



The impact of Pumpkin treatment on TCDD-induced nephrotoxicity in Wister albino rats: biochemical and histopathological studies

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Abstract

Dynamic economical and industrial expansion lead to environmental contamination and pollution with increasing amounts of harmful agents. Recently, dioxins and dioxin-like compounds (DLCs) have received much attention due to their widespread occurrence, high levels of toxicity, and the significant threat these compounds pose to humans. In this study, we investigated renal toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and explained the role of pumpkin seed oil in modulating TCDD toxicity in the kidney of Wister albino rats. Thirty male and female Wister rats were divided into three isolated groups ten animal each. Group 1 (G1) served as normal control, group 2 (G2), rats were received single intraperitoneal dose of TCDD (2µg/kg b.w. dissolved in corn oil), while group 3 (G3), rats were treated with oral pumpkin seed oil (PO) (1.5 ml/kg b.w. day after day) after TCDD treatment (same as group II). Blood samples were collected for biochemical analysis and kidney tissue samples for histological examination. our investigation revealed that, TCDD induced significant increase ($P<0.01$) in urea level and significant increase in creatinine level ($P<0.05$) in comparison to control group. TCDD treated rats showed series of renal histopathological alterations present mostly in renal vasculature associated with severe degeneration and necrosis of renal tubular epithelium. The previous mentioned lesions were reported much more prominent in renal medulla than in renal cortex. Interestingly, PO treated group showed significant increase ($P<0.001$) in urea level and significant decrease ($P<0.05$) in comparison with TCDD group. PO treatment ameliorates these biochemical and pathological changes in renal tissue. In conclusion, exposure to 2, 3, 7, 8-TCDD led to serious toxic effects in renal tissue and treatment with PO could diminish this toxicity to an improved extend.

Keywords: TCDD; Kidney; Pumpkin; urea; creatinine; histopathology

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Introduction

Dioxins are primary examples of persistent organic impurities which induce animals and human toxicity, these compounds have been almost entirely generated by human activity and have left a string of disasters in the wake of their accidental release over the past 200 years (White and Birnbaum 2009). Dioxins are a class of persistent polyhalogenated aromatic hydrocarbons (PHAHs) of which polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), based on biochemical and toxic responses, have identified as among the most globally distributed potent environmental pollutants (Scialli 2001), that accumulate in body due to its lipophilic properties, slow metabolism and excretion (Mohamed, Hegab, and Yousef 2014).

Dioxin compounds are not created intentionally but are formed inadvertently by a number of human and natural activities. These activities include combustion and incineration, forest fires, chlorine bleaching of pulp and paper, certain types of chemical manufacturing as manufacturing of some herbicides and pesticides and other industrial processes (Organization 2016; Erdemli et al. 2020).

Exposure to acute and chronic toxic levels of TCDD in various animal species and man, causes a wide-variety of adverse effects by activating the aryl hydrocarbon receptor (AhR) in a tissue including hepatotoxicity, nephrotoxicity, carcinogenicity, teratogenicity, interference with lipid metabolism, reduction of bone strength, neurobehavioral effects, endocrine disruption, wasting syndrome, thymic atrophy, immunosuppression, developmental and reproductive toxicity (Mohamed, Hegab, and Yousef 2014).

Besides, oxidative stress is an important constituent in the mechanism of toxicity of TCDD, and many studies showed that exposure to TCDD leads to oxidative damage of many tissues such as liver, kidney and testis (Çiftçi 2011; Lu et al. 2009) demonstrated that TCDD and polychlorinated biphenyls induced lipid peroxidation (elevated thiobarbituric acid reactive substances [TBARS]) and reduced imbalance antioxidant enzymes in kidney. It was thought that nephrotoxic effects of TCDD are associated with oxidative stress. Nowadays, many attitudes were directed for using natural plant extracts rich in antioxidant ingredients in various treatments (Ciftci et al. 2012).

Pumpkin (*Cucurbita pepo*) is a leafy green vegetable which belongs to the Cucurbitaceae family, is a native of Asia. However, it is now grown extensively in many of temperate and warm climates of the world (Stevenson et al. 2007; Al-Okbi et al. 2014). Research on pumpkin specially its seeds increased more and more during the last decade due to its complexity of the chemical ingredient and the health benefits (Lestari and Meiyanto 2018). Pumpkin seeds are considered as a suitable source of edible oil (Stevenson et al. 2007). The researchers have so far focused particularly on the three major components of fatty acids (palmitic acid, stearic acid, oleic acid and linoleic acid), phytoestrogens, and tocopherol in pumpkin seeds oil because of its several health benefits such as antioxidant, anti-inflammation, antidiabetic, anticancer, anti-cardiovascular, antihyperlipidemic, and estrogenic-like effect (Lestari and Meiyanto 2018). Therefore, the aim of this study was to investigate whether Pumpkin protect the kidney against the toxic effects of TCDD, through pointed out the potential

benefits of Pumpkin seed oil in improvement of biochemical parameters and restoration of histopathological alterations in kidney tissues in TCDD induced nephrotoxicity rat model.

Material and Methods

Ethical Considerations

This study and all experimental methods were carried out in accordance with the Declaration of the ethics committee's rules "Institutional Review Board" at Assiut University in Assiut, Egypt (approval number 04-2023-100073).

Material

Chemicals and Drugs

TCDD was purchased from Chem-Impex International, Inc. Pumpkin seed oil was purchased from Harraz company for food industry and natural products, Egypt.

Animals

Thirty Wister strain male and female albino rats, weighing 180-220 g obtained from experimental animal house- Sohag University. Rats housed in clean polypropylene cages having four rats per cage and were maintained under temperature-controlled room (27 ± 2 C) with a photoperiod of 12h light and 12h dark cycle. Rats fed a standard pellets diet and water ad libitum throughout the experimental period.

