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Ameliorative Effects of Chitosan and Chitosan Nanoparticles Against 2, 3, 7, 8-Tetrachlorodibenzo-P-Dioxin -Induced Hepatotoxicity in Albino Rats

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Abstract

BIO-NANOTECHNOLOGY employing natural products is a promising approach for discovering and developing new drugs for treating various diseases. Chitosan (CH) is a natural product produced from crustacean shells. Recently, both CH and Chitosan nanoparticles (CH-NP) have been used in various pharmaceutical and biomedical applications owing to their unique properties. The main aim of this study was to determine the efficacy of CH and CH-NP in reducing the negative effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in albino rats. Twenty adult male albino rats were divided into four groups of five rats each: the control (CTRL), TCDD, TCDD + CH, and TCDD + CH-NP groups. The activities of alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT), as well as total bilirubin, direct bilirubin, total protein, cholesterol, and triglycerides, all increased significantly in the TCDD-treated group. The serum albumin levels did not significantly change. Furthermore, histological, histochemical, and immunohistochemical analyses showed marked degeneration of liver tissue.

Conclusion: Many prior dioxin-induced biochemical and histological changes improved when treated with CH or CH-NP, with no statistically significant differences between the CH and CH-NP groups.

Keywords: Dioxin, Chitosan nanoparticles, histopathology, liver function.

Introduction

Dioxin compounds are organic contaminants that are undestroyable and have a negative impact on human health. Dioxins are formed from the burning of chemical waste, including the metal industry, bleaching of paper products via chlorine, combustion of leaded gas, community waste, and the production of pesticides and herbicides [1]. Dioxin compounds include biphenyls, dibenzofurans, and polychlorinated dibenzo-p-dioxins. The continual exposure of 2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD), the foremost toxic substance [2], may induce hepatotoxicity, neurotoxicity, immunotoxicity, teratogenicity, and carcinogenicity [3]. Furthermore, dioxins alter lipid metabolism, leading to some chronic health problems [4]. Dioxin compounds accumulate in fat tissues because of their lipophilic nature [1]. Dioxin intoxication alters lipid metabolism and liver enzymes. However, workers exposed to dioxin compounds had higher serum triglyceride and cholesterol [5].

Natural products are currently being used to discover and develop new medications to treat diseases [6]. Chitosan (CH) is a natural polysaccharide compound produced by the alkaline deacetylation process of chitin and is the main constituent of a crustacean shell. CH is a long polysaccharide that is rich in oxygen with hydroxyl and amino groups and comprises several units of N-glucosamine linked by glycoside bonds [7]. Moreover, CH is a rich source of dietary fiber because it cannot be broken down by human digestive enzymes in the stomach and intestine [8]. It has potential applications in several fields, such as, water purification, agriculture, catalysis, and biomedical materials [9]. Recently, CH has been used in several biomedical and pharmaceutical applications because of its biocompatibility, nontoxicity, and good degradability. Other biological activities of CH have been discovered, including antiviral, antifungal, antimicrobial, antioxidant, antitumor, and chemo-preventive and immuno-potentiating effects in colon cancer [10].

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The use of chitosan nanoparticles (CH-NP) has become of great interest in biomedical and biological applications. However, CH-NP have many ameliorative effects compared with large CH particles, which improve their anticancer and immunomodulatory effects [11] anti-inflammatory, and relative oxygen species (ROS) scavenging activities [12]. Nanoparticles have a potent surface warp, which produces more decay pressure and thus increases their solubility [13]. CH-NP have recently become crucial components of the drug delivery system, where various studies reported that CH-NP can be used as carriers for several reagents, including antibiotics, proteins, anticancer chemicals, and gene drugs [14] CH-NP have been employed as vehicles for insulin delivery by the oral route [15].

Based on knowledge gathered from earlier studies regarding the anti-inflammatory significance of dietary CH and CH-NP, this study aimed to evaluate the ability of CH or CH-NP to protect rats' livers from dioxin-induced hepatotoxicity by minimizing adverse effects and improving tissue regeneration.

Material and Methods

Chemicals

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was obtained from Sigma Chemical Co (St Louis, Missouri, USA.). CH with a low molecular weight (50 kDa, 90% deacetylation degree) and dimethyl sulfoxide were purchased from Fluka Germany Chemical Co. Acetic acid (HOAc), sodium tripolyphosphate (TTP) salt, and sodium hydroxide (NaOH) were obtained from Sigma-Aldrich. The other biochemical analysis Kits were purchased from Human Diagnostics, Egypt.

