Original Communication

# Pro-oxidant effects of a high α-tocopherol dose on kidney antioxidant biomarkers and histopathological aspects

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**Abstract:** The aim of this study is to evaluate the effect of α-tocopherol supplementation at two doses (600 and 1200 mg × kg$^{-1}$) on kidney antioxidant status and the histopathological changes in Wistar rats after 12 weeks of exposure at different diets. Forty rats has been divided into 4 groups of 10 rats each, the control group received basal diet with 5 % fresh sunflower oil (FSO), the second group: 5 % oxidized sunflower oil (OSO), the third group: 5 % OSO supplemented with 600 mg × kg$^{-1}$ α-tocopherol and the fourth group: 5 % OSO supplemented with 1200 mg × kg$^{-1}$ α-tocopherol. In OSO groups, the results showed highly significant increases of LPO (from 31.3 ± 0.9 to 53.8 ± 1.2 nmol of MDA formed/min/mg protein, $p < 0.0001$) with a significant decrease ($p < = 0.001$) of the antioxidant enzymatic activities (CAT, SOD, GPX, GR and G6PDH), body weight (339 ± 9 to 290 ± 3 g) and α-tocopherol levels (13.6 ± 0.6 to 6.5 ± 0.4 μg/mg protein). In OSO groups with 600 mg × kg$^{-1}$ α-tocopherol, an antioxidant effect was found, reflected by a return of the parameters to values similar to those of the control group. However, higher doses of α-tocopherol (1200 mg × kg$^{-1}$) induced a depletion of antioxidant status, α-tocopherol levels (6.0 ± 0.3 μg/mg protein, $p < 0.001$) and a very highly significant rise ($p < 0.0001$) of LPO content (54.86 ± 0.01 nmol of MDA formed/min/mg protein). The kidney tissues also showed changes in glomerular, severe inflammatory cells infiltration, and formation of novel vessels. So, we can conclude that the oxidative stress is attenuated by a moderate administration of 600 mg × kg$^{-1}$ α-tocopherol, while a pro-oxidant effect occurs at 1200 mg × kg$^{-1}$ α-tocopherol.

**Keywords:** Rat, oxidized sunflower, α-tocopherol, kidney, oxidative stress, kidney histopathology

## Introduction

Nutritional or dietary oxidative stress denotes disturbance of the redox state resulting from oxidative load excess or from inadequate nutrient supply favoring pro-oxidant reactions [1]. Lipid peroxidation initiated by free radicals, is considered to be deleterious for cell membranes, and has been implicated in several pathological situations [2]. Thus, oxidative stress plays a critical role in hepatotoxicity, and nephrotoxicity [3], DNA damage that obstructs cells main structure and function and contributes to some degenerative disorders among pathophysiology of several kidney diseases [4].

As far as sunflower oil is concerned, it is known to be widely used in nutrition as a source of essential linoleic acid, and of many staple diets preparation. Nevertheless, due to its high content of polyunsaturated fatty acids, sunflower oil is susceptible to peroxidation, and so free radicals can be formed in high amounts [5].

Oxidative stress represents the bodies' imbalance in the production and the elimination of reactive oxygen species (ROS) [6], which are key mediators of the multi-

ple organ failure and endotoxin induced mortality [7]. Indeed, ROS causes lipid peroxidation of membrane phospholipid which can alter membrane fluidity and impair the different mitochondrial enzymes activities, leading to decreased intracellular energy, cells necrosis and organ failure [7]. In addition, ROS modulates several enzymes as well as membranes receptors, ions channels, lipids kinases, phosphatases transports, and various transcription factors. They are also implicated in virtually all mitochondrial functions from ATP, generation $Ca^{+2}$ buffering, to apoptosis induction. [8]. All this, promote renal cell apoptosis, senescence, decreased regenerative ability of cells, and fibrosis.

These factors have an astochastic deleterious effect on kidney function [9]. In fact, the antioxidant status is an important protective mechanism against ROS, and as many other biochemical systems, their effectiveness vary along with the stage of development, and other physiological aspects of the organism [10]. For instance, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), are considered to be the main antioxidant enzymes that act as defense [11]. The incidence of lipid peroxidation may depend upon both the antioxidant enzymes level, and the fatty acids composition in the organism [12].

Vitamin E (α-tocopherol, αT) is the major lipid soluble vitamin, with a high antioxidant capacity. It plays a fundamental biological role, especially in protecting cells and tissues from oxidative damage, and prevents the formation of toxic oxidation products such as those produced by unsaturated acids [13]. Also, Vitamin E is an effective scavenging lipid radicals recognized as a potent chain-breaking. Moreover, it is also considered to be an antioxidant preventing the production of free radicals reactions [14] in membranes and lipoproteins.

The α-tocopherol occurs in several natural forms, and it is the highest biological active compound responsible for the antioxidant action, in a great part. [15]. A number of studies have demonstrated the potential effect of Vit E on the activity of antioxidant enzymes fatty acids, LPO composition [16, 17].

Its ability to act as a pro-oxidant and increase the peroxidation of lipid is well known in *vitro* [18]. Additionally, a meta-analysis research [19] has demonstrated a dose dependent relationship between Vit E supplementation, and all-cause mortality. In this way, the present study has been designed to investigate (1) nephrotoxicity induced by oxidized sunflower oil diet in rats, (2) the effectiveness of αT to modulate kidney damage, and (3) the effect of high αT supplementation on the structural and functional kidney oxidative stress markers.