Experimental design

Rats were kept underneath strict observation during the week of acclimatization, during this week fecal samples from each group were examined by concentration floatation method. Both sediment and supernatant fluid after centrifugation was examined separately for parasitic eggs and larvae to prove that our experimental animals were free from any parasites, 30 rats were divided into three groups, 10 rats in each group and treated as the follows:

Group 1 (G1): Normal control (n=10): rats were fed on standard rat chow and drinking water.

Group 2 (G2): TCDD (n=10): Rats administered single dose of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) ($2\mu\text{g}/\text{kg}$ b.w.) dissolved in corn oil through intraperitoneal (I/P) injection (Mai et al. 2020).

Group 3 (G3): TCDD+PO (n=10): Rats administered 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (same as in G2), and treated with pumpkin seed oil (PO) at a dose of 1.5 ml/kg b.w. day after day for 8 weeks by gavage (Elhamalawy 2018).

Methods

At the end of experimental period (8 weeks), all experimental animals were decapitated and individual blood samples from each group were taken for assessment of biochemical and histological examination respectively.

Serum biochemical analysis

Blood samples were taken in dry, clean tubes without anticoagulant and kept in an inclined position for 20 minutes at room temperature, then put in refrigerator to avoid glycolysis and complete retraction of blood clot, the samples were centrifuged at 3000rpm for 10 minutes till the clear serum where serum was separated which is carefully collected and stored in Eppendorf tubes at -20°C until estimation of serum chemistry. Serum sample were used for estimation of some biochemical parameters such as Urea & Creatinine.

Urea was determined using the enzymatic colorimetric assay (Fawcett and Scott 1960). A colorimetric (Jaffé-Reaction) test was used to assess creatinine kinetically (without deproteinization) (Bartels and Bohmer 1971). The above-mentioned measured parameters were

counted using industrial kits (Human, Wiesbaden, Germany).

Histopathological examination

Animals were sacrificed after the experiment, and tissue samples from kidneys were collected, dissected, and immediately fixed in 10% formalin for 24 h, dehydrated in a succession of graded alcohols, clarified in xylene, and encapsulated in paraffin (Suvarna and Layton 2013). Tissue sectioning was done at 3µm thickness and stained with hematoxylin and eosin (H&E) (Bancroft, Stevens, and Turner 1996) for histological evaluation. All sections were inspected and photographed using OLYMPUS CX43 microscope and a microscope-adapted OLYMPUS SC52 camera (Department of Pathology and Clinical Pathology, Sohag University).

Morphometric study

Analysis of organ histology was performed by assigning a score depending on the degree of the damage seen in each group in the examined tissue sections for semiquantitative measurements: 0 = no lesions; 1 = mild (1 to 25%); 2 = moderate, (26 to 45%); 3 = severe (> 45%) as described previously (O'Brien et al. 1996; Gibson-Corley, Olivier, and Meyerholz 2013; Hamdin et al. 2019). pathologists evaluated renal tissue specimens blindly. According to the recognized histopathologic categorization for kidney lesions, interstitial lesions, glomerular lesions, vascular lesions, and renal tubular lesions were graded and scoring were assigned according to previous studies (Alsharif et al.). The morphometric was carried out at the morphometric analysis unit, Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Sohag University.

Semithin

Specimens from kidney were collected and immediately fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) at 4°C for 2h. The tissue specimens were post-fixed in 1% osmium tetroxide at 4°C for 2h, dehydrated in ethyl alcohol, cleared in propylene oxide then embedded in epoxy resin (Glauert and Lewis 1998). Semi-thin sections (1µm thickness) were cut by ultramicrotome (Leica Ultracut; Leica, Berlin, Germany), stained with toluidine blue, and examined by light microscope, for general orientation.

Statistical analysis

GraphPad Prism version 5 (GraphPad Software, Inc.) was used to analyze the data. The mean differences were considered to be significant at $P < 0.05$ to indicate a statistically significant difference, and statistical analyses were performed using a one-way ANOVA analysis of variance and a post-hoc Tukey's significant difference test. All results were expressed as mean \pm standard deviation.

Results

Serobiochemical assessment

The serobiochemical results of the present work are shown in Table 1 and Fig.1. As indicated in Table 1, a significant ($P < 0.001$) increase in urea level was detected in TCDD group in comparison to control one (G1). However, there was a significant ($P < 0.001$) decrease in urea level in pumpkin treated group (G3) in comparison to TCDD group (G2).

In addition, there was a significant ($P < 0.05$) increase in creatinine in the TCDD group (G2) in comparison with the control group (G1). Remarkably, Creatinine level was decreased significantly ($P < 0.05$) in pumpkin treated group (G3) in comparison to TCDD group (G2) (Table 1) 1 and Fig.1.

Table (1): Showed the mean and SD of urea & creatinine level in serum of control and TCDD+PO group at the end of experimental period.

Item	Urea (mg/dl)	Creatinine (mg/dl)
Control (G1) (n=10)	45.47 ±1.6	1.33±0.02
TCDD (G2) (n=10)	63.85±2.7 ***	1.47±0.06 *
TCDD +PO (G3) (n=10)	47.87±2.6 ns,###	1.35±0.04 ns,#

Statistical analysis by one-way ANOVA with Newman Keuls multiple comparison test. Data are expressed as means ± standard deviations. Significant differences *versus* the control group are marked by different asterisks while significant differences *versus* TCDD group are marked by different #, all through one-way ANOVA with Tukey's post hoc test: *.#=p<0.05, ***,###=P<0.001

