

## Effect of Vitamin E as $\alpha$ -Tocopherol Acetate on Mercuric Chloride-Induced Chronic Oxidoreductive Stress and Nephrotoxicity in Rats

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### ABSTRACT

Impact of vitamin E against mercuric chloride induced nephrotoxicity in Wister albino rats was studied. Feeding rats with contaminated diet and water with HgCl<sub>2</sub> at a non-lethal dose of (0.20 mg HgCl<sub>2</sub>/kg food or water) every other day for 42 days resulted in significant increase in the serum malonaldehyde (MDA), a biomarker of lipid peroxidation and significant decrease in the reduced glutathione concentration and glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) enzymes activities as compared to the normal group. Simultaneous co-administration of vitamin E (as  $\alpha$ -tocopherol acetate) 100 mg/kg BW/+p.o, every other day for 42 days along with mercuric chloride produced a pronounced cardinal nephroprotective effect against the mercuric chloride induced nephrotoxicity by restoring the normal levels of the estimated biochemical antioxidant parameters. In conclusion, serum biochemical and kidney histopathological findings of the current study highlight the beneficial antioxidant stress ameliorative effects of vitamin E against HgCl<sub>2</sub>-induced nephrotoxicity in rats.

**Keywords:** Mercuric chloride, oxidoreductive stress, nephrotoxicity, Vitamin E, Histopathology

### Introduction

Globally, the prevalence of mercurial intoxication by food and environmental sources has risen in the world. Pollution of soil and water by natural phenomenon such industrial activities, pollution of marine food resources by mercury in the water are among possible resources of mercurial intoxications that caused public health disasters such as those that occurred in Minamata Bay in Japan and in Iraq (1). Mercury is well-known toxicant to human and animal health. Its biological

criteria, complex toxicokinetics and clinical behavior of mercurial intoxication are strongly related to their chemical forms (2). Humans may be exposed to various species of mercury, which includes charged inorganic mercurous (Hg<sup>1+</sup>), mercuric salts (Hg<sup>2+</sup>), neutral elemental metal (Hg<sup>0</sup>) and organic molecules. Exposure to mercury vapor and organic mercurials specifically affect the central nervous system, while kidney is the target organ for inorganic mercury compounds (3-5). The studies of mercury exposure were mainly of inorganic mercury and effects that were observed at relatively low exposure levels were primarily renal. Mercuric chloride (HgCl<sub>2</sub>) is a white, crystalline, heavy, and poisonous powder that is used in antiseptics, antifungal and anti-parasite materials (6). The affected systems and organs by mercuric compounds are nervous system, thyroid gland, reproductive system, kidney, liver, immune, and respiratory systems (1, 7).

Nephrotoxicity is a serious side effect of mercury and is believed to be related to reactive oxygen

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species in the kidney. It is well known that inorganic mercury causes severe kidney damage after acute and chronic exposure (8). It has been shown that HgCl<sub>2</sub> can induce necrosis by oxidative stress and apoptosis in affected organs. Mercuric chloride acts by binding to thiol and sulfhydryl (SH) groups of proteins, causing mitochondrial dysfunction, and impaired cell membrane integrity (9-12) suggest that selenium-binding protein 1 may play a critical role in the pathological processes underlying HgCl<sub>2</sub>-induced nephrotoxicity. Many studies have focused on the possible protective effects of various antioxidant agents on oxidative damage caused by heavy metals (5, 13, 14).

Alpha-tocopherol acetate (Vitamin E) was noticed as the most effective fat soluble antioxidant and had been known to explore a helpful ameliorative role in some disease processes. It protects the body's biological systems due to preventions of lipid peroxidation by scavenging free radicals ability in lipoprotein membranes (15).

It has several naturally occurring forms with d- $\alpha$ -tocopherol having the highest biopotency (16). It is well confirmed that the toxic effects of divalent Hg can be prevented by chelating or enhancing antioxidant defense systems (17). Recently, Kulanthaivel et al. (2018) found that kaempferol may play a significant role in the management of HgCl<sub>2</sub>-nephrotoxicity. Furthermore, the beneficial efficacy of using probiotic bacteria in this type of toxicity has been confirmed (18).