# Materials and methods

## Chemicals

The compounds used for diet formulations were obtained from Sigma Chemical Co., St Louis, MO, USA. Vitamin (AIN-76) mix, Mineral (AIN-76) mix and micro-pulverized vitamin-free casein were purchased from UAR 250, Ville Moisson, Paris-France, α-tocopherol from Merck (Darmstadt, Germany). For the other chemicals and reagents used, they were bought from Boehringer Mannheim (Germany), and ultra-filtrated water was used for all biochemical assays.

## Sunflower oil

Fresh Sunflower oil was obtained from ENCG (National Company of Fat, Algiers, Algeria). It has been heated continuously at 98 ± 2 °C for 46 hours, with air insufflations (40 $L \times h^{-1}$). The acceleration of oxidation was accomplished by vigorous mixing of heated sample with oxygen at an atmospheric pressure [20]. The heated oil was stored in black containers at 4 °C until its use for the diets.

## Experimental animals: Housing and grouping

Forty males Wistar rats aged 3 weeks and weighing about 65 to 75 g, obtained from Pasteur Institute of Algiers were used. The animals were housed in a plastic cage at room temperature of 25 ± 1 °C, with 12 h light/dark cycle.

They have been acclimatized for 10 days before beginning the experiment and have been fed according to formulated diets as illustrated in Table 1. Access to food and water was *ad libitum*. All rats were handled in accordance with the standard guide for the care and use of laboratory animals and its up dated issue [21, 22].

The rats were divided into four groups, the first one received the basal diet (control) with 5% weight fresh sunflower oil (FSO), while the second group received 5% sunflower oil heated as described previously (OSO).The third one received 5% heated sunflower oil supplemented with αT (600 $mg \times kg^{-1}$, $OSOE_1$), while the fourth group received 5% heated sunflower oil supplemented with αT (1200 $mg \times kg^{-1}$, $OSOE_2$). The treatments were added daily to ration until the end of the experiment (12 weeks).

**Samples collection:** The body weight of the rats has been determined weekly during the experimental period (12 weeks). At the end of the experiment, the rats were fasted overnight, anesthetized with 10% chloral hydrate (3 $mL \times kg^{-1}$ BW), and then euthanized with overdose.

**Table 1.** Composition of each experimental diet (/100 g).

| Ingrediets | Diets | | | |
|---|---|---|---|---|
| | FSO | OSO | $OSOE_1$ | $OSOE_2$ |
| Casein (g) | 20 | 20 | 20 | 20 |
| *dl*-Methionine (mg) | 0.16 | 0.16 | 0.16 | 0.16 |
| Agar-agar (g) | 2 | 2 | 2 | 2 |
| Corn (g) | 44.90 | 44.90 | 44.90 | 44.90 |
| Sucrose (g) | 21.94 | 21.94 | 21.94 | 21.94 |
| FSO (ml) | 5 | / | / | / |
| OSO (mL) | / | 5 | 5 | 5 |
| Mineral mixture[a] (mg) | 4 | 4 | 4 | 4 |
| Vitamin mixture[b] (mg) | 2 | 2 | 2 | 2 |
| Energy (kcal) | 392 | 392 | 392 | 392 |

FSO, fresh sunflower oil; OSO, oxidized sunflower oil; $OSOE_1$, oxidized sunflower oil diet supplemented with α-tocopherol (600 mg × $kg^{-1}$); $OSOE_2$, oxidized sunflower oil diet supplemented with α-tocopherol (1200 mg × $kg^{-1}$).
[a] Mineral mixture composition (mg × 100 $g^{-1}$): $KH_2PO_4$ 20.0; $CaCO_3$ 34.6; $CaHPO_4$ 26.55; NaCl 13.70; $MgSO_4 7H_2O$ 3.42; $CuSO_4 5H_2O$ 0.042; $MnSO_4 H_2O$ 0.27; $FeSO_4 7H_2O$ 1.02; $ZnSO_4 H_2O$ 0.15; $CaCO_3$ 0.0008; KI 0.0016.
[b] Vitamins content per kilogram of diet: Vit A 19.800 IU; $D_3$ 2.500 mg; $B_1$ 20 mg; $B_2$ 15 mg; $B_3$ 70 mg; $B_6$ 10 mg; $B_7$ 150 mg; $B_{12}$ 0.05 mg; C 800 mg; E (*dl*-α-tocopherol acetate) 170 mg; $K_3$ 40 mg; PP 100 mg; choline chloride 1.360 mg; folic acid 5 mg; AcPAB 50 mg; biotin 0.3 mg; feed units per rat = 210.

The kidneys were removed immediately after sacrifice, cleaned with ice-cold sodium chloride solution and weighed. Small pieces of kidney were cut and kept in 10 % naturel formalin for histopathological study, and other portions were dipped in nitrogen and stored at –70 °C for oxidative stress biomarkers studies (LPO, SOD, CAT, GPx, GR and G6PDH, total protein and αT levels.

## α-tocopherol level and lipid oxidation of tested oils

The α-tocopherol level of these oils has been measured by a Beckman Model 342 liquid chromatograph (Fullerton, CA, USA) equipped with a Model LC-95 detector (Perkin-Elmer, Norwalk, CT, USA), and a reversed-phase column (Superspher RP-18, 4 µm, 25 mm x 4 mm I.D., LiChroCART HPLC cartridge, Merck) as described by Gimeno et al. [23]. The diene content (µmol × $kg^{-1}$) of the tested oils (FSO and OSO) was determined on the basis of the molar absorption coefficient (29500 L × $mol^{-1}$ × $cm^{-1}$) of the conjugated dienes which were assessed at 234 nm in a hexane solution. Similarly, the peroxide, iode and acid values were determined according to the AFNOR method [24].

