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Protective Effect of Chitosan and Chitosan Nanoparticles on Dioxin-Induced Haematotoxicity and Nephrotoxicity in Male Albino Rats

Hamdy M. A. Hassanein*, Hamdy A. M. Soliman, Fatima M. A. Salem, Fatma Ahmed and Hanan A. M. Okail

Department of Zoology, Faculty of Science, Sohag University, Sohag 82524, Egypt
**Email: hhassanein@science.sohag.edu.eg*

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Abstract: Chitosan (CH) is a natural product produced from the shells of crustaceans. Both CH and Chitosan nanoparticles (CH-NPs) have recently been used in various pharmaceutical and biomedical applications. The present study aimed to evaluate the potency of chitosan or chitosan nanoparticles in reducing the negative effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced haematotoxicity and nephrotoxicity in albino rats. Methods: Twenty adult male albino rats were placed in four groups of five rats each: the control group, the TCDD group (10µg/kg intraperitoneally injected), the TCDD + CH group (intraperitoneal injection of TCDD (10µg/ kg) and an oral dose of CH (200 mg/kg), and the TCDD + CH-NPs group: intraperitoneal injection of a dose of TCDD (10µg/ kg) and an oral dose of CH-NPs (200 mg/kg). For all groups, the experimental period lasted for four weeks. Results: The TCDD-treated group showed a significant ($P < 0.05$) decrease in RBC count, HB, HCT, WBC count, lymphocyte percentage, and PLT, whereas there was a non-significant decrease in MCV, MCH, MCHC, and monocytes. On the other hand, a significant increase in neutrophils and eosinophils was noticed. In addition, several morphological abnormalities in the erythrocyte membranes were observed in the TCDD-treated group. A significant increase in serum urea and creatinine levels and marked histopathological changes in kidney tissue were observed. Administration of chitosan and chitosan nanoparticles could approximately restore the normal hematological parameters and improve the dioxin-induced renal histopathological changes. Although there is no significant difference between CH and CH-NPs groups, CH have seemed to have a better effect on some parameters and vice versa with CH-NPs in some parameters.
The present study concluded that oral administration of CH or CH-NPs to the TCDD-treated animals might play a protective role against dioxin-induced hematotoxicity and nephrotoxicity in rats.
Keywords: Dioxin, Chitosan, Chitosan Nanoparticles, Haematology, kidney function

1. Introduction

Dioxins are a class of permanent polyhalogenated aromatic hydrocarbons. However, among the most widely dispersed and dangerous environmental pollutants are polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) [1]. Dioxins are undesirable byproducts of numerous industrial processes, primarily coming from fuel combustion, waste incineration, and industrial air emissions [2]. Furthermore, because they breakdown gradually in the environment, they remain hazardous contaminants for a very long period. Dioxins enter the human body through the diet, primarily through fish, meat, and fatty dairy products [3]. The most extensively studied dioxin, TCDD, is a polyhalogenated aromatic hydrocarbon toxin found in the environment. TCDD has been shown to accumulate in the body due to its lipophilic properties, slow metabolism, and elimination. TCDD bioaccumulates in fish and animals, which humans consume [4]. Previous research has shown that TCDD can cause a variety of harmful effects in animals, including immunotoxicity, teratogenicity, carcinogenicity, and endocrine disruption [5, 6].

The haematopoietic system is extremely vulnerable to a variety of exogenous substances, including medications, poisons, and heavy metals. To determine the physiological response to a contaminated environment, haematological markers such as haemoglobin concentration, hemocrit value, and blood cell counts can be employed [7]. Analyzing blood parameters is important for risk assessment since they have a high predictive value for toxicity [8]. When high doses of chemical substances are administered, blood parameters frequently change, exhibiting signs of haematological illnesses such as anemia, neutropenia, and thrombocytopenia [9]. Dioxin compounds have numerous adverse effects on the hemobiotic system, and anemia was one of their first hazardous effects in a variety of animal species. TCDD causes apoptosis in the circulating erythrocytes [10]. According to *in vivo* and *in vitro* studies, the negative effects of TCDD are due to its myelotoxic effects on bone marrow, which cause hypoplasia and apoptosis in the cellularity of the bone marrow [6, 11].