Preparation of CH-NP

CH-NP were prepared using the ionic gelation technique [16] with some modifications. In brief, an ionic solution (0.5%, w/v) of clear CH was prepared via continuous magnetic stirring for >0.5 h in dilute aqueous acetic acid (1%, v/v), and its pH was raised to 4.6-4.8 using a titration of NaOH (10 N). At room temperature, the CH-NP were modified by dropwise adding aqueous TPP solution (0.25%, w/v) to the CH solution at a 1:3 ratios. After more than 2 h, a milky colloidal CH-NP suspension resulted from the interaction of the two oppositely charged ions. At 4°C, The CH-NP were precipitated and gathered by centrifugation at 9,000 rpm for 30 min. For CH-NP purification, three rounds of wash were performed in cleaned water with extensive rinsing. The pure CH-NP were collected via centrifugation at 9,000 rpm for 30 min. at 4 °C. The gathered gel-like CH-NP colloids were oven-dried [17] at 40 °C for 3–5-hrs [18] and then preserved at 4 °C until use.

Characterization of CH-NP

The following techniques were used to characterize the prepared CH-NP.

Particle Size and Size Distribution

Using Malvern Zetasizer Nano ZS® equipment, the zetapotential, particle size, and size distribution of the synthesized CH-NP were calculated based on dynamic light scattering (DLS). The characterization analyses were performed in triplicate on diluted CH-NP suspensions in deionized distilled water. At 25 °C, the DLS was measured under a 90° scattering angle [19].

Fourier Transforms Infrared (FT-IR) Spectroscopy Study

FT-IR spectroscopy was used to differentiate the functional groups of CH and the CH-NP powders using the potassium bromide (KBr) pellet method. First, the KBr pellets were ground to a fine powder in an agate mortar. The powders of CH or CH-NP were then mixed separately with the KBr powder at a ratio of 1:10. The resulting mixtures were crushed for 2-4 minutes before being compressed into pellets using a hydraulic press for 1-2 minutes. The FT-IR holder holding the samples received the pellets with caution. The readings were made using Vector22 FTIR spectrometry (Bruker, Germany) within the 400–4000 cm-1 range [20].

Animals

A total of 20 apparently healthy adult male Wistar albino rats weighing 180–200 gm (8 –10 weeks old) were purchased from the animal experimental research unit, Faculty of Medicine, Sohag University, Sohag, for this experiment. All animals were kept in filter-top polycarbonate cages in a room that was free of all chemical contaminants, artificially lit (12 h dark/light cycle), and kept at a constant temperature (25 ± 1°C). The rats were given a supply of commercial diet pellets in addition to fresh and clean drinking water. All animals received humane care in accordance with the standards set forth by the Animal Care and Use Committee of the Faculty of Medicine, Sohag University. The animal experiments were approved (number SOH-2-5-2022-1.

Experimental Design

Albino rats were placed into four separate groups (five rats each). The first group acted as a control (CTRL), the other three groups received an intraperitoneal (IP) injection with a daily dose of 10 µg/kg of body weight (B.W.) from TCDD, according to Abdulkareem and Nanakali [21]. The second group was the dioxin-toxified group (TCDD-treated), the third group (TCDD + CH) was given an oral daily dose of 1.0 ml from 200 mg/kg of B.W. from CH concurrently with the TCDD IP injection, and the fourth group (TCDD + CH-NP) was given an oral daily dose of 1.0 ml from 200 mg/kg of B.W.

from CH-NP [22, 23] concurrently with the TCDD IP injection. After four weeks of continuous experimentation, all the animals were scarified. The blood was withdrawn and stored in vacuum tubes with a clot activator. Blood samples were centrifuged at 3,000 x g for 10 min at room temperature to obtain sera, which were then kept at 20°C until use. Liver tissue were removed from animals during dissection and preserved in 10% formalin at room temperature for histological or immunohistochemical examination.

Biochemical Analyses

All biochemical analyses of liver biomarkers, including the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), as well as the estimation of total protein, albumin, total and direct bilirubin, cholesterol, and triglycerides, were conducted on the collected sera in accordance with the manufacturer's instructions and commercial kit protocols (Human Diagnostics, Cairo, Egypt).