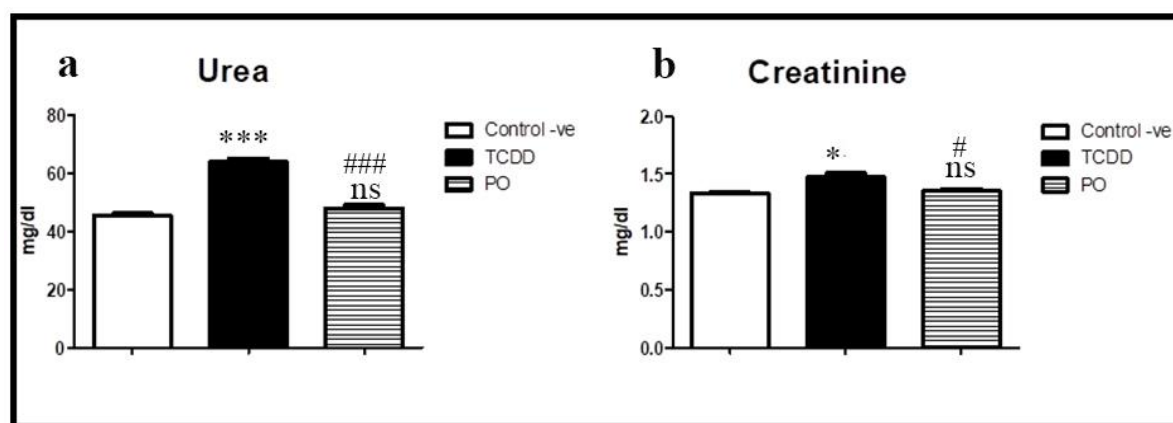


Figure 1: Showed a: urea & b: creatinine level in serum sample of control and PO treated groups. Data are expressed as means ± standard deviations. Significant differences *versus* the control group are marked by different asterisks while significant differences *versus* TCDD group are marked by different #, all through one-way ANOVA with Tukey's post hoc test: *.#=p<0.05, ***,###=P<0.001.

Histopathological assessment

The histopathological examination of kidney sections from experimental groups demonstrated normal kidney histological structures in tissue sections obtained from rats of control negative group (G1), whereas, renal cortices revealed normal glomerular size and structure, basement membrane thickening, proximal and distal convoluted tubules showed typical cellular structures with normal thickening cellular outlines (Fig.2A). Normal renal medullary tubular structure (Fig2.B). Renal histopathological changes reported in TCDD group (G2) were mostly affect renal vasculature with severe degeneration and necrosis of tubular epithelium (Fig.3).

Pathological lesions in medulla were much more prominent than in cortex (Fig.4). In renal cortex, glomerular tuft was congested and atrophied. Bowman's space was dilated (Fig.3A). Renal tubular epithelium exhibited necrobiotic changes (cytoplasm shrinkage and nucleus faded out), while others were of hyalinized wall with epithelial cast inside renal tubules. Intertubular renal capillaries were congested and dilated (Fig.3B). Dilatation and thrombosis were observed in corticomedullary junction vasculature with perivascular edema and mononuclear cell infiltration (Fig.3C)

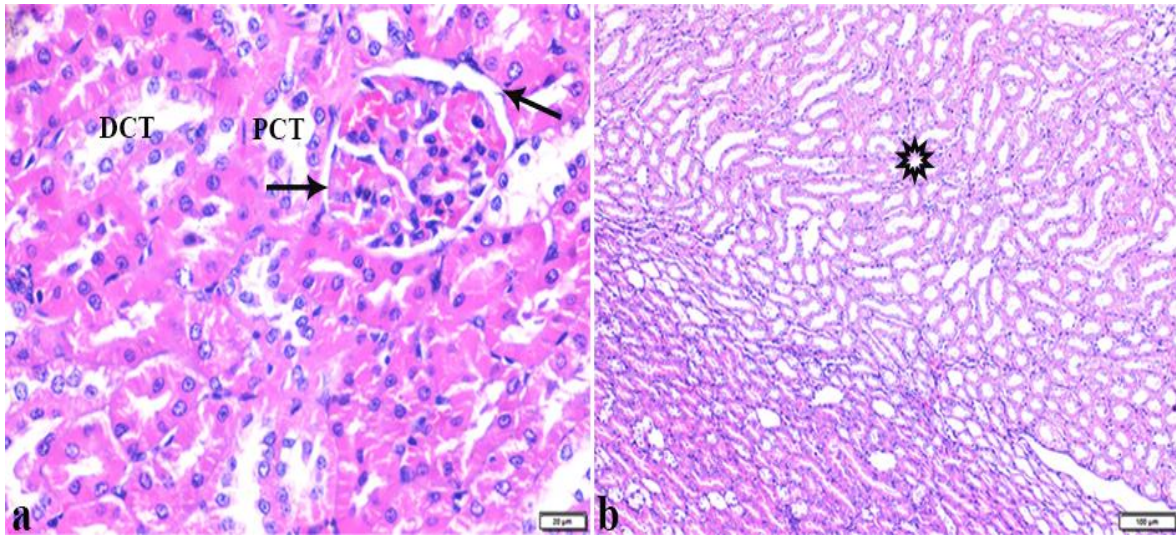


Figure 2: Photomicrograph of renal cortical tissue section from control group showing: (a): normal glomerulus (arrows), normal proximal convoluted tubules (PCT), normal distal convoluted tubules (DCT). (b): normal renal medullary structure (star). HE stains. The bar size is (a=20µm, b=100µm).

