate the efficacy of Vit. E in improving the DPA - induced oxidative stress, histopathological and ultrastructural changes in the liver and kidney of rat fetuses.

2. Materials & Methods

Chemicals

DPA (99% pure) and Vit. E were purchased from Sigma - Aldrich Company (St Louis, MO, USA). They were suspended in corn oil and administrated orally in a non-lethal dose (400mg/kg) for DPA and 100mg/kg for Vit. E during the organogenesis phase i.e., 6^{th} to 15^{th} day of pregnancy. All other chemicals used were of analytical grade.

Animals

Principles of animal care and use were carefully followed during conducting the present study according to the guide for the care and use of laboratory animals approved by the Faculty of Science, Menoufia University, Egypt (Approval No. MNSE2229) and according to the National Institutes of Health guide for the care and use of laboratory animals (NIH publications No. 8023, received 1978). The study was conducted on 75 adults Wistar rats (50 virgin females and 25 fertile males), weighted 170-180 ± 10g and ages 16 ± 1 weeks. The animals were purchased from Helwan Farm, Ministry of Health, Cairo, Egypt. The animals were kept in plastic cages in standard laboratory conditions and had free access to water and a standard diet. For pregnancy induction, female and fertile male albino rats were kept together at a ratio of 2:1 and left overnight. Pregnancy was confirmed by the

presence of sperms in the vaginal smear the next morning and this was considered as the zero-day of gestation. The experiments were conducted following ethical guidelines of the Animal Care and Use Committee of Faculty of Science, Menoufia University, Egypt (Approval No. MNSE2310) and according to the National Institutes of Health guide for the care and use of laboratory animals (NIH publications No. 8023, received 1978).

Experimental design

Pregnant animals were divided into 5 separate groups (10 for each). Animals were comprised of the following groups:

- Group I received 1ml distilled water (Control)
- Group II received 1ml corn oil (as the solvent for DPA and fat-soluble Vit. E) (Corn oil group).
- Group III received Vit. E 100 mg/kg/b.wt. (Vit. E group) (Alsaggaf et al., 2016)
- Group IV received DPA (400 mg/kg/b.wt) (Lenz and Carlton, 1990) (DPA group).
- Group V received DPA and Vit. E (at the above-mentioned doses) (DPA+vit.E group).

On a gestational day of 20, pregnant rats were anesthetized with Halothane (Pharco, Egypt), sacrificed, and dissected to extract fetuses and the liver and kidney were removed for further processing.

Oxidative stress markers and antioxidant enzymes

Tissues from the fetal liver and kidney were used to determine the oxidative stress and antioxidant enzymes. Tissue samples were washed in 0.9% saline and homogenized in 0.2M sodium phosphate buffer (pH 6.2) in an ultrasonic homogenizer. The homogenates were centrifuged, then the supernatant was collected for biochemical analysis of enzymes. All parameters were measured by using a spectrophotometer (Helios Alpha, UNICAM Ltd, England).

Measurement of ROS Level

ROS includes many reactive molecules and free radicals derived from molecular oxygen, which damage DNA and RNA and oxidize proteins and lipids (lipid peroxidation). The most common ROS include O_2-, H_2O_2, HO- and $1O_2$. 50 µL Fluorescent Substrate (H2DCFDA) was added to 200 µL tissue homogenates and changes in fluorescence intensity were detected every 5 min for 30 min on a microplate reader, GENios (Azure Biosystems, Inc, USA), with excitation/emission wavelengths = 485/530 nm. The ROS detection kit uses the cell permeation reagent Dichlorodihydrofluorescein-diacetate (H2DCFDA), which is a fluorescent dye that can measure the intracellular HO-, $H_2O_2$and other ROS activity. After diffusion into the cell, the acetyl group on H2DCFDA is cleaved by intracellular esterase to generate a non-fluorescent compound, which is rapidly oxidized by ROS to highly fluorescent 2',7'-Dichlorodihydrofluorescein. The fluorescence intensity is directly proportional to the level of ROS in the cytosol (Zhang et al., 2018).

Catalase enzyme (CAT) assay

CAT was determined according to Goth (1991). 200µl homogenate was incubated in 1ml H_2O_2 substrate and 1 ml of ammonium molybdate was added to stop the enzymatic reaction. The yellow color complex formed by molybdate and H_2O_2 was measured at 405 nm.

Superoxide dismutase (SOD) assay

SOD was determined according to Beauchamp and Fridovich (1971). The activity of SOD was assayed on its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). One unites of SOD was defined as the amount of enzyme activity that was able to inhibit 50% of the photoreduction of NBT to blue Formosan. Briefly, 200µl of tissue homogenate added to 2300µl sodium phosphate buffer, 100µl NBT, 100µl Na_2Co_3, 100µl EDTA, 100µl L- methionine and 100µl riboflavin. Control was prepared by adding 2300µl sodium phosphate buffer instead of homogenate. The absorbance was measured at 560 nm.

Lipid peroxidation (LPO) assay

LPO refers to the oxidative degradation of lipids. The end products of LPO especially malondialdehyde (MDA) determined according to Ruiz-Larrea et al. (1994). 2 ml of 20% trichloroacetic acid and thiobarbituric acid was added to the homogenate then heated and centrifuged at 3000 rpm for 10 min. The absorbance was measured at 532 nm. Control was prepared by adding distilled water instead of the tissue homogenate.