Histological and Histochemical Examinations

The liver tissue was first treated using the standard histological sectioning procedure after being fixed for 48 h. Liver tissue were routinely embedded in paraffin blocks cut at a thickness of 5 M and mounted on glass slides that had been de-waxed and hydrated. Some of the slides were then stained with hematoxylin and eosin counterstaining according to the procedure formerly outlined by Suvarna et al. [24], and other slides were stained with Masson's trichrome stain for collagen fiber differentiation according to the procedure described previously [25].

Liver damage was scored microscopically and classified as normal, mild, moderate, or severe at scores of 0, 1, 2, and 3, respectively. Histological abnormalities, including sinusoidal dilatation, cellular degeneration, cytoplasmic vacuolization, nuclear pyknosis, hepatic cord destruction, extensive necrosis, and inflammatory cell infiltration [26, 27].

Immunohistochemistry Investigations

An anti-Caspase-3 primary antibody was used following the manufacturer's instructions (ABclonal Co., Catalog No., A11953, USA) for immunohistochemistry to differentiate and localize the Caspase-3 antigen in liver tissues, as described previously [28]. In brief, liver sections were dewaxed, rehydrated, and then treated for 30 min with 3% hydrogen peroxide to stop indigenous peroxidase activity. Finally, they were blocked with 5% normal serum for 15 min. The sections were overnight incubated at 4 ºC with the specified primary antibody, followed by the secondary antibody for activated caspase-3 (1:100, sc-56046, Santa Cruz Biotechnology, CA, 1:500, ab18197, Abcam). Next, the sections were stained with diaminobenzidine and counterstained with hematoxylin. The stained sections were dried using increasing concentrations of ethanol, cleaned in xylene, and mounted using DPX.

The mean area percent of collagen fiber and caspase-3 positive reactions were calculated using image analysis software (ImageJ version 1.46, NIH, USA) for 10 non-overlapping high-power fields of paraphrastic sections of liver tissues from each group.

Statistical analysis

Data were analyzed using GraphPad Prism version 7 (GraphPad Software, Inc.). The statistical analyses were performed using one-way analysis of variance and post hoc Tukey's significant difference test. $P < 0.05$ indicates statistically significant differences. All results were expressed as mean ± standard error.

Results

Characterization of CH-NPs

Particles Size and Size Distribution:

DLS analysis measured the hydrodynamic diameter of the prepared CH-NP in the nanometer range. The size of the CH-NP recorded 216.9 ± 49.11 nm at a polydispersity index of 0.424 (Figure 1 a). The particle size distribution was narrow, ranging from 90 to 270 nm (Figure 1b), and the zeta potential was 29.8 mV, with a zeta deviation of 5.56 mV (Figure 1c).

FT-IR Spectra

The IR absorption spectra show no change in the chemical composition of the samples before and after the ionic gelation process. However, there is a change in the intensity and sharpness of the active band bands. The FT-IR spectra showing the characteristic peaks of both CH and CH-NP are shown in Fig. 2.

The CH spectrum shows combined peaks appearing at the 3422 cm-1 band, which correspond to the stretching vibrations of the NH and OH groups. The band appearing at 1651.06 cm-1 corresponds to the C=O stretch in the amide group, amide I vibration (CONH). Another band appeared at 1586.56 cm-1 is related to the γ (NH2) bending vibration (Fig. 2 b).

The FT-IR spectrum of the CH-NP illustrated in Figure 2a reveals a couple of changes in the positions and intensities of the bands. It was observed that there was a shift from 3422 to 3429.44 cm-1 in the combined peak of the NH and OH stretching vibrations. In addition, the intensity of the (CONH) band decreased and appeared at 1646.36 – 1553.35 cm-1 (mixed (C=O) amide and (C=C) vibrations), and that of the NH2 band decreased and appeared at 1412.91-1385.14 cm-1 (symmetric aromatic ring

stretching vibration (C=C ring)). This indicates the successful crosslinking of the ammonium groups of CH with the polyphosphoric groups of TPP, which enhances the inter- and intramolecular interactions in CH-NP (Fig.2 a).

Biochemical Investigations

The serum activities of ALT, AST, and ALP showed a very significant increase ($P < 0.001$) upon daily dioxin injection compared with the untreated group. The daily dioxin injection in combination with oral CH or CH-NP administration did not restore the normal levels of these enzymes, with an appreciable impact ($P < 0.01$) and ($P < 0.05$) on levels of (ALT and ALP) and AST, respectively, compared with the control rats. Oral administration of CH or CH-NP combined with the dioxin to rats resulted in a substantial decrease ($P < 0.05$) in AST levels and a non-significant drop ($P > 0.05$) in ALT and ALP levels compared with the TCDD-treated rats (Fig. 3).