Humans may be exposed to various species of mercury, which includes charged inorganic mercurous of exposure, toxicity, target organs and ultimately treatment strategies vary according to the species of mercury involved in the exposure.

The current study was designed to evaluate the possible renal pathophysiologic changes due to oxidoreductive stress that chronic exposure to inorganic mercury in low doses may cause at tissue and biochemical levels.

Furthermore, it attempts to assess the possible anti-oxidoreductive stress protective impact of using vitamin E as  $\alpha$ -tocopherol acetate against HgCl<sub>2</sub>

induced lipid peroxidation mediated nephrotoxicity in rats.

## Materials and Methods

### Animals

Twenty four adult male Wister albino rats weighing 250-275 gm were used. They were reared at the animal house of the College of Pharmacy, University of Baghdad, Iraq with the approval of animal rights review committee. They were acclimatized for 1 week on normal diet of pelletized rat chow, with water given *ad libitum* at room temperature (25-28 °C) within a 12:12 hours light and dark cycle before the commencement of the experiment.

### Chemicals

Mercuric chloride (HgCl<sub>2</sub>, 99% purity) was purchased from Sigma Aldrich (Germany) and chosen to induce nephrotoxicity in rats. Vitamin E (DL- $\alpha$ -tocopherol acetate, 500 mg DL- $\alpha$ -tocopherol acetate per ml) was obtained from Merck (Germany).

### Experimental Set up

Experimental rats were randomly divided into four groups, each of six rats as follows:

**Group 1** = Control Negative Group (CN-Gr), received standard diet and normal tap water for 42 days;

**Group 2** = Control Positive Group (E-Gr), treated with vitamin E (DL- $\alpha$ -tocopherol acetate 100 mg/kg BW every other day for 42 days in corn oil;

**Group 3** = (Hg-Gr), received contaminated diet and water with HgCl<sub>2</sub> at a non-lethal dose of 0.20 mg HgCl<sub>2</sub>/kg food or water continuously every other day for 42 days; and

**Group 4** = (Hg+E-Gr), received contaminated diet and water with HgCl<sub>2</sub> at a non-lethal dose of 0.20 mg HgCl<sub>2</sub>/kg food or water with vitamin E (DL- $\alpha$ -tocopherol acetate 100 mg/kg BW in corn oil every other day for 42 days for both.

## Collection and Processing of Rat Tissues

On days zero and 43, blood samples were collected by puncture from the heart in clean test tubes, serum separated by centrifuging the blood samples at 4000 round per minute (rpm) for 5 minutes to obtain the serum for the estimation of malondialdehyde (MDA) level as oxidative stress biomarker, reduced Glutathione (r-GSH) concentration, Glutathione peroxidase (GSH-Px) and Superoxide dismutase (SOD) activities as antioxidant parameters.

Furthermore, at the end of experimental period (43 days), the animals were humanly sacrificed by using 60 mg/kg body weight of sodium pentothal. Kidneys were removed and washed with ice-cold saline and immersed 5 days in 10% neutral buffered formalin, processed in automatic tissue processor and stained with hematoxiline and eosin for histopathologic study.

## Histopathological Studies

Kidneys were fixed for 48 hours in 10% neutral buffered formalin saline. Tissues were embedded in paraffin and sectioned at 5- $\mu$ m thickness using a rotary microtome. Sections were stained with hematoxylin-eosin (H&E) according to Luna, (1968) for light microscopy examination (19).

## Biochemical Analysis

### Assessment of Oxidative Stress Biomarker

Determination of MDA by Thiobarbituric acid reactive substances (TBARS): The proteins in 0.15 mL serum were first precipitated by using sulphuric and phosphotungstic acid and then the levels of MDA were measured in these samples.

Precipitate obtained was incubated with TBA in a water bath at 95 °C for 60 minutes in an environment with oxygen and pH=3.4.