## Fatty acid profile

The fatty acid profile of the oils used in this study has been obtained from the chromatographic analysis of the methyl esters, prepared after a saponification at 60 °C for 30 min with 40 mL × $g^{-1}$ 0.5 N NaOH in methanol, followed by methylation using boron trifluoride-methanol complex. Tricosanoic acid has been employed as internal standard. As far as the esters are concerned, they were extracted into hexane, free of moisture over anhydrous sodium sulphate, and dried under nitrogen.

The fatty acid content of the oil was analysed in a Hewlett–Packard 5890 Series II gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionization detector and a 50 m, i.d. 0.22 mm capillary column (BPX70) with 0.25 mm film thickness (SGE, Austin, Texas, USA). Three samples from the fresh oil and from heated oil were analysed. The total altered fatty acid content in oil was calculated, taking into account the amount of chromatographed fatty acids [25].

## Oxidative stress biomarkers in kidney

Malondialdehyde (MDA) levels have been measured in kidney according to thiobarbituric acid, reactive substances (TBARS) assay described by Buege and al.,(1976) [26]. The samples suspended in phosphate buffer saline (pH 7.4), have been mixed with butyl-hydroxytoluene BHT-TCA solution (1 % w/v BHT dissolved in 20 % TCA), then centrifuged at 1000 g for 10 min. The reaction mixture containing the supernatant collected from the centrifugation, 0.5 N HCl and 120 mM 2-thiobarbituric acid (TBA) in 26 mM Tris, has been heated at 80 °C for 15 min. After cooling, MDA production expressed in nmol MDA × $mg^{-1}$ of protein, and using an extinction coefficient of $1.56.10^5$ $M^{-1} \times cm^{-1}$ the absorbance of the resulting chromospheres has been measured at 532 nm with a BIORAD UV-visible spectrophotometer (Smart Spec 3000).

Kidney Glutathione peroxidase (GPx) (E.C.1.11.1.9) level was determined according to Paglia and Valentine method [27]. The Oxidized glutathione generated by the conversion of NADPH to NADP+, under the glutathione reductase action (GR), and after adding cumene hydroperoxide, has been monitored spectrophotometrically at 340 nm for 2 min.

A GPx activity has been expressed in nanomoles of GSH oxidized per minute and per milligram of protein.

Superoxide dismutase (SOD) (E.C.1.15.1.1) activity, definited as units per milligram of protein, is based on the inhibition of superoxide radical reaction with pyrogallol [28]. It has been determined by measuring the velocity of oxidized pyrogallol formation. The reaction medium in

which it is formed, contains Tris buffer (50 mmol×$L^{-1}$, pH 8.2), pyrogallol (24 mmol×$L^{-1}$) and catalase (30 mmol×$L^{-1}$). For the absorbance measures, they have been changed at 420 nm for 2 mn. Catalase (CAT) (E.C.1.11.1.6) activity has been assayed by the method of Aebi [29], which is based on the disappearance of $H_2O_2$ at 240 nm. One unit of the enzyme is defined as 1 µmol of hydrogen peroxide consumed per minute, and the specific activity was reported as micromoles of $H_2O_2$ decomposed per mg of protein. Glutathione reductase (GR) (E.C.1.6.4.2) activity was evaluated at 37 °C and 340 nm, following the oxidation of NADPH by glutathione disulfide (GSSG) [30]. One EU is defined as the enzyme oxidation of 1 mol of NADPH per min at 25 °C and at optimal pH (8.0). Glucose-6-phosphate dehydrogenase (G6PDH) (E.C.1.1.1.49) catalyzes the conversion of G-6-P to 6-phosphogluconate. Its activity was assayed by measuring the rate of NADH formation, followed spectrophotometrically at 340 nm [31].

### Total protein assay

The protein content of supernatants has been spectrophotometrically estimated, according to Lowry method, using bovine serum albumin as standard [32]. αT level has been measured following the absorbance according to the method of [33], and expressed as µg×$mg^{-1}$ of protein.

### Histopathological studies

The kidney has been dehydrated in graded serial of alcohol and embedded in paraffin wax. Five micrometer thick sections have been cut and stained by hematoxylin and eosin (H&E). In addition, ten field areas have been examined for histopathological changes.

The examination was done using a light microscope (Olympus BX50) with a camera (Olympus E-410). The histopathological alterations in kidney have been scored as follows; normal appearance (–), mild (+), important (++), and severe (+++).

### Statistical analysis

The normality of data distributions and the homogeneity of variances have been tested using Kolmogorov-Smirnov and Levene tests respectively. Concerning the mean parameters of the different groups, they have been compared to those of control group using Dunnett tests. Some comparisons between experimental groups have also been performed using Student t-tests. The results are expressed as means ± SE (SE: standard error of mean), and have been considered as significant at ($p < 0.05$). The statistical analysis has been established using Statistica 10, StatSoft Inc., Tulsa, OK 74104, USA.

## Results

### Fresh and oxidized Sunflower oil status

The chemical features of fresh and oxidized sunflower oil, are presented in Table 2 and 3. As observed in (Table 2), heating fresh sunflower oil causes its alteration by the depletion of αT (2 vs 0 µg×$mL^{-1}$), and by the higher increase of peroxides and hydro peroxides values (1 vs 258 mmol×$kg^{-1}$).

In addition, the fatty acid composition is also affected by heating, while the polyunsaturated fraction, mainly C18:2 (Table 3) is oxidized, and its proportion has been reduced by 2/3. Thus, polar esters appear in the oxidized sunflower oil.

### Body and kidney weight

The effect of OSO and αT on body and kidney weight, is illustrated in Figures 1A and 1B respectively. The body weight of the OSO group has decreased significantly compared to the FSO group (Dunnett test, $p < 0.001$).