Histopathological preparation

Parts of the liver and kidney were fixed in 10% neutral formalin and proceeded for paraffin blocks. 5 µm thick paraffin sections were stained using Harris' hematoxylin and eosin according to Suvarna et al. (2018). Histological sections were examined and photographed using an Olympus microscope (BX41, Japan).

Transmission electron microscopic preparation

Small specimens of fetal heart and kidney were excised and rapidly fixed in 2.5% glutaraldehyde for 24h. Then the tissues were washed in 0.1M phosphate buffer, postfixed in 1% osmium tetra-oxide at 4^{o}C, washed in phosphate buffer, and dehydrated in ascending grades of ethanol then transferred to propylene oxide solution for clearing. The specimens were then infiltrated in a mixture of clearing agent and embedding medium and embedded in epoxy resins. Semithin sections of 1µm thickness were stained with toluidine blue for examination with a light microscope. Ultra-thin (50nm) sections were cut, mounted on copper grids, and stained with uranyl acetate and lead citrate (Kuo, 2007). The grids were examined and photographed with a JEOL electron microscope (TEM-1400Plus, Japan), Electron Microscope Unit, Alexandria University.

Statistical analysis

The values of ROS, MDA, SOD, and CAT were measured in fetal liver and kidney, and the mean and standard error (SEM) for all groups were calculated. Differences between groups were analyzed using a one-way ANOVA test using a statistical package of social science (IBM SPSS) statistics software for windows, Version 22 (IBM Corp., Armonk, NY USA) followed by an LSD test for multiple comparisons. The significances were expressed as $P < 0.05$ and highly significant at $P < 0.001$.

3. Results

Oxidative stress markers

- **Total ROS**

Total ROS was measured in both fetal liver and kidney homogenate. The ROS levels were highly increased in the liver and kidney of rat fetuses from the DPA group compared with the control. While administration of Vit. E with DPA suppressed the ROS levels which showed a highly significant decrease compared with the DPA group (Fig. 1a).

- **Liver & kidney CAT**

The CAT activity was significantly decreased in the liver and kidney homogenates of rat fetuses from the DPA group compared with the control group but after Vit. E administration a highly significant increase in its activity was shown when compared with the DPA group (Fig. 1b).

- **Liver & kidney SOD**

A marked highly significant decrease in SOD activity was presented in the DPA group when compared with a control group. Treatment with Vit. E after DPA resulted in a significant increase in comparison with DPA treated group (Fig. 1c).

- **Liver & kidney LPO**

LPO induction by DPA was measured by the production of MDA in fetal liver and kidney homogenates (Fig. 1d). The MDA contents in the fetal liver and kidney of the DPA group showed a highly significant increase compared with the control group and a highly significant reduction in the combined group compared with the DPA group.