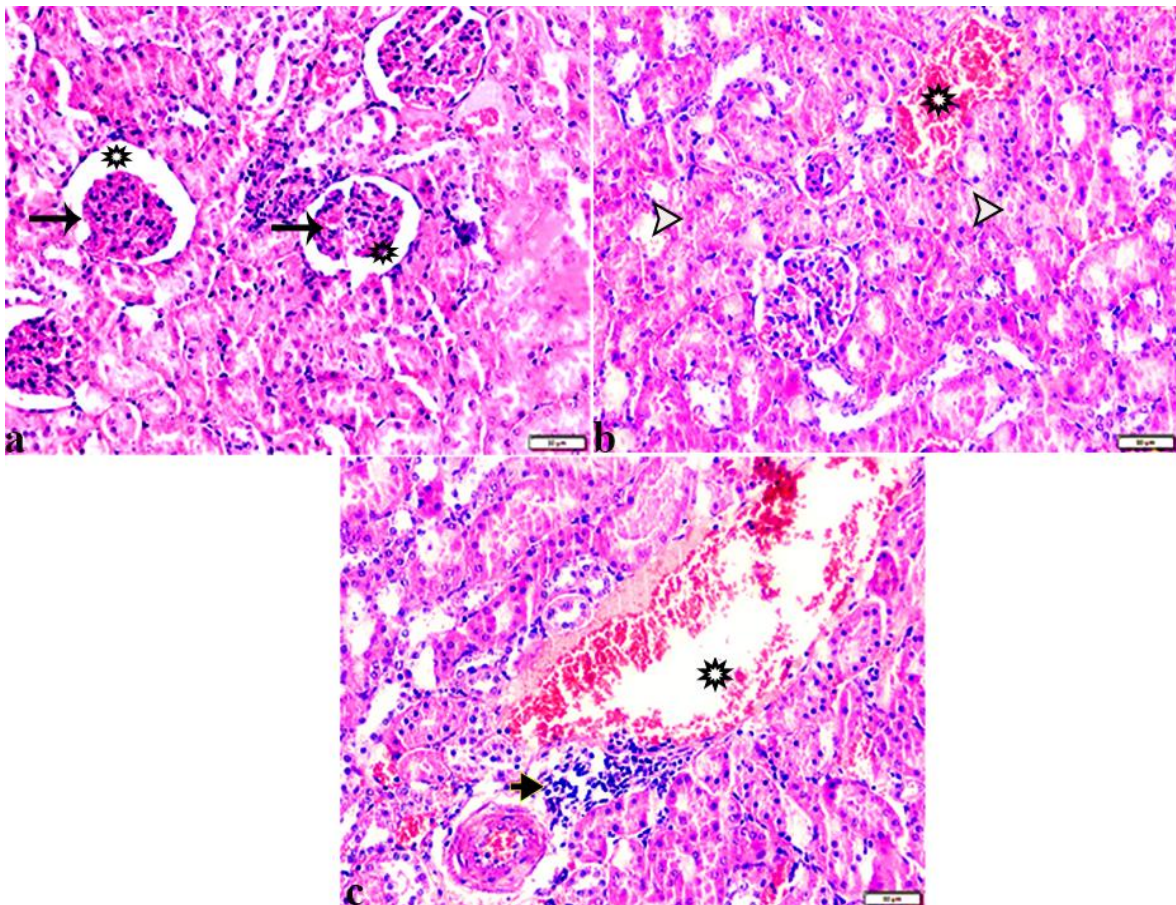


Figure 3: Photomicrograph of renal cortical tissue section from TCDD group showing: (a): atrophy of glomerular tuft (arrows) and widening of bowman's space (stars). (b): congestion of intertubular capillaries (star), necrobiotic changes of renal tubular epithelium (karyorrhexis and lysis) (arrowheads). (c): dilatation and thrombosis of arcuate vein with thickened wall (star), perivascular edema and mononuclear cell infiltration at the corticomedullary junction (arrow). HE stains. The bar size is 50µm

In medulla, renal tubular epithelium showed coagulative necrosis and atrophy. There were intertubular connective tissue proliferation and hemorrhage (Fig.4A). Renal single or multiple renal cysts (Fig.4B) were observed in renal medullary area. Also, the wall of renal tubules was hyalinized and showed cystic dilatation (Fig.4C). In other cases, desquamated epithelium obliterated tubular lumen with inflammatory cell infiltration, epithelial cast occupied lumen of renal tubules (Fig.4D). Microscopic examination of renal tissue from TCDD+ PO treated group revealed nearly normal glomerulus with more or less normal renal tubules (Fig.5A).

Distal convoluted tubular epithelium exhibited little degree of desquamation. In medulla, there was mild dilatation of renal tubules with intertubular inflammatory cell infiltration (Fig.5B). Renal pelvis showed more or less normal histological architecture (Fig.5C).

Histomorphometrically, the kidney indicated significant ($P<0.001$) different type of cell damage in their tissue from the TCDD group, as compared with the control group. On the other hand, Pumpkin treatment (TCDD+PO) showed significant ($P<0.001$) improvement as compared to TCDD group.