Also the supplementation of diet with 600 mg×$kg^{-1}$ αT has restored the body weight of OSO fed rats. By contrast, the fact of increasing the dose αT at 1200 mg×$kg^{-1}$ has not improved rat's growth. Kidney weight for the OSO fed group has dropped compared to the control group, but not significantly ($p = 0.84 >> 0.05$). In the same way, the supplementation with αT at 600 mg×$kg^{-1}$ ($OSOE_1$) and at 1200 mg×$kg^{-1}$ ($OSOE_2$) does not show any change in kidney weight ($p = 0.996$ and 0.975 respectively).

**Table 2.** Lipid oxidation markers and α-tocopherol content of tested oils.

| Parameters | I | II |
|---|---|---|
| Iodine value | 130 | 82 |
| Acid value (KOH mg×$g^{-1}$) | 0.1 | 4.2 |
| Peroxides value (mmol×$kg^{-1}$) | 1 | 258 |
| Hydroperoxides (mmol×$kg^{-1}$) | 10 | 232 |
| Ethylenic diketones | 0.6 | 2.8 |
| α-tocopherol (µg×$mL^{-1}$) | 2 | 0 |

I: Fresh sunflower oil; II: Oxidized sunflower oil.

**Table 3.** Fatty acids composition of tested oils expressed as the percentage of total fatty acids content.

| Parameters | I | II |
|---|---|---|
| Polar esters (%) | 0.00 | 43.3 |
| Non altered fatty acids (%) | 100.0 | 56.7 |
| 14:00 | 0.05 | nd |
| 16:00 | 5.62 | 5.62 |
| 18:00 | 3.57 | 4.32 |
| 18:1 ω9 | 27.5 | 25.5 |
| 18:2 ω6 | 61.6 | 19.9 |
| 18:3 ω3 | 0.19 | 0.25 |
| 20:00 | 0.23 | 0.11 |
| Saturated fattyacids | 9.48 | 10.1 |
| Monounsaturated fatty acids | 27.5 | 25.5 |
| Polyunsaturated fatty acids | 61.8 | 20.1 |

I: Fresh sunflower oil; II: Oxidized sunflower oil
nd: not detected

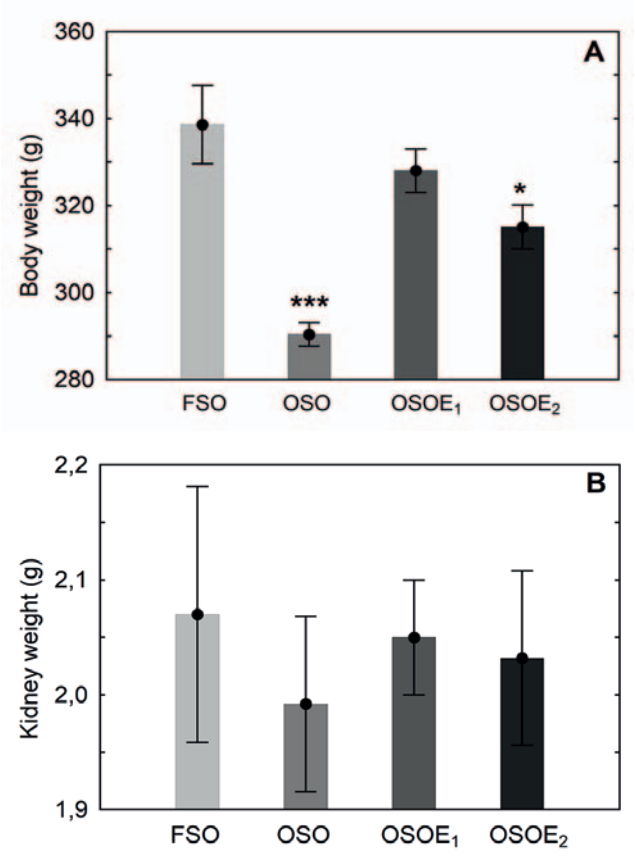


**Figure 1.** Effect of oxidized sunflower oil (OSO) supplemented with α-tocopherol at 600 and 1200 mg×kg$^{-1}$ on body and kidney weights (mean ± standard error, 5 replicates for each bar), (*p < 0.05, **p < 0.01, ***p < 0.001 between control and treatment groups).

## Vitamin E

In OSO and OSOE$_2$ groups exposed rats (Figure 2), a very important decrease is noted in αT levels as compared to the control group (p <0.001), whereas supplementation with αT at 600 mg×kg$^{-1}$ in the diet shows recovery of this antioxidant level, but remains significantly lower compared to the control group (p = 0.001).

## Lipid peroxidation

Figure 3 shows a highly significant increase in the concentration of lipid peroxidation in rats exposed to OSO (p <0.0001) compared to control rats FSO. The addition of αT at 600 mg×kg$^{-1}$ OSE$_1$ to rats, offers a protection against OSO, and induces lipid peroxidation in rat kidney, as evidenced by the significant decrease in LPO formation values, which remains comparable to those of the control group (p = 0.59).

An increase of the dose of αT at 1200 mg×kg$^{-1}$ OSE$_2$ group, does not enhance the protection lipid peroxidation; inversely, the level of LPO has risen very significantly (p <0.001) as for the oxidized group (OSO).

## Antioxidant enzymes activities

The results obtained from the evolution of antioxidant enzymes activities, namely CAT, SOD, GPx , G6PDH and GR in kidney of experimental animals, are presented in Figure 4 (A to E).