Dioxin toxicity led to a substantial ($P < 0.001$) rise in the serum levels of total protein and a non-significant ($P > 0.05$) increase in albumin when compared to the control. TCDD intoxication coupled with CH or CH-NP administration did not change significantly ($P > 0.05$) the total protein and albumin levels when compared with the CTRL. There was a highly significant ($P < 0.01$) drop in the total protein level, while the level of albumin showed insignificant change ($P > 0.05$) compared with the TCDD-treated group (Fig. 4).

After TCDD injection, the serum levels of total bilirubin increased significantly ($P < 0.01$), whereas those of direct bilirubin increased significantly ($P < 0.05$) compared with the control. However, the total and direct bilirubin levels showed insignificantly ($P > 0.05$) change from the control level after TCDD administration along with CH administration compared with the TCDD group. Treatment with CH-NP plus the injection of TCDD resulted in a substantial ($P < 0.05$) decrease in the serum levels of total bilirubin and direct bilirubin compared with the TCDD effect (Fig. 4).

The TCDD-treated rats exhibited a very high significant increase ($P < 0.001$) in cholesterol levels and a significant increase ($P < 0.05$) in serum triglyceride levels compared with the untreated rats. Compared with the CTRL group, the injection of TCDD along with CH or CH-NP administration revealed a considerable change ($P < 0.05$) in serum cholesterol level and no statistically significant effect ($P > 0.05$) in triglyceride concentrations. However, the injection of dioxin along with the treatment of CH or CH-NP led to a highly significant ($P < 0.01$) drop in the cholesterol level and insignificant decrease ($P > 0.05$) in the triglyceride concentration compared with the group that received TCDD (Fig. 6). In all tested parameters, there is no significant difference between CH and CH-NP groups (Fig. 4).

Histological Investigations

Following 4 weeks of treatment, histological investigations of liver sections from all experimental animal groups are presented in Figure 5. In the control liver tissue, hepatocytes were organized in hepatic cords with scattered sinusoids in between hepatocytes, as displayed in Figure 7a. However, liver tissue from the TCDD-treated group exhibited disrupted hepatic lobule structure, vacuolation, disarrangement of hepatocytes, and a large necrotic area of cells with pyknotic nuclei (Fig. 5b). The TCDD-treated group that received CH (Figure 5c) or those that mixed CH-NP (Fig. 5d) showed an enhancement in the liver tissue where the liver tissue had less cellular degeneration and an undamaged structure that included some normal nuclei compared with the group receiving TCDD treatment, but some apoptotic and necrotic hepatocytes with vacuolation were still observed.

The scoring of liver injury in the liver sections revealed a significant ($P<0.001$) damage of hepatocytes in the TCDD-treated group in the form of severe necrosis, cellular and hepatic cord degeneration, and cytoplasmic vacuolation, as well as nuclear pyknosis and inflammatory cell infiltration, compared with the control group. On the other hand, CH or CH-NP treatment abrogated liver injury and led to a significant reduction ($P<0.05$) in liver injury scores in rats compared with the TCDD-treated group (Fig. 5 e).

Histochemical Investigations

Using Masson's trichrome staining, the number of collagen fibers in liver tissue from all groups was determined (Fig. 6). In the TCDD-treated rats, liver sections demonstrated a marked increase in the collagen fibers around the portal vein branches, which displayed an extremely thick epithelium surrounding the central veins and irregularly random fibers found between the hepatic cells (Fig. 6 b). The fibers of collagen in the liver tissues of dioxin mixed with CH (Fig. 6 c) or CH-NP-treated mice (Figure 6 d) were approximately comparable to those found in control mice (Fig. 8a). There was a significant increase ($P<0.001$) of the area percentage of collagen fibers in the TCDD-treated group compared with the control group. Rats in the CH or CH-NP groups showed a significant decrease in the collagen fiber area percent ($P<0.05$) as compared to TCDD-treated group (Fig. 6 e).