The colored complex that occurred was refrigerated to room temperature.

Then the complex was taken into n-buthanole phase. At the end, the complex of MDA-(TBA) was measured by using Shimadzu UV-1201V spectrophotometry at 532 nm. TEP (1,1,3,3-tetraethoxypropane) was used as standard MDA.

### Assessment of Antioxidative Stress Biomarkers

Serum activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) and glutathione (reduced form) of all groups were analyzed on Randox diagnostic's kits by automated chemistry analyzer.

### Statistical Analysis

Data were analyzed as one-way ANOVA using SPSS software version 18 (SPSS Inc. Chicago, IL, USA). Least significant difference (LSD) was used to separate means at  $P \leq 0.05$ . Data were expressed as mean $\pm$ SEM.

## Results

### Oxidative - Antioxidative Stress Biomarkers

On day 0, there were no significant statistical differences ( $P \leq 0.05$ ) between all groups regarding the serum levels of each of MDA, reduced glutathione, and activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) (Table1).

**Table 1. Serum malondialdehyde (MDA) concentration, reduced glutathione content, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in rats on day 0<sup>1</sup>**

Variable	Treatment			
	CN-Gr	E-Gr	Hg-Gr	Hg+E-Gr
MDA (mmol/L)	0.53±0.19 <sup>A</sup>	0.52±0.13 <sup>A</sup>	0.54±0.12 <sup>A</sup>	0.53±0.18 <sup>A</sup>
SOD (u/mgHb)	11.01±1.26 <sup>A</sup>	10.97±1.88 <sup>A</sup>	10.53±1.15 <sup>A</sup>	10.33±1.10 <sup>A</sup>
GSH-Px (mu/mgHb)	26.15±0.69 <sup>A</sup>	26.23±0.19 <sup>A</sup>	25.95±1.11 <sup>A</sup>	25.32±0.49 <sup>A</sup>
R-GSH (μ mol/gHb)	5.08±0.03 <sup>A</sup>	5.02±0.07 <sup>A</sup>	5.06±0.09 <sup>A</sup>	5.05±0.06 <sup>A</sup>

Mean±SEM, n=6. <sup>1</sup>Each value not sharing a common letter superscript is significantly different ( $P\leq 0.05$ ).

Whereas, on day 43 there was a significant statistical differences ( $P\leq 0.05$ ) between both NC-Gr and Hg+E-Gr comparing to the Hg-Gr (Group 2) regarding the estimations of MDA concentration and SOD activity.

Prominent and significant statistical decreases ( $P\leq 0.05$ ) in the activity of GSH-Px were seen between Hg-Gr comparing to the remaining groups on this day (Table 2).

**Table 2. The protective role of Vitamin E against the toxic effects of mercuric chloride on the serum levels of each of malondialdehyde (MDA), reduce form glutathione (r-GSH), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) on day 43<sup>1</sup>**

Variable	Treatment			
	NC-Gr	E-Gr	Hg-Gr	Hg+E-Gr
MDA (μmol/L)	0.60±0.19 <sup>B</sup>	0.52±0.13 <sup>C</sup>	1.88±0.16 <sup>A</sup>	0.66±0.41 <sup>B</sup>
SOD (u/mg Hb)	10.56 ±1.11 <sup>A</sup>	10.95±1.15 <sup>A</sup>	7.52±1.11 <sup>B</sup>	9.88±1.19 <sup>A</sup>
GSH-Px (mu/mgHb)	24.13±0.70 <sup>A</sup>	24.88±0.17 <sup>A</sup>	13.57±0.19 <sup>C</sup>	18.75±1.11 <sup>B</sup>
r-GSH (μmol/gHb)	6.08±0.03 <sup>A</sup>	6.10±0.05 <sup>A</sup>	3.10±0.05 <sup>C</sup>	4.95±0.06 <sup>B</sup>

Mean±SEM, n= 6. <sup>1</sup>Each value not sharing a common letter superscript is significantly different ( $P\leq 0.05$ ).