A highly significant decrease is observed in the activities of CAT (p <0.001), SOD (p <0.001), GPx (p <0.001),

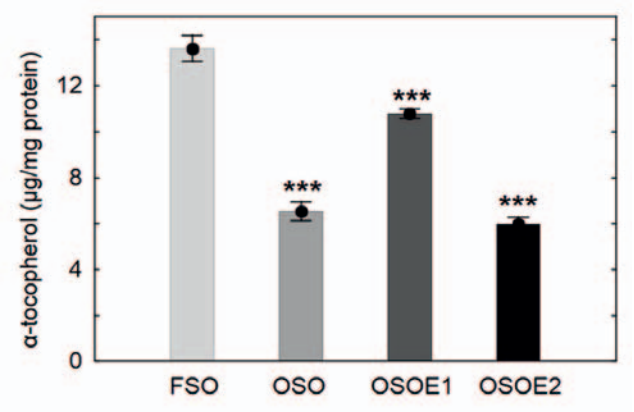


**Figure 2.** Effect of oxidized sunflower oil (OSO) supplemented with α-tocopherol at 600 and 1200 mg×kg$^{-1}$ on α-tocopherol level in kidney (mean ± standard error, 5 replicates for each bar), (***p <0.001 between control and treatment groups).

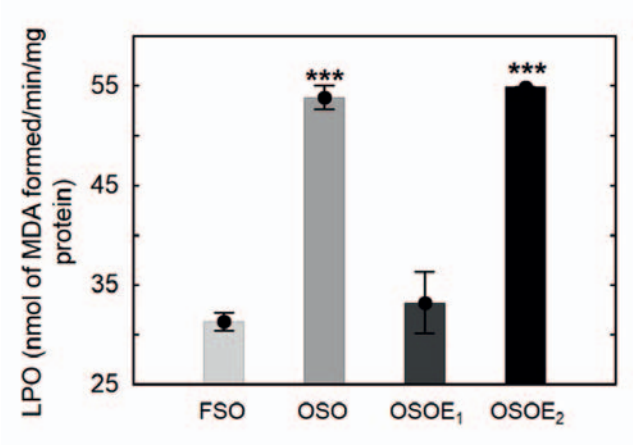


**Figure 3.** Peroxidation markers (mean ± standard error, 3 replicates for each bar) in the kidney of different treated groups (***p <0.001 between control and treatment groups).

G6PDH ($p < 0.001$), and GR ($p = 0.001$) for OSO feed rats compared to the control group.

Upon simultaneous administration of OSO and αT at 600 mg×kg$^{-1}$, the antioxidant enzyme CAT activities increase significantly, but remain lower than those of the control group ($p = 0.03 < 0.05$). Though, the mean levels of SOD, GPx, G6PDH, and GR activities stay comparable to the control group ($p = 0.07$, 0.99, 0.86 and 0.20 respectively).

Conversely, the treatment with OSO at αT 1200 mg×kg$^{-1}$ induces a very significant reduction of CAT ($p < 0.001$), SOD ($p < 0.001$), GPx ($p = 0.001$), G6PDH ($p < 0.001$), and GR ($p < 0.001$) activities as shown in Figure 4 (A to E), compared to the control group.

## Histopathological findings

Figure 5 (A to D) and Table 4 show histopathogical alterations, and score in male rats kidneys exposed to different diets OSO, $OSOE_1$ and $OSOE_2$, compared to the control FSO.

A normal histopathological structure of glomeruli and tubules has been recorded in kidneys of control group rats (FSO) as demonstrated in (Figure 5 A and Table 4). Nevertheless, severe histopathological alterations including edema, congestion of blood vessels, glomerular atrophy, and inflammatory cells infiltrations are noted in rats kidneys exposed to oxidized sunflower oil (OSO group) (Figures 5B(x10) and 5B(x40), Table 4).

The administration of αT at 600 mg×kg$^{-1}$ ($OSOE_1$ group) has, on the contrary, displayed a remarkable improvement in the renal injuries towards the normal histoarchitecture of the kidney, a regeneration of some glomerular, and has reduced inflammatory cells infiltration as observed in (Figure 5 C and Table 4).

However, the rats exposed to 1200 mg×kg$^{-1}$ of αT ($OSOE_2$ group) have presented an important blood vessels congestion, with severe inflammatory cells infiltration, edema and degenerative changes in glomerular, also an alteration of renal structure tubules, including novel vessels formation. (Figures 5D(x10) and 5D(x40), Table 4).

# Discussion

It is obvious that kidneys play a pivotal role in the regulation of various chemicals [34], and the oxidative stress can produce alteration of kidneys functions [35]. Indeed, vit E is effective in scavenging free radicals thus preventing lipid peroxidation in a membrane [36].

In the present study, 5 % OSO has reduced significantly the body weight which may be due to the cellular damage caused by the increased lipid peroxidation, and to the ROS production [37]. Our findings are similar to those obtained by Ahmed and Mahmood (2012) [38].

Nevertheless, supplementation of OSO with αT at 600 mg×kg$^{-1}$ has prevented weight loss in agreement with the observations of [39] and [35] who have reported that vitamin E improves daily food intake and body weight. Whereas, at a higher dose of αT (1200 mg×kg$^{-1}$), there is no significant change in growth similar to the result of [40], which has not shown any significant difference in the weight gain of fish fed diet, containing either fish oil or swine fat, supplemented with 1500 mg of αT acetate per kg of diet.

Furthermore, we have not observed a significant decrease in the kidney weight for the different groups, as reported by Osim et al. [41] and Rouaki et al. [42] with oxidized palm and sunflower oil respectively.

Changes in activity of antioxidant markers, such as CAT, SOD, GPx, GR, G6PDH, and αT were accompanied by an intensification of lipid peroxidation process, considered as an index of oxidative stress severity.