Nephrotoxicity, one of the most common health problems, is brought on by being exposed to chemicals or medications [12]. Renal failure causes a severe decrease in the excretory capacity of the kidney, resulting in a buildup of nitrogenous waste in the blood [13]. Renal failure, either acute or chronic, is a fatal pathophysiological condition induced by TCDD [14]. The direct nephrotoxic effects of TCDD include oxidative stress, which is one of the major risk factors for acute kidney injury and leads to proximal tubular toxicity, phospholipid breakdown, and mitochondrial dysfunction [15].

Chitosan, a natural product active compound derived from the complete or partial deacetylation of chitin, is known to have a wide range of biological activities, including antioxidant[16], antidiabetic [17], anti-inflammatory activities [18], antibacterial [19], drug delivery [20, 21], and immunoenhancing [19, 22]. Recently, CH-NPs have drawn a lot of interest from researchers because of their potential applications, particularly in medicine [23, 24]. Additionally, the haematoprotective properties as well as the immunostimulatory activity of CH-NPs have been established as well [25-28], and CH-NPs could be a powerful nephroprotective agent [29].

Studies of the haematoprotective and nephroprotective effects of CH are rare. The current study aims to assess the ameliorative effect of CH or CH-NPs on the alteration of haematological and biochemical parameters, as well as kidney histopathological changes, caused by TCDD in male rats.

2. Materials and method

2.1. Chemicals

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was obtained from (Sigma Chemical Co.). Low molecular weight chitosan (50 kDa, 90% deacetylation degree) was obtained from (Fluka Germany Chemical Co.). Nano-chitosan was prepared at the Faculty of Science, Sohag University, Sohag Governorate, Egypt. Kits for other biochemical assays were purchased from Human Diagnostics and Biodiagnostic in Egypt.

2.2. CH-NPs Preparation

Ionic gelation was used to prepare CH-NPs, albeit with some modifications [30]. In a brief, CH-NPs were prepared by dropwise adding an aqueous sodium TTP solution into the CH solution at a ratio of 1:3 with continuously magnetic stirring at room temperature. Then, the CH-NPs were precipitated, collected and purified. In accordance with [31] and [32], the CH-NPs gel-like colloids were collected, oven dried at 40 °C for 3-5 hrs, and then kept at 4 °C until use or analysis.

2.3. Animals

A total of 20 healthy adult male Wistar albino rats of 8-10 weeks old and weighing approximately 180–200 gm purchased from the Animal Experimental Research Unit, Faculty of Medicine, Sohag University, Sohag, for this experiment. All animals were housed in filter-top polycarbonate cages in a chemical-free room that was artificially illuminated (12 h dark/light cycle) and thermally controlled (25±1◦C). The rats were fed on constant supplies of standard pellet diet, fresh and clean drinking water were supplied *ad-libitum*. All animals were received humane care in compliance with the guidelines of the Animal Care and Use Committee of the Faculty of Medicine, Sohag University. The animal experimentation was approved under number (Sohag-2-5-2022-1).

2.4. Experimental design

After 7 days of acclimation, the rats were randomly allocated into four experimental groups (5 animals /group), as follows: Group 1: The control group, which was fed the basal diet. Group 2: TCDD–treated group, which was fed the basal diet, and received a daily dose of TCDD (10 µg/kg B.W.) through intraperitoneal (IP) injection according to Abdulkareem and Nanakali [33]. Group 3: the TCDD + CH group, which was fed the basal diet, received a daily dose of TCDD (10 µg/kg B.W.) along with an oral dose of CH-NPs (200 mg/kg), according to Toz and Değer [34]. Group 4: TCDD + CH-NPs group, which was fed the basal diet, and received a daily dose of TCDD (10 µg kg/B.W.) concurrently with an oral dose of CH-NPs (200 mg/kg), according to Elsonbaty et al. [35]. For all groups, the experimental period lasted for four weeks. At the end of the experiment, the rats were euthanized 24 hours after receiving the last treatments. The whole blood samples were collected in tubes with anti-coagulant (EDTA) for the complete blood count (CBC) test and poikilocytosis. In addition, blood was drawn and kept in vacuum tubes with clot activator. To obtain the sera, these samples were centrifuged at 3,000 x g for 10 minutes at room temperature, and the sera were then stored at -20 °C until use. The kidney tissues were excised from the animals for histological and histochemical studies.