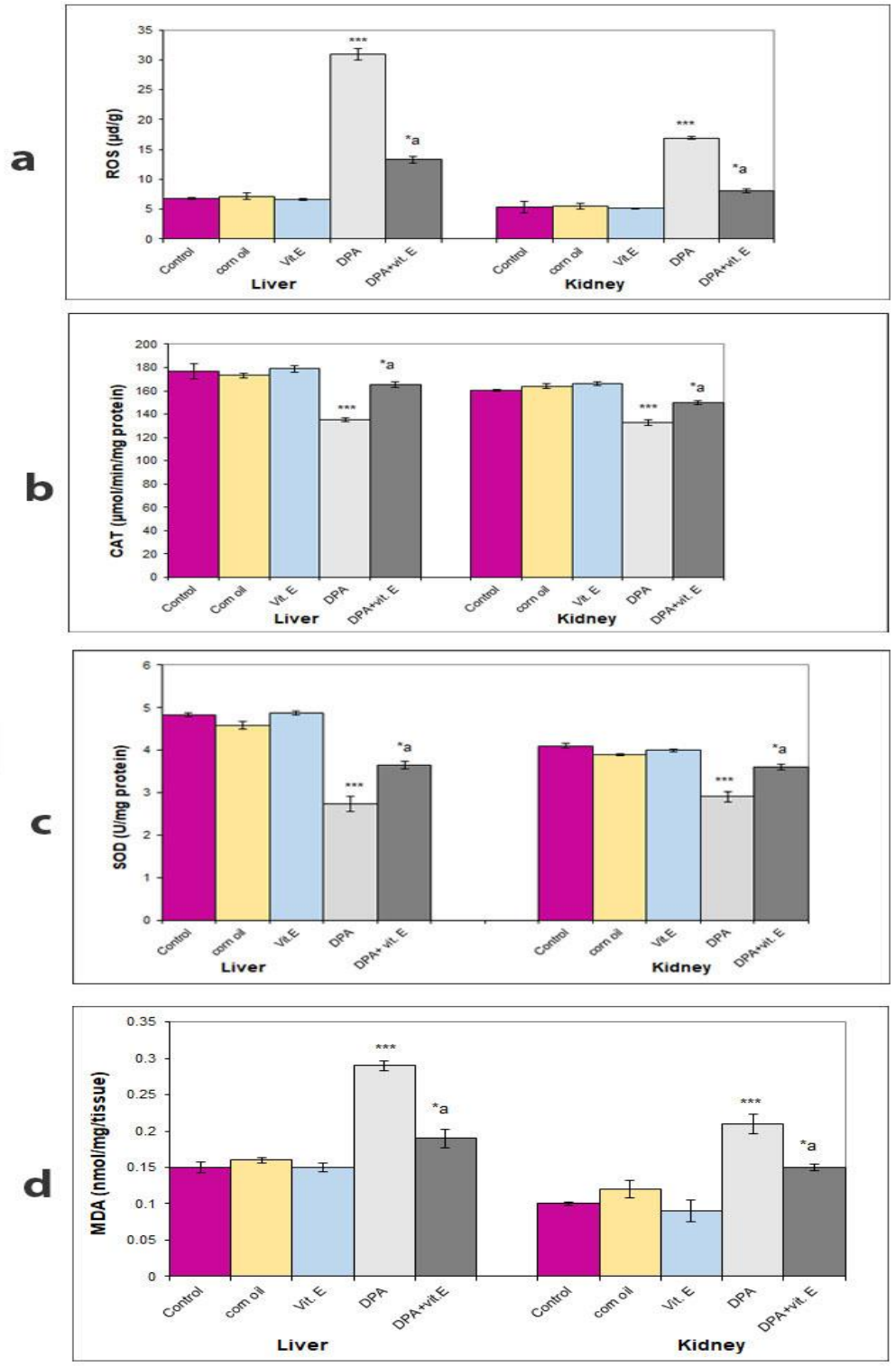

Figure 1. Graph showing effect of prenatal administration of DPA and Vit. E on ROS level, CAT and SOD enzyme activities, and MDA level in fetal liver and kidney homogenate. **(a)** ROS, **(b)** CAT, **(c)** SOD and **(d)** MDA. Asterisks (***P> 0.001, *P>0.05) refer to the P-value compared with the control group. a= highly significant (P<0.001) compared with the DPA group.

Histopathological observations:

A- Liver

The fetal liver from control, corn oil, and Vit. E groups showed well-preserved cytoarchitecture with normal central vein, hepatocytes, sinusoids, and few megakaryocytes (Figs. 2a-c). The liver sections from DPA treated group displayed some histopathological changes including cytoplasmic vacuolation of most hepatocytes, loss of cellular arrangement, widening, and congestions of central veins, hemorrhage, and hepatic necrotic areas occupied by leucocytic infiltration. Moreover, the central veins appeared ruptured in some areas, and its lumen continuous with the sinusoid. Also, some hepatocytes lost their nuclei while others contained pyknotic nuclei, erythrocytes congestion in the portal vein and new bile ductules formation were seen (Figs. 2d-f). The fetal liver tissue of the combined group displayed normal structure including normal shape and size of the central veins, healthy arranged hepatocytes between sinusoids (Fig. 2g).

B- Kidney

The fetal kidney of control, corn oil, and Vit. E groups showed the normal histological structure of the renal corpuscles and the renal tubular cells. Also, both subcapsular and medullary zones were clear (Figs. 3a-c). Examination of the fetal kidney from the DPA treated group revealed degenerative changes mainly in the renal tubules represented by vacuolated cytoplasm of the renal lining epithelium, degenerated brush border, and dilation of the lumen. The glomeruli of the renal corpuscles appeared shrunken with a wide capsular space while others were empty. Congestion in the blood vessels, tubular hyalinization, and hemorrhage was also seen (Figs. 3d-f). The renal tissue from the combined group displayed normal renal corpuscles and renal tubules without signs of inflammation except little epithelium vacuolation (Fig. 3g).