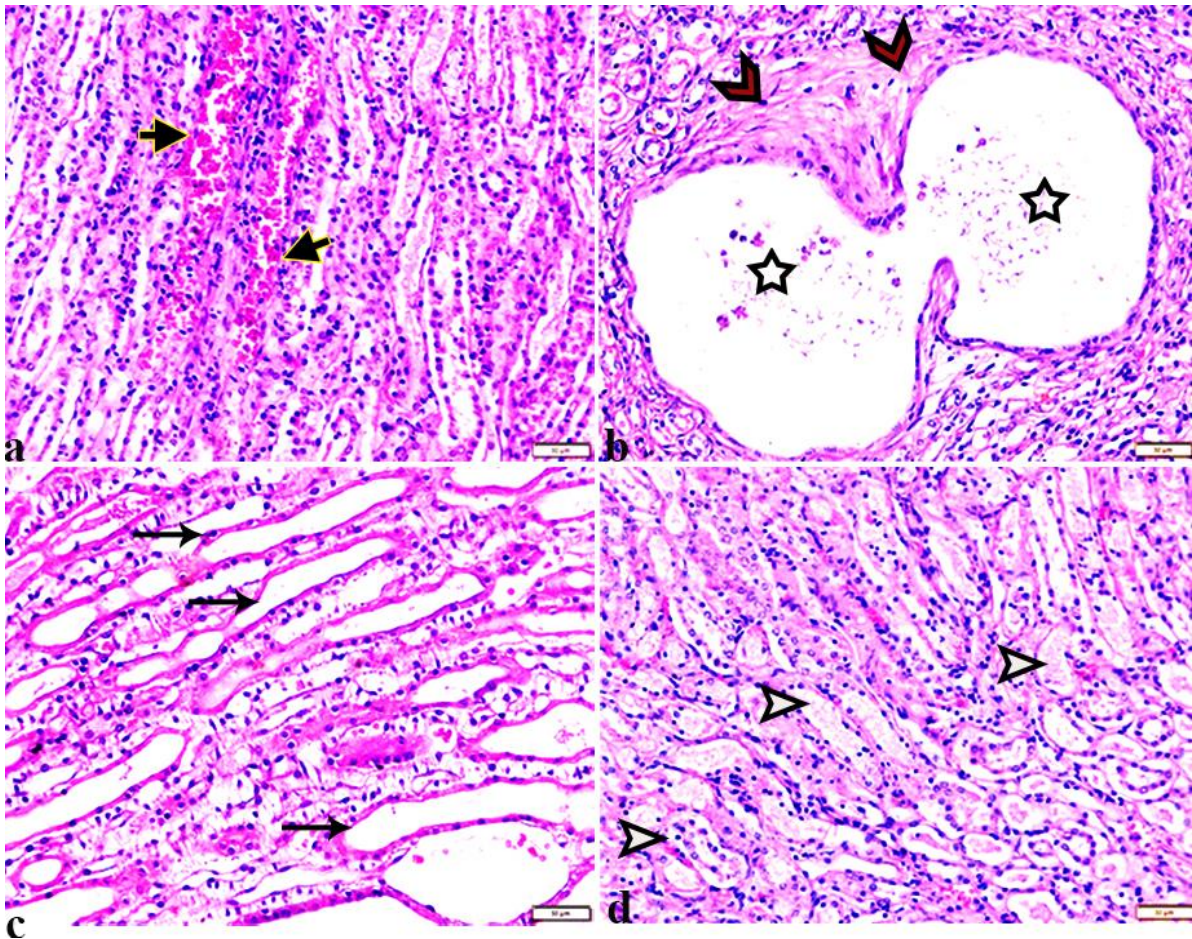


Figure 4: Photomicrograph of renal medulla tissue section from TCDD group showing: (a): congestion of intertubular capillaries (arrows). (b): renal cyst (stars) surrounded by dense layers of fibrous connective tissue (arrowheads). (c): cystic dilatation and hyalinization of renal tubular wall (arrows). (d): desquamated renal tubular epithelium which obliterate its lumen with mononuclear cells infiltration (arrowheads). HE stains. The bar size is 50µm.