### **Histopathological Findings**

No histopathological lesions and normal tissue architecture were seen in the kidneys of both Control Negative (CN-Gr) and Control Positive Groups (E-Gr) (Figure 1 A). Meanwhile, kidneys of Hg intoxicated rats in (Hg-Gr), showed multifocal areas of coagulative necrosis of the distal part of the proximal convoluted tubules, mainly in the pars recta and in the outer stripe of outer medulla, respectively. Furthermore, nephropathies with wide range of reversible lesions like nephrosis, foci of hydropic tubular degeneration, thickened tubular basement membrane and scattered dilated tubules containing hyaline casts were noticed.

Frank, massive and diffuse cellular necrosis was observed in the proximal tubules of kidneys from rats treated with HgCl<sub>2</sub> alone. Obviously, the necrotic cells in this area were appeared in advance stages of karyolysis and cytoplasmolysis, fragmentation and dissolution.

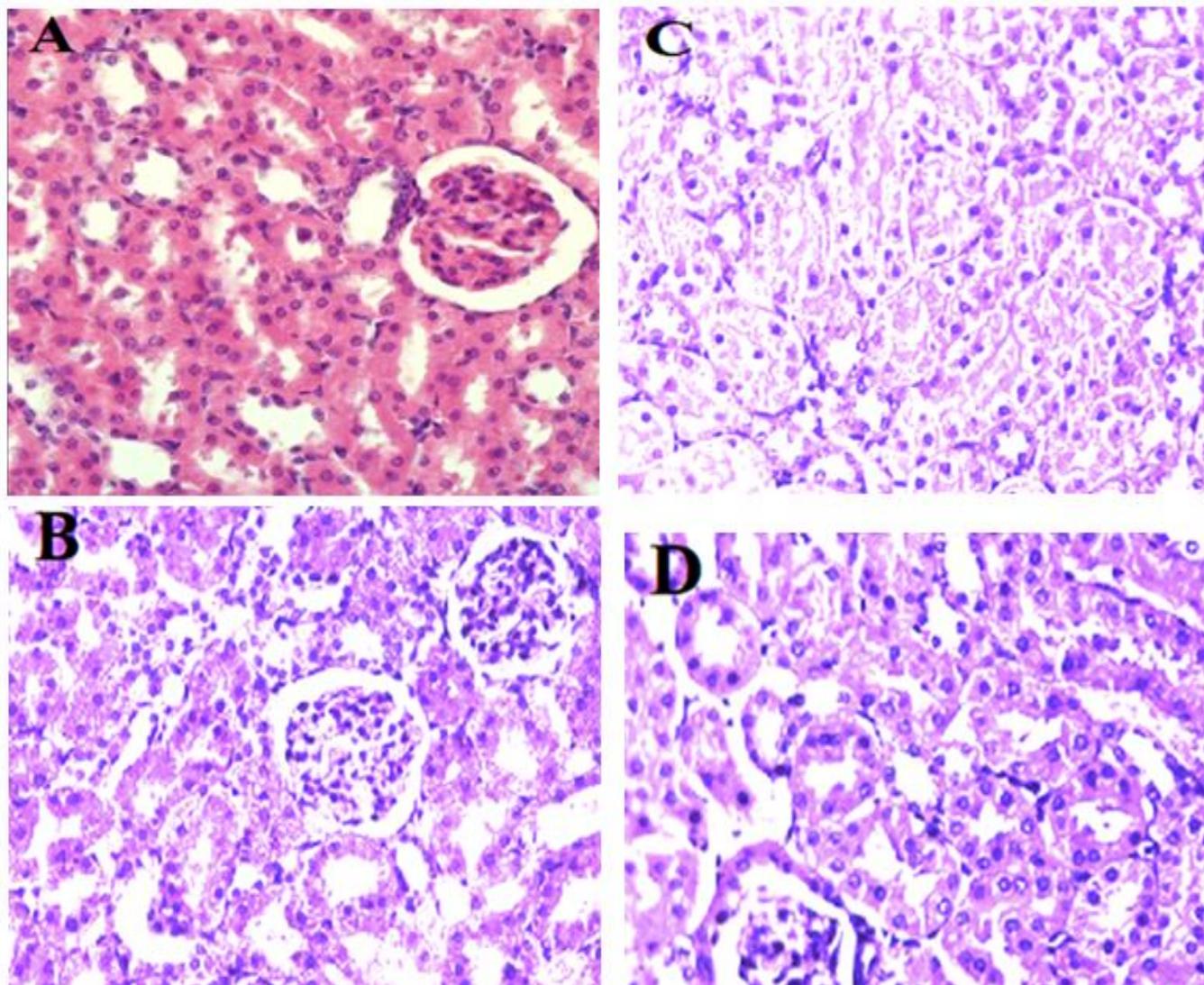
In addition, the lumen of some tubules were filled with numerous dead cells, fragments of degenerative cells, sometimes membrane-bound, and desquamated cellular debris were shed into the lumen of the endothelium tubular cells together with multiple hyaline casts formation. Infiltrated inflammatory cells in the form of