Lipid peroxidation (LPO) content is significantly higher in OSO group, the same way just as mentioned in previous studies [43, 44]. So these results are associated with a significant reduction of αT levels (Figure 2), and a decreased enzyme activities (Figure 4, A to E). Elmaddawy and Gad (2012) [45] have demonstrated that the impairment in the kidney function markers coincides with a significant increase in LPO product. However, the reduction of αT level may result from its use in the process of free radicals scavenge [46]. Sies. [47] , Banudevi et al. [37] confirmed that αT reacts effectively with organic lipid radicals production in the process of LPO.

Histopathology has stained kidney sections of OSO group, and has shown a significant loss of normal architecture that is reflected by blood vessels congestion, with in-

 

flammatory cells infiltration, edema and glomerular atrophy (Figures 5B(x10) and 5B(x40) and Table 4). These lesions corroborate the various changes of biochemical parameters, related to kidney functions.

Some researchers like Osim et al. [41] have indicated that peroxides contained in oxidized oil cause congestion, hemorrhage and tubular atrophy of the kidneys. This effect may be related to reactive oxygen species resulting from membranes lipid peroxidation, which leads to a loss of membranes integrity and enzymes activity, and a decrease of its fluidity, inducing eventually, cell death [48]. On the other hand, a drastic decrease in CAT

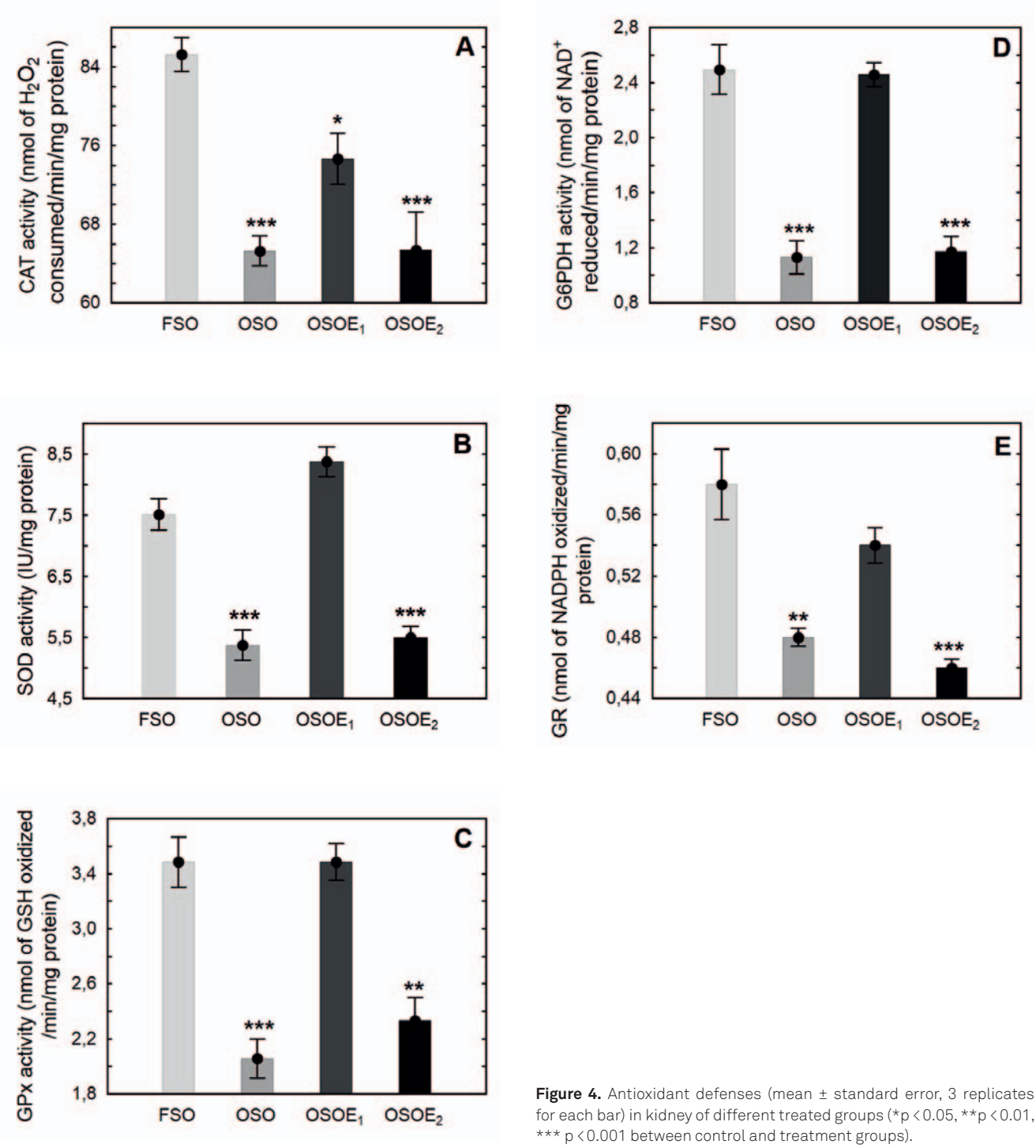


**Figure 4.** Antioxidant defenses (mean ± standard error, 3 replicates for each bar) in kidney of different treated groups (*$p < 0.05$, **$p < 0.01$, *** $p < 0.001$ between control and treatment groups).