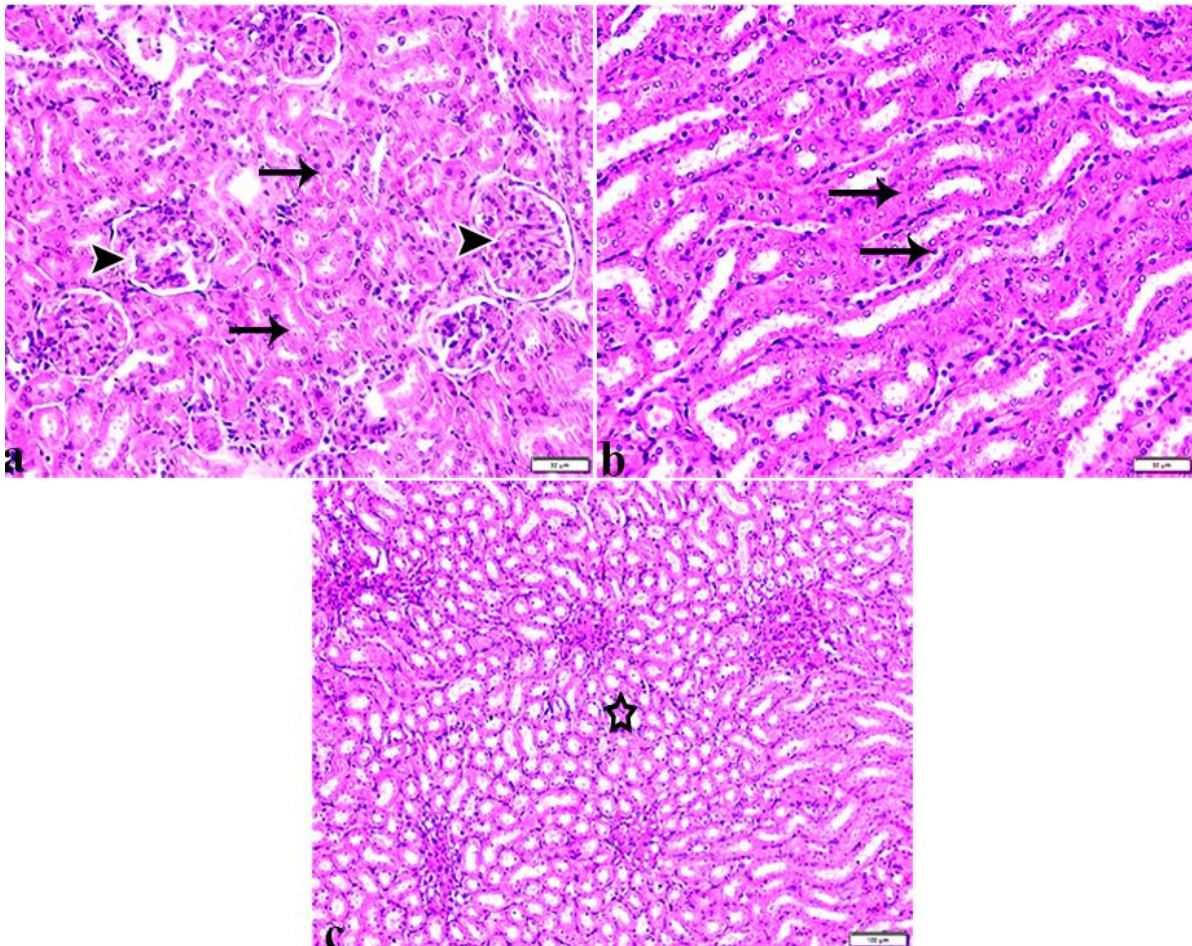


Figure 5: Photomicrograph of renal tissue section from TCCD group treated with PO showing (a): normal cortical renal tissue presents in normal glomerular size and structure (arrowheads), more or less normal cortical renal tubules (arrows). (b): mild dilatation of medullary renal tubules with intertubular inflammatory cell infiltration (arrows). (c): more or less normal renal pelvis (star). HE stains. The bar size is (a&b=50 μ m, C=100 μ m).

Semithin

Semithin section examination of renal tissue in the control group showed a normal histological structure in the form of healthy cortex, medulla and corticomedullary junction (Fig.7). In TCDD group, cortex showed proliferation of glomerular tuft, congested blood vessels and prominent vacuolation of renal tubular epithelium (Fig.8A). while in medulla, renal tubular membrane was thickened (Fig.8B) with intertubular fibrosis (Fig.8D). Corticomedullary junction exhibited dilated

and congested blood vessels with perivascular fibrosis (Fig.8C). On the other hand, renal tissue in PO group showed that Glomerulus was more or less normal with marked intertubular inflammatory cell infiltration (Fig.9A). Corticomedullary junction exhibited normal arrangement and structure with slight mononuclear cell infiltration. (Fig.9 A). In medulla, renal tubular epithelium exposed mild necrotic changes with intertubular inflammatory cell infiltration (Fig.9B).

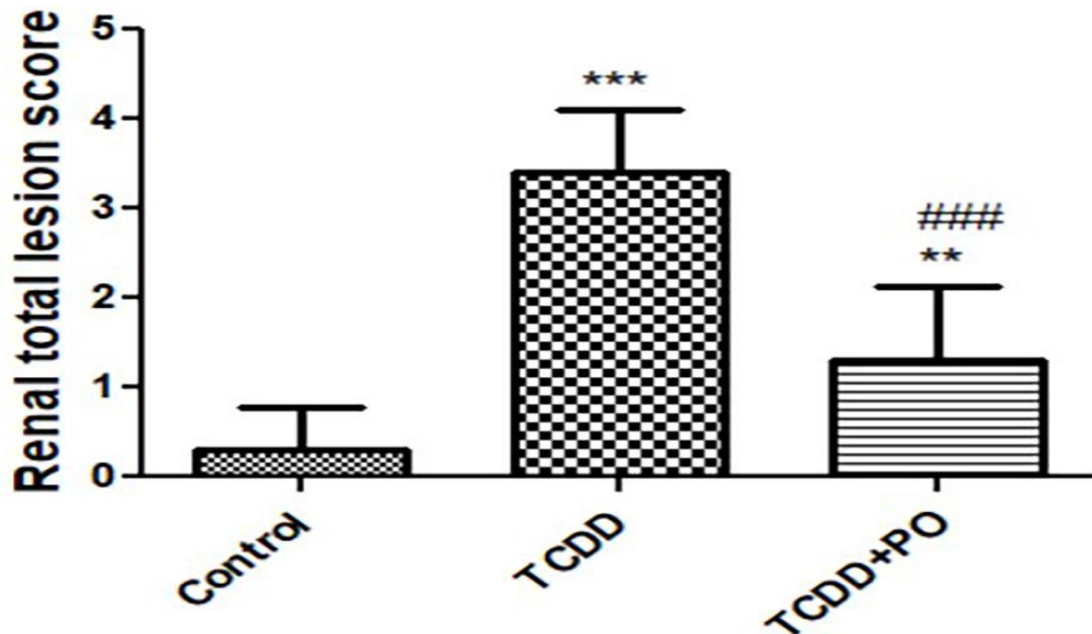


Figure 6. Histomorphometry graph showing semiquantitative measurements of total lesion scores recorded in kidney tissue sections among the experimental groups: Data are expressed as means \pm standard deviations. Significant differences *versus* the control group are marked by different asterisks while significant differences *versus* TCDD group are marked by different #, all through one-way ANOVA with Tukey's post hoc test: ** $p \leq 0.01$, ###*** $p \leq 0.001$).