mononuclear cells mostly lymphocytes were infiltrated into the renal interstitial connective tissue (Figure 1 B, C).

On the contrary, kidneys of Hg intoxicated rats that treated simultaneously with vitamin E (Hg+E-Gr), revealed mild to moderate damages exclusively, characterized by vacuolization of some renal convoluted tubules and rarely early signs of necrosis (pyknosis) in few proximal convoluted tubules where seen in limited areas of kidney.

There was a significant reduction in lesions of epithelial and nuclear changes typically associated with tubular necrosis, observed only in the animals that were continuously treated with Vitamin E (Figure 1 D). This was evidenced by preservation of tubular morphology compared to the group treated with HgCl<sub>2</sub> alone which almost appeared to have ghost like appearance with no nuclear staining and no endothelial outlines. Statistically significant differences were observed in kidneys of vitamin E treated and control groups comparing to the Hg intoxicated group.

The kidneys lesions of all groups involved in this study are summarized in Table 3.



**Figure 1. Histopathological findings in kidney. (A) Negative Control group (NC-Gr) and positive control group (E-Gr), reveals normal tissue architecture. (B) Mercuric chloride treated group (Hg-G), nephropathies are characterized by nephrosis, multifocal intracytoplasmic hydropic and tubular degeneration, and a few hyaline casts are present in the distal part of the pars recta between the vascular bundles. (C) HgCl<sub>2</sub> treated group (Hg-G), first part of pars recta showing pyknosis, karyorrhexis, karyolysis and cytoplasmolysis as advanced nuclear and cytoplasmic features of coagulative necrosis together with mild degree of mononuclear cells infiltration. D- Kidney of rat from (HgCl<sub>2</sub>+Vitamin E) treated group (Hg+ E-Gr), a significant reduction in lesions were observed only in the rats that were continuously treated with vitamin E during the course of Hg intoxication, there is pronounced preservation of tubular morphology and frank and significant decreases in the degenerative and nuclear and cytoplasmic necrotic features**

**Table 3. Histopathologic scoring lesions in kidneys of Control Negative Group (CN-Gr), vitamin E treated Group (E-Gr), HgCl<sub>2</sub> intoxicated Group (Hg-Gr) and HgCl<sub>2</sub>+vitamin E treated Group (Hg+E-Gr) at the end of the experimental period**

Lesions	Treatment			
	NC-Gr	E-Gr	Hg-Gr	Hg+ E-Gr
Nephrosis	-	-	++++++	++
Pyknosis in the endothelium of the proximal convoluted tubules	-	-	++++++	+++
Karyorrhexis	-	-	++++++	+++
Karyolysis	-	-	++++++	++
Cytoplasmolysis	-	-	++++++	++
Infiltration of inflammatory cells	-	-	++	+

### Discussion

According to its broad ability to process blood filtration, the kidney is a favorite organ for several pollutants including mercury (20). Previous studies indicated that chronic exposure to low concentration of mercury causes tissue or organ damage (21). Obviously, excessive production of reactive oxygen species (ROS) due to mercuric chloride treatment causes oxidative stress (22) and renal failure (17).