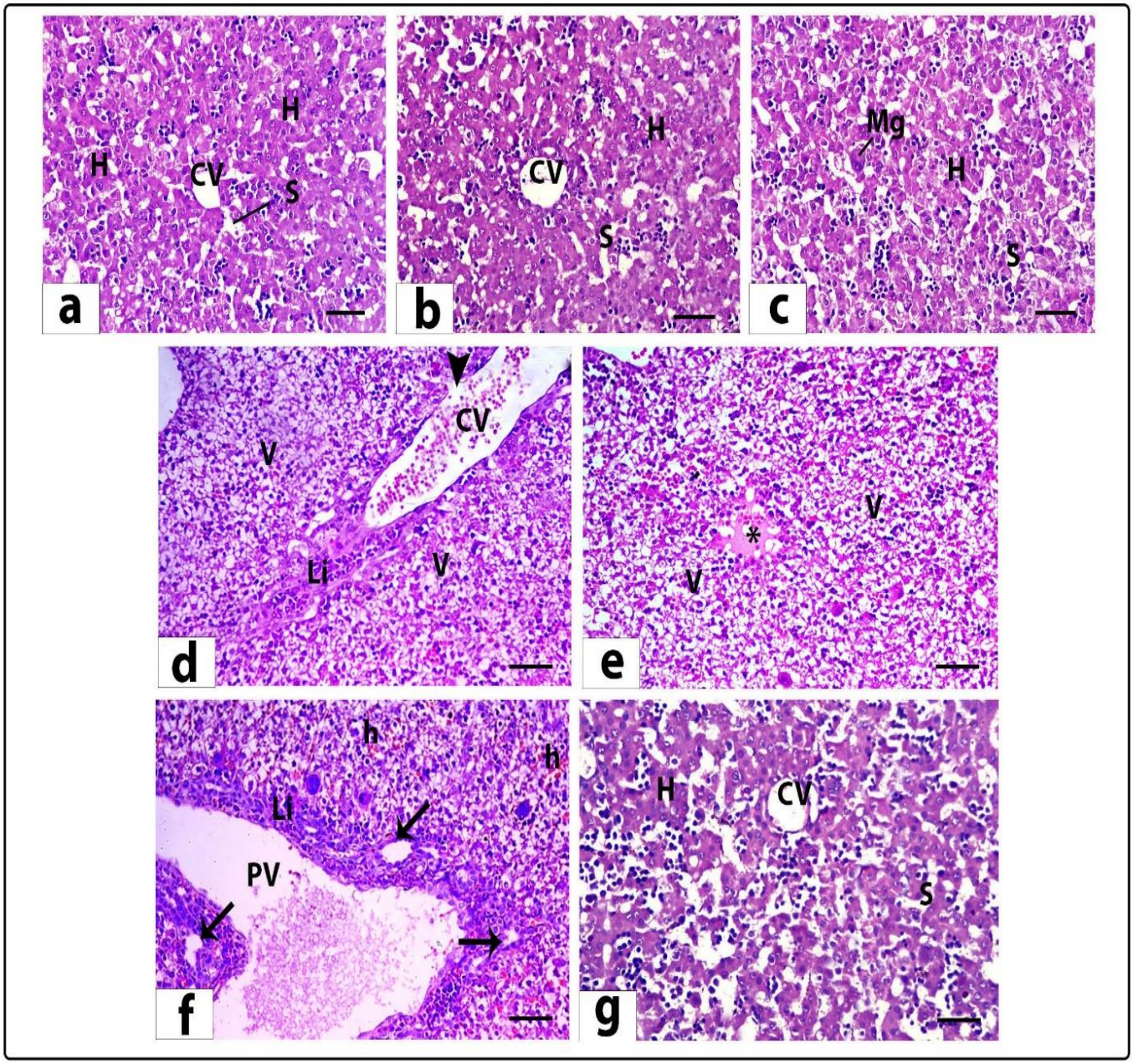

Figure 2. Photomicrographs of liver sections of 20- day old rat fetuses showing: (a) control, **(b)** corn oil, **(c)** Vit. E, **(d-f)** DPA and **(g)** DPA and Vit. E groups. H, Hepatocytes; CV, central vein; S, Sinusoids; Mg, Megakaryocyte; V, Vacuolation; Li, Leukocytic infiltration; Arrowhead, Ruptured central vein; *, Necrotic area; h, hemorrhage; PV, Portal vein; Arrow, bile ductulus formation. Scale bar=0.059mm. H&E stain.