2.5. Haematological assays

A complete blood picture was presented (CBC), including, total red blood cell count (RBC), haemoglobin (Hb), haematocrit (HCT), and RBC indices [mean cell volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC)]. Total white blood cell (WBC) count and differential percentages; lymphocytes, monocytes, neutrophils, and eosinophils; and platelets (PLT) count were also included. All these parameters were determined by using an automated method with the fully automated haematology analyzer (HA-Vet Automatic Hematology Analyzer, Belgium; S/N HA3DM004).

2.6. Erythron profile (poikilocytosis)

The blood films were immediately performed after collection, stained with Leishman stain, selected, coded, and scored blindly for erythrocyte alterations according to Al-Sabty et al. [36].

2.7. Biochemical analysis

The concentrations of serum urea and creatinine were determined by utilizing the procedures described in [37] and [38] respectively.

2.8. Histological and Histochemical Examinations

The kidney tissues of rats from each group were fixed for 48 hrs in 10% formalin at room temperature, After that, the slides were prepared and stained with Harris's haematoxylin and eosin in accordance with the method previously described by Bancroft & Gamble [39] and Masson trichrome stain for collagen fibers as previously mentioned [40].

2.9. Statistical analysis

GraphPad Prism version 7 (GraphPad Software, Inc.) was used to analyse 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 ± standard error.

3. Results

3.1. Haematological Results

The results of the present study revealed that administration of dioxin for four weeks showed a highly significant ($P < 0.01$) decrease in RBCs count, hemoglobin, haematocrit and a significant decrease ($P < 0.05$) in white blood cells count, lymphocytes percentage and platelets in relative to the normal control group (Table 1). Oral administration of CH or CH-NPs to dioxin treated groups showed also significant change ($P < 0.05$) in RBCs count, HB, HCT, and a non-significant increase ($P > 0.05$) in WBC count and the PLT in comparison to the control group. In contrast, CH or CH-NPs treatment of the TCDD-treated group showed signs of improvements in the previous haematological parameters with significant changes ($P < 0.05$) when compared to the TCDD-treated group (Table 1).

On the other hand, non-significant changes ($P > 0.05$) in MCV, MCH, MCHC and monocytes % were observed in control and all treated groups. While, TCDD-treated group showed a highly significant ($P < 0.001$) increase in the neutrophils % and significant ($P < 0.05$) increase in eosinophils % compared to control group. Whereas, oral administration of CH or CH-NPs to TCDD treated group showed a non-significant change ($P > 0.05$) in the % of neutrophils and eosinophils compared to both control and TCDD-treated groups. In all the evaluated hematological parameters, there is no significant difference between CH and CH-NPs groups (Table 1).

Table 1: Effect of CH or CH-NPs on the haematological parameters in male rats treated with TCCD.

Parameter	Control	TCDD	TCDD + CH	TCDD + CH-NPs
RBCs [10^6/mm^3]	8.66±0.06	7.68±0.14^{a*}	7.93±0.07^{b}	7.82±0.13^{b}
HB [g/dl]	15.15±0.15	13.8±0.1^{a*}	14.50±0.07^{b}	14.3±0.13^{b}
HCT[%]	49.7±0.51	43.25±0.35^{a*}	47.83±0.8^{b}	44.59±0.80^{ab}
MCV [fl]	59.3±0.71	57.9±0.86	61.32±0.18	58.53±1.08
MCH [pg]	17.73±0.13	18.14±0.24	18.32±0.09	18.5±0.31
MCHC [g/dl]	30.54±0.39	31.32±0.34	29.7±0.27	32.76±0.81
PLT [10^3/µL]	802.7±16.3	727±52.7^{b}	823±67.3^{b}	793.5.5±30.4^{b}
WBCs [10^3/µL]	8.7±0.72	5.96±0.15^{a*}	7.4±0.41^{b}	7.21±0.97^{b}
Lymphocytes [%]	87.44±1.5	77.2±2.2^{a}	82.63±0.3	84.30±1.9
Monocytes[%]	9.8±0.78	10.2±0.73	10.08±0.61	10.3±1.6
Neutrophils[%]	1.8±0.5	7.8±1.4^{a*}	6.1±0.2^{a}	5.6±1.5^{a}
Eosinophils [%]	0.2±0.2	1.4±0.24^{a}	1.5±0.24^{a}	1.5±0.20^{a}

- Significance (a): relative to the control group.
- Significance (b): relative to the TCCD group.
- Significance: $P < 0.05$, highly significance (*): $P < 0.01$, very highly significance (**): $P < 0.001$.