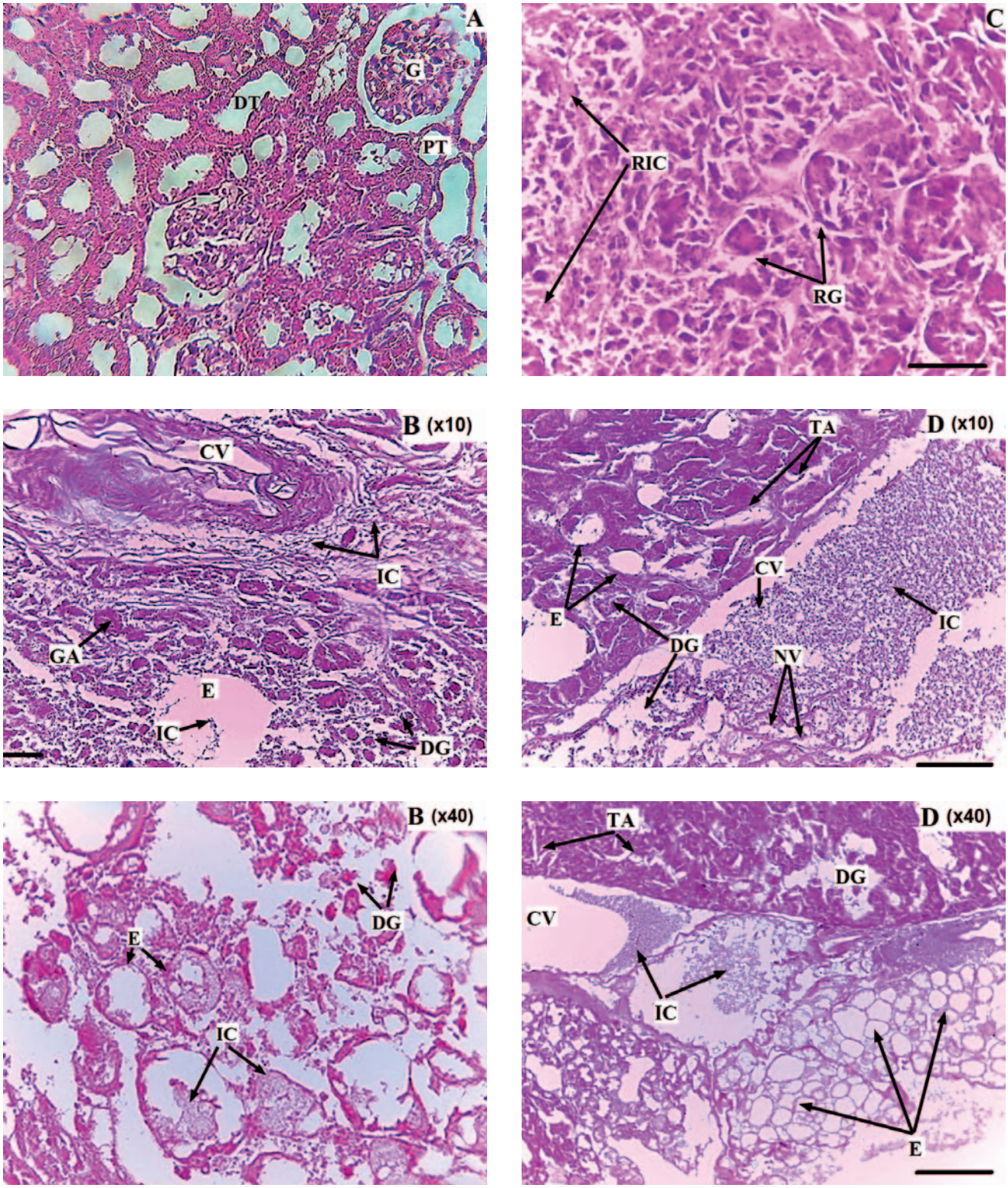


**Figure 5.** Light micrograph of rat kidney showing : (5A) normal histological structure glomerulus (G), proximal tubules (PT) and distal tubules (DT) in fresh sunflower oil (FSO) group, (5B) glomerulus atrophy (GA), congestion of blood vessels (CV) and edema (E) in oxidized sunflower oil (OSO) group, (5C) remarkable improvement in the histological appearance with a considerable regeneration of glomeruli (RG) and a reduction of inflammatory cells infiltration (RIC) in oxidized sunflower oil diet supplemented with 600 mg × $kg^{-1}$ α-tocopherol (OSOE1) group, (5D) severe degenerative changes in glomerulus (DG), severe edema, formation of novel vessels (NV), and congestion vessels with severe inflammation cells infiltration (IC) in oxidized sunflower oil diet supplemented with 1200 mg × $kg^{-1}$ α-tocopherol (OSOE2) group. All figures with bar = 50 μm except 5B(x10) and 5D(x10) whith bar = 200 μm).

**Table 4.** Grading of the histopathological changes in kidney sections of different groups.

| Groups | Glomerulus atrophy | Congestion of blood vessels | Inflammatory cells infiltration | Edema | Degenerative changes in glomerulus | Alteration of tubules structure |
|---|---|---|---|---|---|---|
| *Control FSO* | – | – | – | – | – | – |
| *OSO* | +++ | ++ | ++ | ++ | +++ | ++ |
| *OSOE$_1$* | – | – | – | – | – | + |
| *OSOE$_2$* | – | +++ | +++ | +++ | +++ | +++ |

+++ Severe; ++ important effect; + mild; – null
FSO, fresh sunflower oil; OSO, oxidized sunflower oil; OSOE$_1$, oxidized sunflower oil diet supplemented with α-tocopherol (600 mg × kg$^{-1}$); OSOE$_2$, oxidized sunflower oil diet supplemented with α-tocopherol (1200 mg × kg$^{-1}$).

and SOD activities ($p < 0.001$) has been observed, as shown in Figures 5 A and B. This could be due either to a loss of the cells expressing these enzymes, as to direct effect of reactive oxygen species on the enzymes, or to a direct inhibition of oxidized oil causing impairment of the antioxidant function, in accordance with those reported by Ozden et al. [49] in kidney and liver rats exposed to methiocarb.