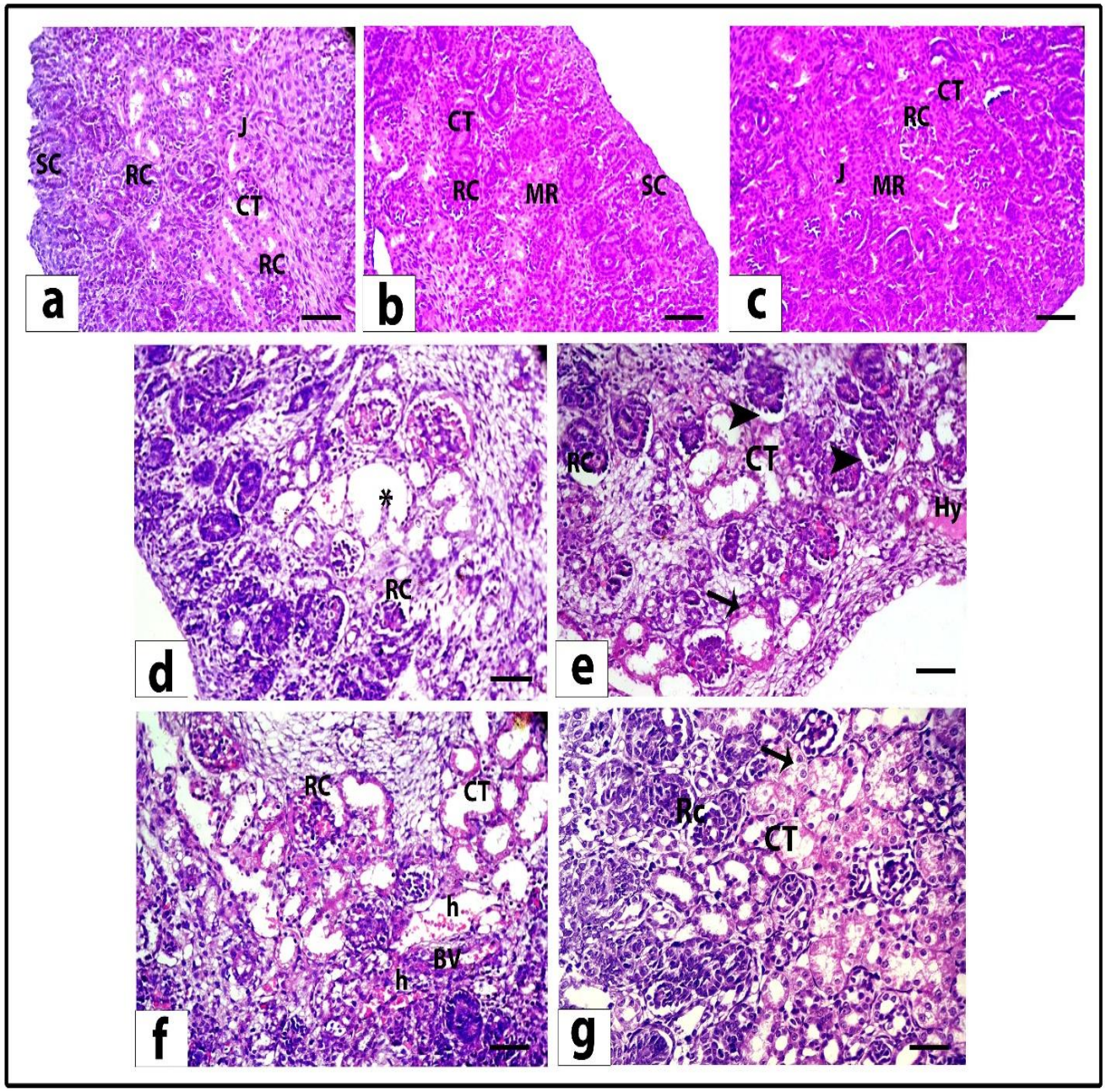

Figure 3. Photomicrographs of kidney sections of 20- day old rat fetuses showing: (a) control, **(b)** corn oil, **(c)** Vit. E, **(d-f)** DPA and **(g)** DPA and Vit. E groups. SC, Subcapsular zone; RC. Renal corpuscle; CT, Convoluted tubules, MR, Medullary rays; J, Juxtamedullary; *, degenerated renal corpuscle; Arrowhead, shrunken renal corpuscle; CT, dilated convoluted tubules; Arrow, vacuolated cytoplasm of the renal lining epithelium; BV, congested blood vessels, Hy, tubular hyalinization; h, hemorrhage. Scale bar=0.059mm. H&E stain.

Transmission electron microscope observations:

A- Liver

The hepatocytes of control, corn oil, and Vit. E groups displayed normal ultrastructure. The hepatocytes contained euchromatic nuclei with prominent nucleolus and regular nuclear envelope alongside various mitochondria with well-arranged cristae, many rough endoplasmic reticula (rER), and glycogen granules. Normal Kupffer cells were also seen in the sinusoids. Hepatocytes of DPA treated group showed many degenerative signs including degenerated cytoplasm, many pyknotic, irregular, and shrunken nuclei, and swollen mitochondria. The heterochromatin of some nuclei aggregated on the nuclear envelope. The rER appeared swollen and their stacks were fragmented. The Kupffer cells displayed rarified cytoplasm, vacuoles of varying sizes, and many lysosomes. Numerous hematopoietic cells were also seen. The iron precipitate was also seen in some hepatocytes. Almost hepatocytes lost their intact shape. The hepatocytes from DPA + Vit. E group didn't show any change in the ultrastructure of the nuclei and the internal organelles.

B- Kidney

TEM investigation of the fetal kidney from the control group displayed a normal figure of proximal convoluted tubules, distal convoluted tubules cells, and glomeruli. The glomeruli possessed normal podocytes with many foot processes, wide urinary space, and a thin glomerular basement membrane. The epithelial cells lined the proximal convoluted tubules showed round nuclei with a peripheral distribution of chromatin and many microvilli forming brush borders. The electron-dense cytoplasm contained many mitochondria between the basal infoldings and rER. The distal convoluted cells revealed few microvilli, large basal rounded nuclei, various mitochondria, and rER. The renal cells of both corn oil and Vit. E groups didn't show any ultrastructure changes. Ultrastructural examination of the fetal kidney of the DPA treated group showed thickening and fragmentation in the podocyte process, urinary space narrowing, and thickening in the basement membrane. Most podocytes nuclei were shrunken and pyknotic with abnormally clumping of chromatin. The nuclei of both proximal and distal convoluted tubule cells were shrunken and electron-dense with clumping chromatin while other nuclei appeared fragmented or with the irregular nuclear envelope. There was a marked degeneration of mitochondria which appeared swollen. The cytoplasm contained numerous lysosomes and the brush border was fragmented and absent in some proximal tubule cells. The glomeruli of the combined group showed amelioration in the ultrastructure compared with DPA treated group. Also, the renal cells showed normal nuclei, mitochondria, and a well-arranged brush border.