Furthermore, mercury generates hydrogen peroxide and so contributes to renal failure. Oxidoreductive stress is defined as an imbalance between the production of ROS and their elimination by antioxidant systems. This imbalance causes severe damage of important biomolecules and organs (23). The administration of HgCl<sub>2</sub> brings about renal dysfunction, which is evident in the current study by profound decrease in glutathione concentration and severe degenerations and coagulative necrosis in the renal tubules especially the proximal convoluted tubules (PCTs). Inorganic mercury (HgCl<sub>2</sub>) has been shown to affect the morphology and function of these tubules primarily (24).

The obvious histopathologic changes in our study, may ultimately lead to decrease in glomerular filtration rate and an increase excretion of  $\gamma$ -glutamyltransferase ( $\gamma$ -GGT) which has been used as an indicator of nephrotoxicity (25). In the present study, mercuric chloride caused multiple renal histopathological changes like nephrosis, which appear as hydropic degenerative lesions composed

of accumulation of variable size water droplets with hazy boundaries in (PCTs) endo-thelium. Furthermore, hyaline degeneration and hyaline casts' formation were noticed as consequences of lipid peroxidation induce endothelial desquamation. Advance coagulative necrosis was seen as irreversible lesion in the endothelial linings of the too. The latter lesion was noticed in three patterns of nuclear changes (pyknosis in the nuclei of endothelium of the proximal convoluted tubules, karyorrhexis and karyolysis) together with cytoplasmolysis.

These changes might be due to the excessive formation of reactive oxygen species induced by mercuric chloride as consequences of lipid peroxidation mediated endothelial desquamation. The oxygen radicals attack the cell membrane and lead to disintegration of cell membrane as a result of lipid peroxidation (26).

On the other hand, the deterioration in the tubular histologic architecture can be ascribed to the decline in the mitochondrial function that follows the oxidative stress. The released free radicals are expected to attack the mitochondrial membrane resulting in loss of the ability of the mitochondria to generate ATP and subsequently to interfere with Na/K active pump, which in turn lead to hydropic degeneration. Ultimately, degeneration of PCTs is associated with loss of the brush border designation and the endothelial cell polarity (27). The mechanisms mediating renal cell death induced by

nephrotoxicants involve ATP depletion, oxidative border membrane, and cell polarity (27). Kidney histopathological changes were milder in the vitamin E plus mercuric chloride-treated group. These results are in agreement with the findings of (28) who found that post-treatment of vitamin E showed more protection in an experimental study of rat mercurial nephrotoxicity compared to pre-treatment due to the partial protection on oxidative stress parameters.

Our light microscopic histopathological findings were harmonized with the entire oxidoreductive/antioxidoreductive findings of biochemical examinations. The source of ROS generation in the current study is the structural alteration of essential macromolecules of the renal convoluted endothelial cells (DNA, protein, and lipids) by irreversible  $\text{HgCl}_2$  reactions, which generate derivatives, such as malonaldehyde and may be hydroperoxides that propagate oxidoreductive renal damages. In the current study, tubular coagulative necrosis has been attributed to oxidative damage (2) and (29). This is due to the high affinity of critical molecules such as albumin, glutathione, and cysteine for sulfhydryl groups that affects their normal functions (9). Our study showed that severe cellular coagulative necrosis involving primarily the pars recta of proximal convoluted tubules was observed in rats treated with  $\text{HgCl}_2$  only). The lesions observed in the group of rats given  $\text{HgCl}_2$  alone were localized in the S3 segment of the proximal tubules. It has been reported that  $\text{HgCl}_2$  affects the S3 segments in the cortico-medullary junction (30). As the time of  $\text{HgCl}_2$  exposure, had increased the injury notices to involve both S1 and S2 segments of the proximal convoluted tubules. The renal damage caused by  $\text{HgCl}_2$  was histopathologically ameliorated by vitamin E co-administration with  $\text{HgCl}_2$ . The co-administration of vitamin E as free radical scavenger with  $\text{HgCl}_2$  restored the altered indices to near normal levels by preventing or greatly retarding the oxidation of easily  $\text{HgCl}_2$  oxidizable phospholipid cell membrane. Unfortunately, this study has showed that  $\text{HgCl}_2$  caused damaged to the normal collagen content of the kidneys which was not restored by vitamin E treatment. The latter finding is completely coinciding with previous work of (24). In the current study, vitamin E participates in the restoring of reducing form glutathione the master antioxidative precursor of several primary enzymatic systems. Among these enzymes, GSH-Px, which has at least eight-glutathione dependent