As for the significant decline in GPx activity (Figure 5 C), it might be ascribed to a reduction in the level of substrate gluthation, and an increase in the level of peroxide [50].

Thus, the balance of this enzymes system can be essential to remove superoxide anion, and peroxides generated in kidney [37], in agreement with earlier findings of [13] and [42].

The GR activity is lower in kidney (Figure 4 E), suggesting inadequate levels of reducing equivalents NADPH as proposed by Banudevi et al. [37]. A significant reduction of G6PDH activity (Figure 5 D) indicates a failure to maintain GSH levels. Our results are similar to those indicated by Rouaki et al. [42]. So according to [51], this result leads to a diminution of the NADPH production and eventually, to a decrease of blood antioxidant levels, even though the supplementation at 600 mg×kg$^{-1}$ of αT has shown a significant increase in renal antioxidant status and αT level, with a fundamental drop in LPO, demonstrating the stress stabilization through αT activity.

These data are in agreement with a number of reports, which have shown the increased activity of intracellular antioxidant enzymes and renal levels of αT, correlated with a decrease in lipid peroxidation [52, 53]. The antioxidant property of αT is indeed, not only to scavenge ROS, but also to up- regulate antioxidant enzymes through the control of gene expression or the activity of antioxidant enzymes [54]. Furthermore, the oxidative stress effects are, at least, partially normalized, exhibiting a significant protective effect in all aspect, as it is obvious from reversal of histopathological alteration.

It has been confirmed by kidney sections showing a remarkable improvement in the histological appearance, with considerable regeneration of some glomerular, and a reduction of inflammatory cells infiltration (Figure 5 C and Table 4).

These results have been reported earlier, by Rouaki et al. [42] in heart cells, suggesting nontoxic properties of OSO if a moderate dose of αT is incorporated in the diet.

Some investigations have demonstrated that αT supplementation, inhibits lipoprotein oxidation and intraglomerular macrophage infiltration [55], as [56] who has reported a nephroprotectivity effect by an attenuation of renal structural and functional alterations.

Whereas, at a high dose of αT 1200 mg×kg$^{-1}$ a significant increase in LPO with an important drop of antioxidant biomarkers, such as CAT, SOD, GPx, GR and G6PDH activities, and also αT levels, have been confirmed in Eder et al. study [57]. The significant decrease in αT levels may be explained by 2,5,7,8 tetra methyl 2(29 carboxyethyl)-6 hydroxychroman (α-CEHC), which is another metabolite of αT. Its excretion enhances when a specific plasma level of RRR-αT exceeds.

The intact chromatin structure of this metabolite indicates that (α-CEHC) is derived from αT, which does not react as an antioxidant. Thus, excretion of (α-CEHC) may be used as an indicator of an adequate or excess αT supply [58]. When a higher dose was administered, the level of αT degradation was importantly reduced. The absorption is a function of the ingested dose, with a decrease in the efficiency of absorption of higher intakes in humans and rats experiments [59]. Then, [54] has reported that higher dose of αT for longer periods of time increased the level of LPO, followed by a significant reduction in the activity of antioxidant enzymes. Upon encountering ROS, αT within lipids becoming oxidized, forming αT radical, there is an increase in LPO, a process known as tocopherol mediated peroxidation [60]. Moreover, kidney sections of OSOE$_2$ group have shown severe lesions compared to the OSO

group, as revealed by the congestion of more bloods vessels with acute inflammatory cells infiltration, more edema, the formation of novel vessels, degenerative changes in glomerulus, and an alteration of tubules structures. These findings have been confirmed by the significant increase in the peroxidation markers, such as LPO and the decrease in renal antioxidant biomarkers, which suggest a pro-oxidant effect of αT.

*In vitro* studies [61, 62], high dose of αT has a pro-oxidant effect due to its reaction with other peroxyl radicals, or with polyunsaturated fatty acids in the LDL [63]. This reaction leads to an accumulation of hydroperoxides with a conjugated diene structure [60]. Similarly, [64] has found a pro-oxidant effect of vit E at high doses (100 and 200 nmol), after cells incubation of aortic smooth muscle, following the reduction of the mRNA levels of SOD. At the higher dose of vit E, a pro-oxidant activity has been shown in LDL, isolated from healthy volunteers and from patient, with a defect in the αT transfer protein (α-tTP) gene.

As a conclusion, OSO enhances significantly LPO levels, but the specific activities of antioxidant enzymes were significantly reduced in kidney cells, indicating an imbalance between free radical production and an antioxidant defense mechanism.

On the other side, a moderate dose of αT (600 mg×kg$^{-1}$), conversely, has restored antioxidants enzymes activity, accompanied by a decreasing in LPO level, and an improvement of histopathological changes, while a 1200 mg×kg$^{-1}$ of αT induces a pro-oxidant effect revealed by a significant increase and decrease in LPO content, and an antioxidant activity respectively, which have been accompanied by a tissue damage resulting in nephrotoxicity.

The pro-oxidant effect of a dose at 1200 mg αT per kg of diet has been demonstrated in this study. This αT level is higher than those inducing an antioxidant effect and cited in the literature.

In addition, this pro-oxidant effect was observed on the biochemical parameters and particularly, on the renal histological parameters not yet studied at the chosen doses of α-tocopherol. However, further studies are needed to estimate the real dose of α-tocopherol from which this prooxidant effect appears in kidney and other organs.

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## Conflict of Interest

The authors declare that there are no conflicts of interest.

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