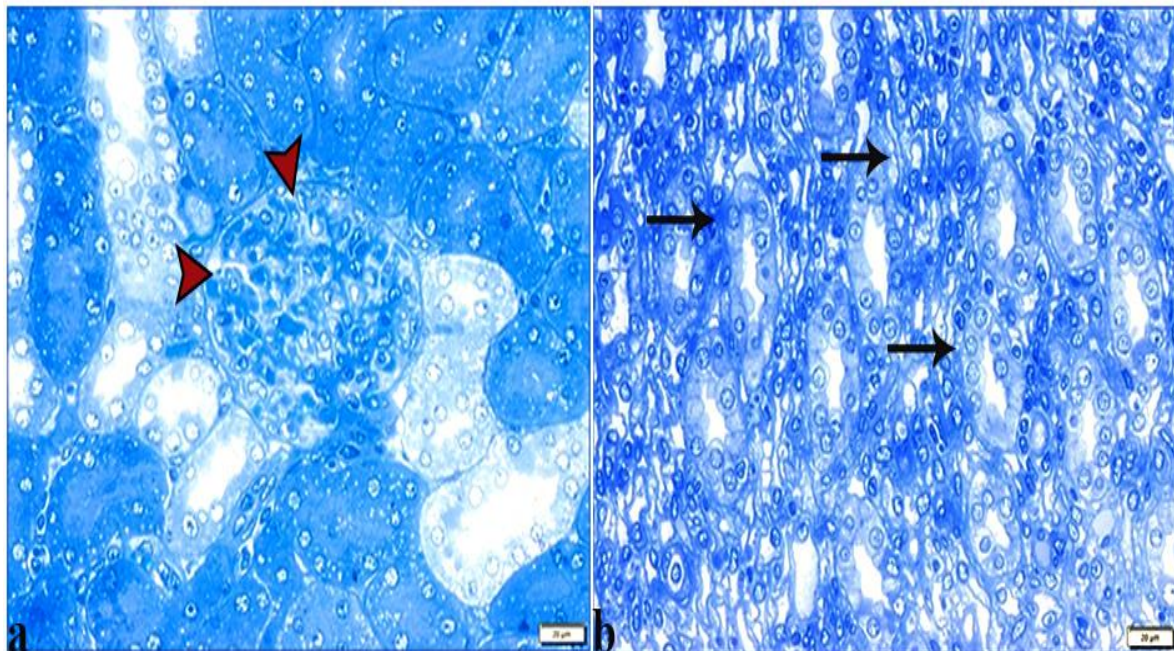


Figure 7: Photomicrograph of renal tissue semithin section from control group showing: (a): normal glomerular structure and (b): normal renal tubular architectures. (Toluidine blue stain, the bar size is =20µm).

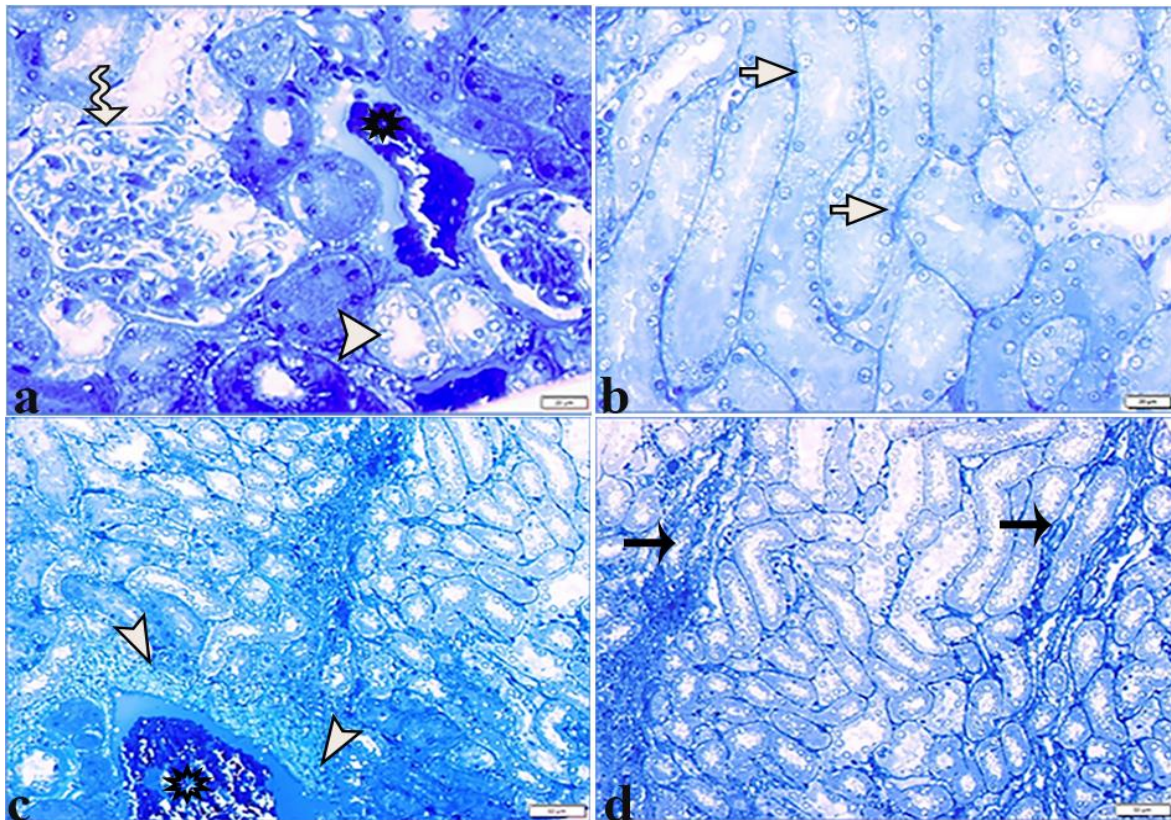


Figure 8: Photomicrograph of renal tissue semithin section from TCDD group showing: (a): glomerular proliferation (Zigzag arrow), congested cortical blood vessels (star), vacuolated epithelium of renal tubules (arrowhead). (b): thickened renal tubular membrane (arrowheads). (c): Dilated and congested blood vessels (star), with perivascular fibrosis (arrowheads). (D): medullary intertubular fibrosis (arrows). (Toluidine blue stain, the bar size is (a&b=20 μ m, c&d= 100 μ m).