Immunohistochemical Investigations

The rats treated with dioxin had more caspase-3 immunoreactivity in their liver tissue than the rats in the control group, as shown by the intense brown stain in Figures 7a and 7b. In contrast to the TCDD-treated group, rats given CH or CH-NP displayed a

notable reduction in the amount of caspase-3, as seen by the decreasing intensity of the brown stain (Fig. 7 c&d). Statistically, the TCDD-treated group showed a significant increase ($P<0.001$) of the area percentage of the caspase 3 positive reaction compared with the control group. In contrast to the TCDD-treated group, rats treated with CH or CH-NP showed a significant decrease ($P<0.05$) area percent of caspase 3 positive reactions compared to the TCDD-treated group (Fig. 7 e).

Discussion

CH and CH-NP are commonly used for various purposes, but are essentially used in biomedical applications [29]. The present investigation aimed to assess the ameliorative role of oral CH or CH-NP administration against dioxin-induced toxicity. For this study, an oral dose of CH and CH-NP (200 mg/kg) was selected because it has been demonstrated in earlier studies to detoxify blood circulation, restore altered liver function, and enforce antioxidant and oxidative stress defense mechanisms [22, 23]. Toz and Değer [25] found that this dose of orally administered CH was successful in eliminating lead from the blood circulation in lead toxicity-induced rats, whereas Elsonbaty et al [23]. reported that this dose of CH-NP had a beneficial effect on liver toxicity induced by diethylnitrosamine.

Many researchers are interested in synthesizing nanoparticles of bioactive ingredients to enhance their biological activity, especially those used as antioxidant reagents [29]. The modified products of nano-synthesis have higher bioavailability, bio-distribution, and sensitivity, with reduced toxicity than their parent reagents owing to their larger surface areas and unique chemical and physical properties [29]. As the avoidance of the use of organic solvents for nanoparticle preparation is preferable for biological applications [30], a simple ionotropic gelation process using a very dilute organic solvent was selected to modify CH-NP. The safe, controlled, and fast method used relies on the ionic interaction between molecules with opposite charges, the phosphate groups in the TPP solution (polyanions) and the amino groups in the CH solution (polycations), both being continuously magnetically stirred at ambient temperature [16, 31].

The obtained CH-NP have particles that range in size from 216.9 ± 49.11 nm, which is expected to enhance the anti-oxidant effectiveness in vivo [32]. Biodegradable nanoparticles usually lie between the 10 and 1000 nm size range, and nanoparticles that meet a size range of 150–300 nm are usually considered safe and biocompatible for biological applications [33]. Moreover, the zeta potential of our scanned particles recorded +29.8 ± 5.56 mV, which meets the range of nanoparticle biocompatibility (from +15 to -30 mV) [33]. The FT-IR spectra clarified the active groups in CH and its modified nanoparticles and indicated the successful ionotropic gelation process, which enhanced the inter- and intramolecular interactions of the nanoparticles. This enhancement is in good agreement with previous findings [20, 34, 35], which displayed similar shifting in the active groups of CH-NP. Owing to their small size, nanoparticles can be distributed systemically upon ingestion by passing through body cell barriers, thus, they can be easily ingested in the blood circulation and taken up by the body's vital organs [36].

It is well known that alterations in liver enzymes indicate hepatocyte degeneration. In this study, the TCDD-treated group exhibited altered liver function with substantial elevations in the levels of AST, ALT, and ALP. These elevated enzyme levels were confirmed by visible histopathological changes, these findings are consistent with those of many other authors who studied dioxin-induced hepatotoxicity in mice [37, 38]. However, the histopathological examination in the current study revealed cell degeneration, severe necrosis, and cytoplasmic loosening of hepatic cells, similar findings were reported by Türkez et al. [39]. In the present study, the toxic effects of dioxin on the liver might be due to reactive oxygen species (ROS) formation and a reduction in antioxidant enzyme activity. This suggestion was confirmed by many authors [38, 40, 41] who suggested that ROS produce lipid peroxidation chain reactions that disrupt the lipid bilayer of hepatocyte plasma membranes, resulting in severe cellular damage and the release of cytosolic enzymes into the bloodstream. In the current study, treatment of TCDD-treated rats with either CH or CH-NP demonstrated negligible improvement and did not reduce the increase in ALT and ALP levels, despite the presence of some histopathological changes in liver sections. This may be due to the inadequate length of the experimental period, which may explain the incomplete improvement. These results were in disagreement with those of many authors who reported that the inhibition of free radicals can occur by treatments containing CH, whether as large particles [42], or nano-particles [32, 43], this is due to the positive effect of CH on the reduction of free radical accumulation. In a recent study, treatment with silymarin, CH-NP, or silymarin-CH-NP significantly improved ALP, ALT, and AST enzyme levels in rats treated with CCl4 [20].