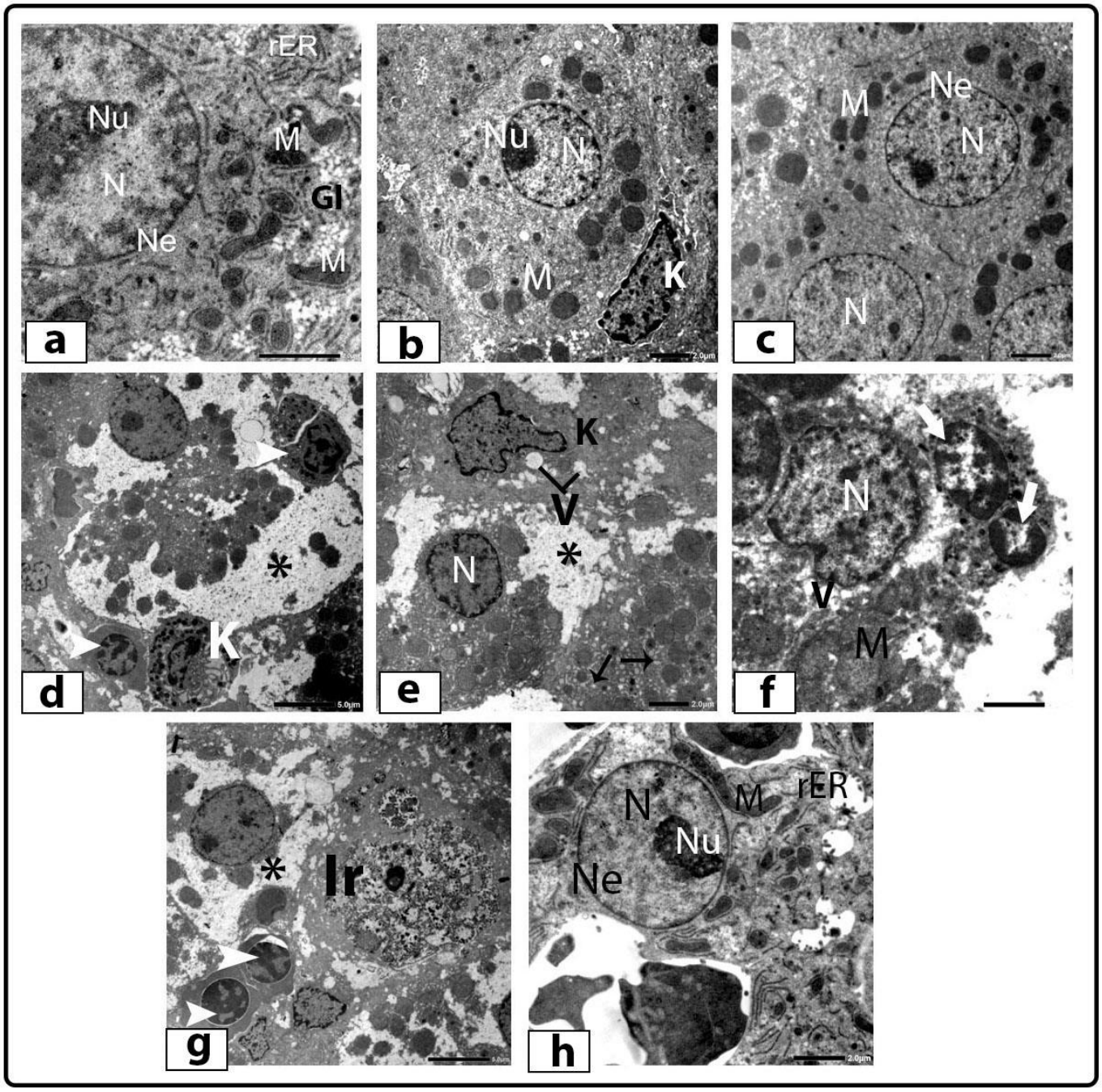

Figure 4. Electron micrographs of 20-day old rat fetuses' hepatocytes. (a) control, **(b)** corn oil, and **(c)** Vit. E groups showing normal nuclei (N); nucleolus (Nu), regular nuclear envelope (Ne), mitochondria (M), glycogen granules (Gl), rough endoplasmic reticulum (rER), and normal Kupffer cell (K). **(d-g)** DPA group showing rarified cytoplasm (*); Kupffer cells (K) containing vacuoles (V) and many lysosomes; fragmented and swollen rER (Black arrow); irregular (N) and Pyknotic nuclei (White arrow); swollen mitochondria (M); hematopoietic infiltration (White arrowhead); and iron precipitate (Ir) **(h)** DPA and Vit. E group showing normal organelles. Scale bar= 2µm (a,b,c,e,f,h) and 5µm (d,g).