3.2. Erythron profile (abnormalities of RBC's)

The blood smears of the control group showed normal red blood cells, which have a biconcave disc shape and an area of pallor in the center when viewed microscopically, as shown in Figure 1. In the blood films of TCDD-treated rats there were several morphological abnormalities in the erythrocyte membranes, including tear drop like cells, sickle cells, acanthocytes, where the red blood cells develop an irregular cell surface with numerous projections, and kidney-shaped cell anisocytosis. Oral administration of CH or CH-NPs to the dioxin-treated group showed signs of improvement in the number of altered RBCs.

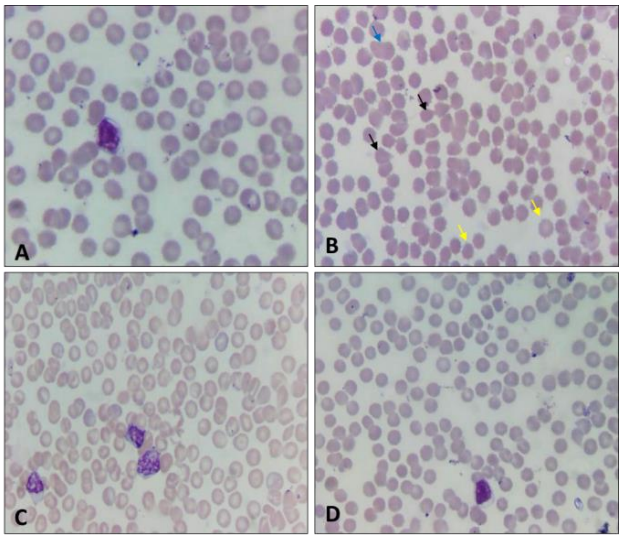

Fig. 1. Photomicrographs of blood film smears of male rats from the control, TCDD, TCDD + CH, and TCDD + CH-NPs groups stained with Leishman stain. **(A)** control group showing a normal red blood cell with a biconcave disc shape **(B)** The TCDD-treated group showed drop like cells (black arrow), acanthocytes (yellow arrow), and kidney-shaped cell anisocytosis (blue arrow). The TCDD + CH treated group **(C)** and the TCDD + CH-NPs group **(D)** had a significant decrease in the number of altered RBCs when compared to the dioxin-only group. X1000 (i.e., oil immersion objective lens)

3.3. Biochemical Results

Table 2 demonstrated that dioxin intoxication increased the serum levels of urea and creatinine significantly ($P < 0.01$) when compared to the control. However, the serum level of urea showed a non-significant increase ($P > 0.05$), but the serum level of creatinine showed a significant increase ($P < 0.05$) after treatment of CH or CH-NPs with dioxin when compared to the control. In contrast, administration of CH or CH-NPs to the TCDD-treated group for four weeks caused a significant drop ($P < 0.05$) in the serum level of urea and a non-significant decrease ($P > 0.05$) in the serum level of creatinine compared to the TCDD treated group. There is no significant difference between CH and CH-NPs groups (Table 2).

Table 2: Effect of CH or CH-NPs on the kidney function of male Albino rats treated with TCDD.

Parameter	Control	TCDD	TCDD + CH	TCDD + CH-NPs
Urea (mg/dl)	25.25±2.6	38.3±1.9[a*]	31.6±0.8[b]	30.2±1.5[b]
Creatinine (mg/dl)	0.48±0.04	1.1±0.05[a*]	0.82±0.09[a]	0.95±0.03[a]

- Significance (a): relative to the control group.
- Significance (b): relative to the TCCD group.
- Significance: P < 0.05, highly significance (*): P < 0.01, very highly significance (**): P < 0.001.