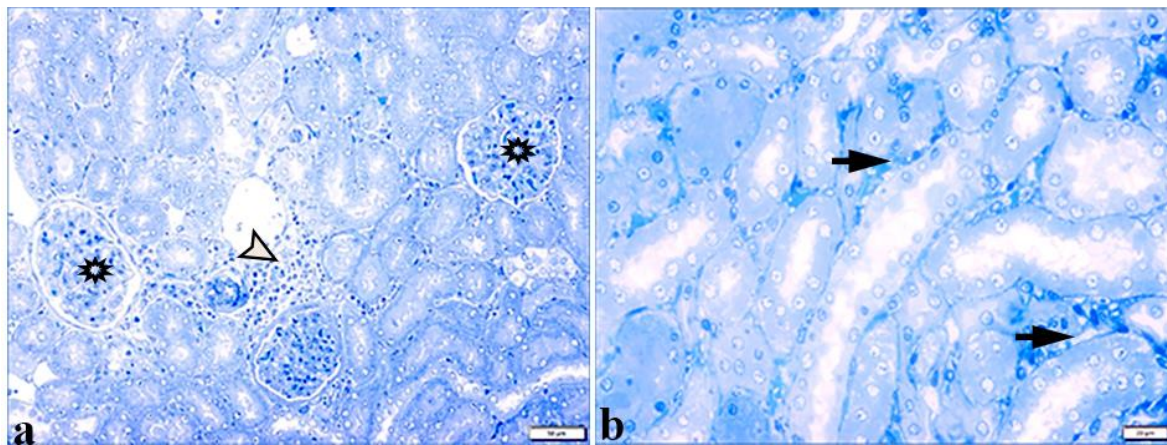


Figure 9: Photomicrograph of renal tissue semithin section from TCDD treated with PO showing: (a): More or less normal glomerulus (stars), marked intertubular inflammatory cell infiltration (arrowhead). (b): Mild necrotic changes of renal tubules with intertubular inflammatory cell infiltration (arrows). (Toluidine blue stain, the bar size is =20 μ m).

Discussion

It was known that TCDD has nephrotoxic affect in various animal species and human. This aspect of TCDD toxicity could be studied for risk assessment and

mitigation. Our study was carried out to assess biochemical and histopathological changes in experimental rats with nephrotoxicity induced by TCDD.

In this study, kidney function tests recorded significant increase in blood urea and creatinine concentration at TCDD group. These results agree with Al-Musawi et al, 2021 (Al-Musawi et al. 2021) as renal insufficiency occurs due to disturbance in Na-K pump of nephron due to epithelial cell detachment from tubules and destruction of renal parenchyma and these changes were prominent in histopathological examination of cortex and medulla. Dioxin enter the body in the food then enter circulatory system to adipose tissue and liver cells. In liver TCDD ties to protein aryl-hydrocarbon (Ah- receptor) then tie to secondary protein. Ah- receptor- nuclear translocation.(Ah- rnt), this protein converted protein- dioxin complex in cell nucleus causing multiple disease like nephropathy, cancer, immunosuppression & hydronephrosis (Al-Musawi et al. 2021). Histopathological examination of renal tissue revealed that TCDD was mostly affect renal vasculature with severe degeneration and necrosis of tubular epithelium, these results are in agree with previous studies (Erdemli et al. 2020; DOĞAN et al. 2023) In cortex, glomerular tuft was atrophied, showed podocyte injury as discussed elsewhere (Al-Musawi et al. 2021). It may be marred by mitochondrial dysfunction. Podocyte injury is a key feature of protein-uric glomerular disease and progress to tubulointerstitial injury through proteinuria-induced tubular cell injury. Podocyte injury usually causes a morphological pattern of focal segmental glomerulosclerosis (FSGS) (Jefferson and Shankland 2014). The pathological lesions in the medulla were shown to be significantly more prominent, as demonstrated in previous literatures, which discussed the administration of TCDD and measured the antibody immunoreactivity against CYPIA and activation of Ah-r

dependent gene on outer zone of the medulla which cause the tissue damage(Fujiwara et al. 2008). Renal tubular epithelium of both cortex and medulla exhibited necrobiotic changes (cytoplasm shrinkage and nucleus faded out), while others were of hyalinized wall with epithelial cast occupied lumen of renal tubules, these results agreed with previous studies (Al-Musawi et al. 2021; DOĞAN et al. 2023). Also, the wall of renal tubules was hyalinized and showed cystic dilatation and sometimes leading to form single or multiple renal cysts. In other cases, desquamated epithelium obliterated tubular lumen with inflammatory cell infiltration. Interstitial fibrosis with mononuclear cells infiltration due to that TCDD develop inflammatory process by increased proinflammatory interleukins and free radical generation which cause destructive change in tissue, these results are in agree with previous studies (Calkosinski et al. 2014; Humadi 2019; DOĞAN et al. 2023). In corticomedullary junction, arcuate artery was dilated and thrombosed with perivascular edema and lymphoid cell infiltration. Finally, it is clear that TCDD exposure can cause an imbalance in oxidant/antioxidant system and histological damage in renal tissue of rat.

Pumpkin seed oil (PSO) treatment under TCDD-induced toxic circumstances significantly protected the kidney tissue structure as it decreased the extent of renal tissue damage and interstitial fibrosis and also restored kidney functions as urea and creatinine level returned to its normal level. This protective effect of PSO extract could be the result of direct free radical scavenging properties (Gurel et al. 2005). Pumpkin has been shown to have protective effects against oxidative stress in various tissues as kidney, liver, brain and testes in

vivo and in vitro studies (Amara et al. 2008; Morakinyo, Achema, and Adegoke 2010). Protective role of PSO was confirmed by Paul et al, 2020 (Paul et al. 2020) who found that treatment with PSO markedly alleviated formaldehyde induced pathological changes in liver, kidney and brain in mice. Phenolic compounds such as polyphenols flavonoids as well as vitamins and zinc in pumpkin are attributed factors for its antioxidant activity by neutralizing free radical generation (Amara et al. 2008; Morakinyo, Achema, and Adegoke 2010). In addition, PSO is also rich in zinc which plays an important role in the structure of proteins and cell membranes and protect against damage (Bataineh, IH, and Al-Alami 2002).

Conclusion

The intoxication of rat by administrating 2,3,7,8-tetrachloro-dibenzo-p-dioxin causes changes in both renal tissue and its blood function tests. Dioxins significantly affect the structure of the kidney, which negatively affects its function, mainly in the scope of the kidney function tests. It has been observed that the administration of pumpkin seed oil to TCDD-treated rats results in a reduction in these changes.

Conflict of interest

The authors declare that they have no competing interests

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