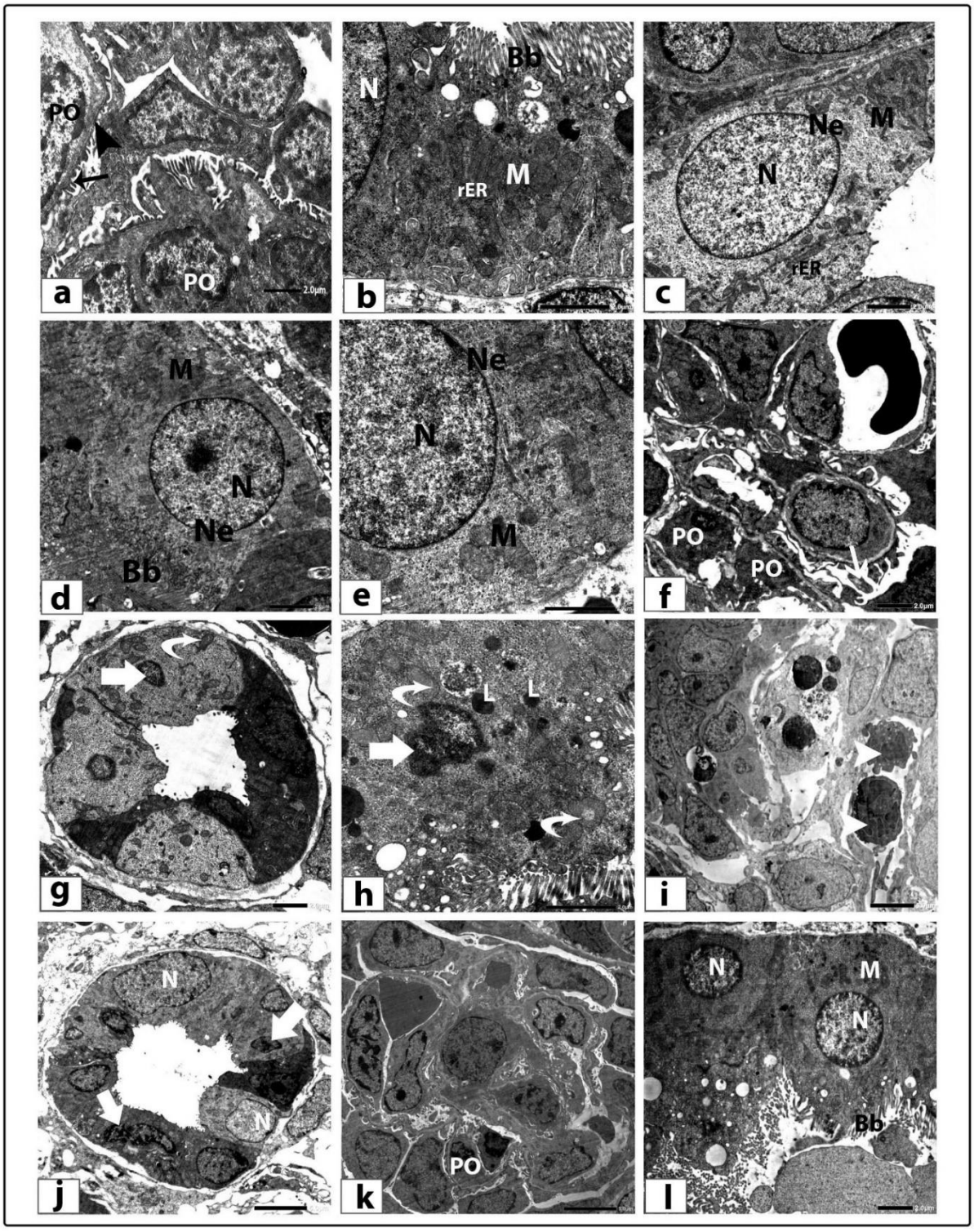

Figure 5. Electron micrographs of 20-day old rat fetuses' renal cells. (a-c) control group showing **(a)** podocytes (PO) and their foot processes (Arrow), thin basement membrane (Black arrowhead), **(b)** proximal convoluted tubule cell with normal nucleus (N), mitochondria (M), rough endoplasmic reticulum (rER) and brush border (Bb), and **(c)** distal convoluted tubule cells with a nucleus (N), nuclear envelope (Ne) rough endoplasmic reticulum (rER) and mitochondria (M). **(d)** corn oil and **(e)** Vit. E groups showing normal renal cells. **(f-j)** DPA showing **(f)** glomerulus with thick and fragmented podocyte process (Arrow), narrow urinary space, and shrunken and pyknotic podocytes nuclei (PO). **(g-h)** proximal convoluted tubule cells have shrunken, electron-dense, and fragmented nuclei (Thick arrow), swollen mitochondria (Curved arrow), and numerous lysosomes (L). **(I)** hematopoietic infiltration (Arrowhead). **(J)** degenerated distal convoluted tubule cells with irregular (N) and degenerated nuclei (Thick arrow). **(k&l)** DPA and Vit. E group showing amelioration in the renal cells. Scale bar= 2µm (a,b,c,d,e,f,g,h,l) and 5µm (I,j,k).

4. Discussion

The results of this study confirm the developmental toxicity of commercial DPA as described in preliminary studies. However, several important new observations deserve discussion. It has been observed in this study that DPA administration to pregnant rats during the organogenesis phase induced many biochemical, histopathological, and ultrastructural changes in the developing liver and kidney. However, these changes were significantly reduced by treatment with 100 mg/kg of Vit. E. Treatment with Vit. E effectively improved the DPA-induced depletion in CAT and SOD levels, indicating the hepato- and nephroprotective effects of Vit. E against DPA intoxication. Prenatal DPA administration caused high levels of liver and kidney oxidative damage, as evidenced by a significant elevation of ROS and MDA concentration and depletion of CAT and SOD activities in both fetal liver and kidney homogenates. However, Vit. E markedly inhibits DPA-induced oxidative stress in the liver and kidney of rat fetuses. The increased production of ROS induced by DPA exposure could be an important factor in the development of the internal malformations observed in the fetuses. High levels of ROS can produce DNA, membrane, and enzyme function damage, leading the cells into an adaptive state of hyperplasia and/or hypertrophy (Zangar, et al., 2004; Brocardo et al., 2011).

This decrease in both fetal liver and kidney SOD activities could be due to the overproduction of ROS as evident from the increased LPO levels due to DPA administration. However, some studies have indicated that superoxide radicals can also inhibit CAT activities and the increased H2O2 levels resulting from CAT inhibition could finally inhibit SOD activity. Thus, increased formation of MDA could be due to both increases in ROS production and SOD inhibition (Gultekin et al., 2001). Lodovici et al. (1997) reported that DPA at a dose of 0.14-mg/kg-day/10 days produced free radicals which can induce genetic damage through increasing 8-OH-2-deoxyguanosine levels in rat hepatocytes. Also, Masubuchi et al. (2000) stated that DPA was cytotoxic in vitro at concentrations ranging from 10 to 500 μM in cultured rat hepatocytes.

Previous studies confirmed that Vit. E improved the oxidative stress status by increasing the activity of CAT and SOD enzymes and reducing the formation of MDA in the liver and kidney of both rat fetuses (Weshahy et al., 2007; Salehzadeh et al., 2020) and adults (Kongkham et al., 2013; Ghlissi et al., 2014; Shams et al., 2020). Also, it has been reported that Vit. E significantly decreased the levels of free radicals induced by Aluminium (El-Demerdash, 2004) and lithium (Omar et al., 2016) in rats. Moreover, Vit. E ameliorated CAT activity and significantly decreased the MDA and H2O2 levels in both serum and liver tissue of rats exposed to electromagnetic fields (Eid et al., 2015). In addition, Vit. E decreased the level of MDA caused by lead or noise (El Sheikh et al., 2014), contrast (Kongkham et al., 2013), and colistin methanesulfonate in rat renal tissue (Ghlissi et al., 2014). Antioxidants are known to decrease oxidative radical-induced responses. Vit. E (α-Tocopherol) is a vital antioxidant that prevents LPO by converting lipid peroxyl radicals into a tocopheroxyl radical (Al-Attar, 2011).