3.4. Histological Results

Histological observation of kidney sections from various experimental animal groups after four weeks of treatment is shown in Figure 2. The control kidney tissue comprises renal corpuscles with well-developed glomeruli and Bowman's capsules, as well as renal tubules (Figure 2A). The kidney sections from TCDD-treated rats revealed severe histopathological changes, such as an expansion of the Bowman's space, shrinkage of most of the glomeruli, loss of the proximal convoluted tubules' brush border, necrotic lesions, swollen cells, and degenerative changes in the lining epithelial cells of the renal tubules (Figure 2B). There were a few tubules with mildly dilated and necrotic lesions in the lining epithelial cells of the renal tubules in both the TCDD + CH-treated group (Figure 2C) and the TCDD + CH-NPs (Figure 2D).

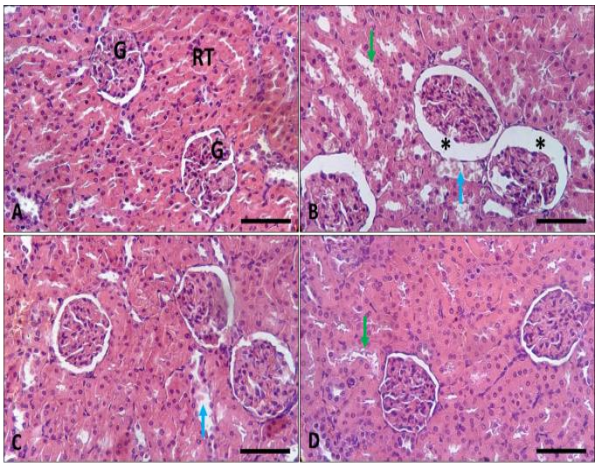

Fig. 2. Photomicrographs of kidney tissue sections of male rats from the control, TCDD, TCDD + CH, and TCDD + CH-NPs groups stained with H&E **(A)** The control group showed renal corpuscles with well-developed glomeruli (G) and Bowman's capsules, as well as renal tubules (RT). **(B)** TCDD-treated groups showing shrinkage in the majority of the glomeruli and the Bowman's space is widened (*). Some tubules had epithelial lining cells that were enlarged (blue arrow). Numerous renal tubules displayed extensive degeneration, and cellular debris filled their lumens (green arrow). **(C)** TCDD + CH treated group and **(D)** TCDD + CH-NPs group showing improvement in kidney tissue with slightly swollen renal tubule cells (arrow) and a return to a probably nearly normal renal appearance with normal or near-normal glomeruli and tubules. Scale bar: 50µm

3.5. Histochemical Results

The Masson Trichrome stain revealed that there was little interstitial connective tissue around the blood vessels and no collagen content surrounding the glomeruli in the control sections (Figure 3A). The amount of collagen of the renal tissues increased in the TCDD-treated group, where collagenous bundle fibres were abundant in the tissue and around the renal corpuscles, as well as in the intertubular gaps and intraglomerular spaces, and around the blood vessels (Figure 3B). The rat's renal tissue treated with dioxin combined with CH or CH-NPs revealed a moderate reduction in the collagen fiber content and was present only in the periglomerular space and in the intertubular regions when compared with the dioxin group (Figure 3 A and B).