stress, proximal tubule cell death, loss of the brush enzyme forms (GSH-Px1–GSH-Px8) (31) was triggered and activated. Consequently, this activated enzyme plays an important role in inhibiting the process of lipid peroxidation (destroying the peroxides) and, therefore, protects cells from oxidoreductive stress damage (18). On the other hand, superoxide dismutase (SOD) metalloenzyme the most important and most powerful detoxification enzyme in the cell that catalyzed the dismutation of the highly reactive superoxide anion to  $\text{O}_2$  and to the less reactive species  $\text{H}_2\text{O}_2$  (32) was significantly activated by vitamin E supplementation. This SOD activation, in turn, prevents free radicals protein oxidation (33) and subsequently decreases the coagulative necrosis damage.

It can be concluded from the current study that  $\alpha$ -tocopherol acetate plays a significant role in the management of nephrotoxicity via triggering and restoring the anti-oxidant status of  $\text{Hg}$ -intoxicated rats. However, further studies are needed to be conducted to find out the molecular aspects of nephro-protective role of vitamin E alone and/or in combination with selenium and other agents to ascertain their potential.

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### Conflict of Interest

The authors declare that there is no conflict of interest.

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## تأثير استخدام فيتامين هاء كألفا-توكوفيرول اسيتيت على حالة الجهد التأكسدي الاختزالي الناشيء عن المحدث كلوريد الزنبيق والمتسبب في حالة التسمم الكلوي في الجرذان

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### الخلاصة

صممت هذه الدراسة لمعرفة تأثيرات فيتامين هاء على حالة التسمم الكلوي المستحدث تجريبياً في الجرذان باستخدام جرعة غير مميتة من مركب كلوريد الزنبيق مقدارها 0.2 ملغم/كغم علف او ماء بين يوم واخر مع او بدون تجريع الجرذان فمويافيتامين هاء وجرعة منه مقدارها 100 ملغم/كغم وزن حي ولفترة 42 يوماً. لقد تسبب كلوريد الزنبيق بعد 42 يوماً بارتفاع المؤشر الحيوي لحالة الاجهاد التأكسدي الاختزالي المسمى المألون داي الديهايد وانخفاض في تركيز الكلوتاثيون المختزل وقلّة في فعالية انزيمي كلوتاثيون بيروكسيديز وسوبر اوكسايد دسميوتيز المضادين لأكسدة الدهون وللجهد التأكسدي الاختزالي وقد سببت أيضاً تغييرات نسيجية تنخرية وتنكسية في كلى الجرذان المعاملة به. في نفس الوقت اظهر الاستخدام المتزامن لفيتامين هاء مع كلوريد الزنبيق انخفاضاً معنوياً في مؤشر أكسدة الدهون وارتفاعاً مهماً احصائياً في فعالية الانزيمات مضادات الجهد التأكسدي الاختزالي وتقليلاً في الاضرار والافات النسيجية المرضية الناشئة عن التسمم الكلوي بالزنبيق في الجرذان.

كلمات مفتاحية: فيتامين هـ، ألفا توكوفيرول، الجهد التأكسدي، كلوريد الزنبيق، التسمم الكلوي