Taken together, the evidence presented in the current study has shown that imbalance in the antioxidative scavenger-promoted generation of MDA, resulting in the appearance of hepatic and renal injury, is consistent with histological and ultrastructural assessment. It has been reported that DPA induced nephrotoxicity in both Syrian hamsters and Sprague-Dawley rats which was dose-dependent (Lenz and Carlton 1990). Also, DPA affects the nephron of the developing rat kidney and causes cystic dilation in the corticomedullary zone after prenatal exposure (Crocker et al., 1972). Eknoyan et al. (1976) also, reported that administration of 2.5% DPA in the diet for 3-6 weeks

to female rats caused morphological alterations and gross cysts in the corticomedullary region of the kidneys. There was cystic dilatation of the collecting ducts, proteinaceous casts in the dilated tubules, and focal dilatation in the cortical collective ducts and distal tubules. Moreover, it has been shown that oral administration of 333 and 1000 mg/kg b.wt./d DPA to Fischer rats of both sexes increase liver, spleen, and kidney weights, and anemia was observed in the high dose group in both sexes. Also, dilatation, degeneration, or necrosis of renal tubules in the corticomedullary was seen in both doses (Yoshida et al. 1989). Furthermore, a brown coloration of tissues, central lobular necrosis in the livers, increased interstitial nephritis in the kidneys was seen in both sexes of rats treated with 1.0% DPA (791 mg/kg-day for males and 978 mg/kg-day for females) (Lenz and Carlton, 1990).

Another study by Krohmer (1992) showed the oral toxicity of DPA in Sprague-Dawley rats at 9.6, 96, 550, and 1200 mg/kg b.wt per day in males and 12, 110, 650, and 1300 mg/kg b.wt per day in females. The kidneys and livers were dark and enlarged in animals of each gender who received high doses, and about 60% of the females at the high dose had dark and/or enlarged livers. Histopathological examination revealed an increased incidence of hematopoiesis and pigment in the liver and kidneys. Also, administration of 1.7, 94, 444, or 926 mg/kg-day for males and 2.1, 107, 555, or 1101 mg/kg-day for females of Swiss-derived CD-1 mice for 90 days, was associated with the appearance of brownish-yellow pigment and extra-medullary hematopoiesis in the liver and kidney which was severe at highest doses (Botta, 1992). Rodwell (1993) reported that DPA administration to Sprague-Dawley rats in the diet for 90 days at doses of 40, 115, or 399 mg/kg-day in males and 46, 131, or 448 mg/kg-day in females, resulted in brown pigment in the proximal convoluted tubules of the kidney, hepatocytic hypertrophy and brown pigments in the Kupffer cells of the liver.

Moreover, it has been found that DPA was a cytotoxic agent under the experimental conditions, Pseudoenergetic mitochondrial swelling and uncoupling of mitochondrial oxidative phosphorylation, depleting cellular ATP were seen when rat hepatocytes incubated with 0-, 10-, 25-, 50-, 100-, 250-, or 500-µM DPA (Masubuchi et al., 2000). It has been shown that oxidative stress, caused by the overproduction of ROS, is thought to be involved in the development of liver and renal diseases (Zhu and Fung, 2000; Shimizu et al., 2005).

The present results revealed that oral administration of Vit. E improved the histopathological changes in fetal liver and kidney. Following these findings, Alsaggaf et al. (2016) confirmed that Vit. E reduced the histological changes induced by aluminum chloride in the rat fetal liver. Also, Vit. E was found to be a good ameliorative against histopathological alteration in the liver of male rats exposed to electromagnetic field (Eid et al., 2015) and have a cytoprotective effect in the liver of rats with chronic CCL4-induced liver cirrhosis (Sclafani et al., 1986). Also, Shokrzadeh et al. (2012) revealed that Vit. E, A, and C have a protective effect against diazinon-induced hepatotoxicity in rats, as it increased the antioxidant status.

Previous results by Osfor et al. (2010) and El Sheikh et al. (2014) support the current findings that Vit. E supplementation was reported to be beneficial in reducing and slowing progressive kidney diseases. Furthermore, Vit. E supplementation resulted in improvement of the histological changes including perivascular edema and interstitial lymphocytic cell reaction in the kidney of rats treated with lithium (Omar et al., 2016). Also, Vit E administration could restore the histopathological changes against fipronil-induced hepatorenal injury (Abdel-Daima and Abdeen, 2018), amikacin, (Selim et al., 2017), formaldehyde (Bakar et

al., 2015), doxorubicin (Shoukry et al., 2018), and colistin methanesulfonate induced nephrotoxicity in rats (Ghlissi et al., 2014). The antioxidant activity of Vit. E owing to its ability to break the chain of free radical reactions by allowing free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids (Pascoe et al., 1987). Therefore, Vit. E administration protects the kidney and liver tissue against peroxidative damage through decreasing ROS production, improving mitochondrial respiration, preventing cell lysis, and maintaining adenosine triphosphate level (Hu et al., 1989; Al-Attar, 2011; Nowak et al., 2012)

Conclusions

DPA affects the liver and kidney of the developing rat during the organogenesis period. DPA-induced structural changes in the liver and kidney may be due to oxidative stress. However, these changes were significantly reduced by treatment with 100 mg/kg of Vit. E. So, Vit. E supplementation could be used as potential antioxidants against DPA-mediated oxidative damage in the fetal liver and kidney.

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Data availability.

All data generated or analyzed during this study are included in this published article (are available from the corresponding author on reasonable request).

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