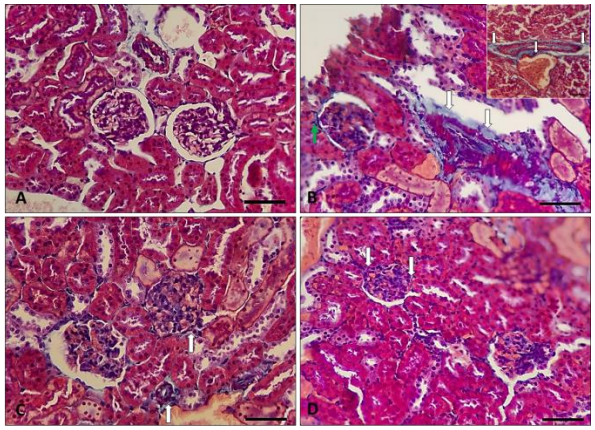

Fig. 3. Photomicrographs of kidney tissue sections of male rats from the control, TCDD, TCDD + CH, and TCDD + CH-NPs groups stained with Masson trichrome stain. (A) The control group shows little interstitial connective tissue and few collagen fibers surrounding the renal tubules. (B) The kidneys of the TCDD-treated group show an increased amount of collagen in the interstitial connective tissue (white arrow), periglomerular in the space (green arrow), and in the intertubular regions. The (C) TCDD+CH group showed a moderate decrease in collagen fiber content and was present only in the periglomerular space in the intertubular regions. (White arrow) (D) In the TCDD + CH-NPs group, collagen fiber content decreased moderately. Scale bar: 50µm

4. Discussion

CH and CH-NPs are commonly used for various purposes but essentially for biomedical applications. In the present study, dioxin treatment induced toxicity through alteration of haematological parameters as well as renal structure and function in rats. The haematological parameters, plasma membrane of red blood cells, and kidney structure and function, exhibited evidence of improvement after oral administration of CH or CH-NPs to the TCDD-treated group.

Haematological indices are crucial for diagnosing many diseases, including infections [41]. The current haematological findings demonstrated that the treatment of TCDD causes haematotoxicity as evidenced by a considerable drop in RBC count, HB concentration, and HCT in male rats. These findings are consistent with an earlier study [42], which found reduction in RBCs, HB concentration, and HCT may have a negative

impact on the haematopoietic system, reducing the supply of RBCs through decreased production and/or an accelerated rate of removal from circulation. In addition, Tanuja and Nivedita [43] found that chronic low doses of TCDD (0.00005 g/Kg) to Swiss albino mice caused a highly significant decrease in RBC, HCT, Hb, MCV, MCH, and MCHC. In the present study, the decrease in RBC count, Hb concentration, and HCT may be due to the apoptotic cell death in circulating erythrocytes and the inability of bone marrow to counteract the accelerated rate of destruction caused by the myelotoxic effect of TCDD on bone marrow [6].

According to the present findings, TCDD-treated animals showed a significant decrease in the WBC count and % of lymphocytes, with an increase in the proportion of neutrophils and eosinophils compared to normal ones. These findings concur with those of Mohamed et al. and Abd El-Nasser et al. [6, 11]. A number of mechanisms have been proposed to explain the negative effects of TCDD on total and differential leukocytes; Frazier et al. [44] linked this condition to the immunological suppressive effect of TCDD on bone marrow stem cells via a process that is either directly or indirectly related to the activity of oestrogen. According to Murante and Gasiewicz [45], the ability of bone marrow to create prolymorphocytes is decreased as a result of the myelotoxic effects of TCDD, which interfere with the processes of differentiation and/or proliferation of hemopoietic stem cells. Moreover, thrombocytopenia and leucopenia are caused by the loss of stem cells and the bone marrow's inability to create new blood cells [46]. In contrast, the present results are inconsistent with those of Tanuja and Nivedita [43], who recorded a significant increase in WBC count in the TCDD treated group. They hypothesised that the reason for this increase might be TCDD exposure-related haematological system dysfunction.

Furthermore, the observed structural defects in the erythrocyte membranes that resulted in an overabundance of acanthocytes were confirmed by the destruction of RBCs in the TCDD-treated group as evidenced by altered haematological results. However, spiculated echinocytes may be the result of xenobiotic-induced changes in membrane composition and the release of intracellular substances [47, 48]. Similarily, the transformation of discocytes into echinocytes can also be seen upon exposure to lead acetate [49] or metal oxide nanoparticles [50]. Also, Owonikoko et al. [51] mentioned the erythro-morphometry assessment of Wistar rats exposed to cement dust. The disruption of the antioxidant systems, due to increased free radical production, and lipid peroxidation, may cause RBC membrane damage, including a decline in membrane transporter activity and changes in membrane permeability [52, 53].

In this study, oral administration of CH or CH-NPs to the TCDD-treated group resulted in significant improvements in haematological parameters when compared to the dioxin-treated group. Meshkini et al. [54], reported similar results, stating that dietary supplementation with 0.25% CH for up to 56 days could improve rainbow trout haematological parameters against stress resistance. The potential of the CH-NPs to promote bone marrow activity which significantly increased the total WBC count, RBC count, HB and platelet count and reversed the myelosuppressive effects of cyclophosphamide [26]. On the other hand, the present results are inconsistent with previous studies; Kim et al. [55] how found that treatment of CH at doses of 500, 1000, and 2000 (mg/kg/day) had no effect on the rat's haematological markers. Similarly, the levels of PLT, MCV, MCH, and MCHC in Hanwood calves did not significantly change after receiving therapy with CH [56]. In addition, Kisadere et al. [57] hypothesised that the values for haematological parameters in rats exposed to cadmium had not changed significantly after the administration of CH oligosaccharide. Additionally, in the cadmium-treated rats, it somewhat reduced the lymphocyte count and slightly ameliorated the increased WBCs [58].

The results demonstrated that dioxin treatment caused nephrotoxicity, as shown by an increase in serum levels of urea and creatinine as well as perceptible histopathological changes in the kidney tissue. The findings are consistent with earlier research on the histopathological changes in kidney tissue brought on by dioxin administration, along with an increase in serum urea and creatinine levels. Lu et al.[14] found that The administration of 10 µg/kg/day 2, 3, 7, and 8-TCDD to male rats for 12 days resulted in renal tubular cell damage, which was characterised by moderate swelling and flattening of proximal tubular epithelial cells, as well as tubulointerstitial congestion [29]. Also, [59] recorded significant increases in serum creatinine and blood urea nitrogen levels, indicating renal functional impairment. Moreover, the observable biochemical findings might be attributable to kidney damage, as evidenced by the marked histopathological changes observed in the dioxin-treated group's kidney during the current study. These alterations could be brought on by the production of free radicals, which leads to lipid peroxidation and the breakdown of the membrane structure, which in turn damages the structural integrity of the nephron [14, 15]. TCDD treatment increased lipid peroxidation and induced significant alterations in the antioxidant enzymes in the kidney, confirming the link between TCDD-induced kidney toxicity and oxidative stress [15].

The TCDD-treated animals with the administration of CH or CH-NPs displayed improvement in the histopathological evaluations and substantial changes in serum urea level and slight decrease in serum creatinine level when compared to the TCDD group. These results agree with a previous study by Chou et al. [60], which indicated that oral administration of low-molecular-weight CH (165 or 825 mg/kg/day) for 13 days to rats with gentamicin-induced nephropathy doses improved serum creatinine and blood urea nitrogen levels along with renal tissue. Similarly, Aboulthana and Ibrahim [29] reported that CH had a promising effect in the prevention of renal damage and oxidative stress induced by lithium injection as a decreased urea, creatinine, and blood nitrogen urea levels, with significantly increased antioxidant enzymes in the renal tissues. Also, CH exhibited efficacy against oxidative stress through reducing the lipid peroxidation levels associated with an increase in activities of the antioxidant enzymes [34, 61].

Fibrosis in the kidneys is typically regarded as the final stage of organ failure before function deterioration [62]. In the present study, the TCDD-treated group showed a marked

increase in collagen content in the intertubular spaces, and around the renal corpuscles and blood vessels. This finding is consistent with the findings of Ciftci et al. [15], who reported that TCDD-induced nephrotoxicity impairs kidney function, increases inflammation, and causes fibrosis, with significantly increased collagen deposition as a result of increased free radical levels. However, the administration of CH or CH-NPs to TCDD-treated rats resulted in a significant decrease in collagen fiber content; this protective effect could be attributed to CH's antioxidant and antifibrotic properties [63, 64].

5. Conclusion

The current study found that dioxin-induced hematotoxicity resulted in alterations in hematological parameters and RBC shape abnormalities, as well as a disorder in renal structure and function. Oral administration of CH or CH-NPs to the TCDD-treated group resulted in improvement in the evaluated haematological and biochemical parameters and the histological structure of the kidney. This indicates that CH or CH-NPs have the potential to be hematoprotective and nephroprotective and there is no significant difference between CH and CH-NPs against TCDD toxicity. Also, this study showed that the effect of particle size was minimal and unlikely to be of statistically significance, and this is consistent with the previous study [65].

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