In this study, dioxin administration resulted in a considerable increase in the total protein level and an insignificant increase in the albumin level. The increase in serum levels of total protein and albumin may be due to changes in protein synthesis and/or metabolism [44]. The treatment of TCDD-treated rats with either CH or CH-NP resulted in substantial alleviation of the serum total protein level. These findings are similar to those of Abdel-Kader et al.

[40], revealing that TCDD causes hepatic damage by increasing serum total protein and globulin levels while decreasing serum albumin and the albumin/globulin ratio. The same author also demonstrated that CH alleviated most dioxin's biochemical and histological hepatotoxic effects. In addition, Aranaz et al. [45]. discovered that CH reduced plasma albumin levels in hepatic damage caused by CCl4 in rats. Interestingly, Yahya et al. [42] reported that the intake of CH before or after thioacetamide intoxication improved liver markers, including albumin.

In this study, the TCDD-treated group exhibited a substantial increase in the levels of total and direct bilirubin, consistent with the findings of previous studies [46-48]. TCDD affects heme production and breakdown, causing porphyria and jaundice. [49] Furthermore, increases in bilirubin levels are related to hepatic jaundice [50]. It was reported that an increase in serum bilirubin can be produced by hemolysis of RBCs through rapid disintegration of red blood cells [50], which is consistent with our previous study [51]. In contrast, the oral administration of TCDD-treated rats with either CH or CH-NP alleviated total and direct bilirubin. The findings are in line with earlier research by Eshak and Osman [52], who noted that CH administration to rats exposed to bisphenol A alleviated the elevated levels of total bilirubin caused by bisphenol, and Kim et al. [53], who discovered that CH consistently decreased elevated levels of total bilirubin caused by cadmium-induced toxicity in rats. According to a recent study [20], all treatments given to CCl4-treated rats that included silymarin, CH nanoparticles, and silymarin-CH nanoparticles led to a significant decrease in total bilirubin.

In our findings, the TCDD-treated group showed an altered lipid profile with a substantial increase in serum levels of triglycerides and cholesterol, where the disturbance of lipid metabolism is due to damage to membrane integrity [54-56]. Ohbayashi et al. [57] also revealed that time- and dose-dependent effects were responsible for the increased liver weight of rats and the increase in total cholesterol and phospholipid levels in their serum following TCDD treatment. Additionally, Kakizuka et al. [58] proposed that dioxin accumulation in the liver alters the levels of bile acids in the blood, promotes their excretion, and disrupts their formation, causing a change in lipid homeostasis. According to other human health studies, there might be links between exposure to dioxin and health impacts at the level of lipid metabolism, such as triglyceride accumulation [5]. Nevertheless, the administration of CH or CH-NP to the TCDD-treated group resulted in substantial reductions in the serum levels of triglycerides and cholesterol. Similar results were reported by Abdelhakim et al. [59], who discovered that rats fed a diet high in fat showed significantly lower levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol when treated with CH and CH nanoparticles. Additionally, numerous studies have revealed that CH has a strong effect on decreasing plasma lipid levels in rats [60] and humans [61]. In addition, Moon et al. [62] explained the impact of hypocholesterolemia and the potent ability of dietary CH to reduce plasma lipid levels via a mechanism involving an increase in hepatic CYP7A1 activity. However, the most crucial mechanism for removing cholesterol from diabetic rats' bodies is liver-based bile acid conversion [63]. Moreover, CH significantly decreased the levels of hepatic triglycerides and cholesterol in diabetic rats. This suggests that high-molecular-weight CH may have a greater effect on glucose and lipid metabolism in diabetic rats than low-molecular-weight CH [64].

It is well known that excessive abnormal deposition of extracellular matrix because of untreated inflammation or injury causes liver fibrosis [65]. In the present study, liver sections of TCDD-treated rats showed a marked increase in collagen content. A similar result was reported by Duval et al. [37], who found that TCDD induced liver fibrosis by increasing liver collagen staining and serum transaminase levels in mice fed enriched fat. In contrast, Lamb et al. [66] reported that TCDD toxicity did not impair fibrosis, suggesting that TCDD-mediated changes in extracellular matrix remodeling may prevent collagen deposition and the progression of fibrosis. However, this histochemical investigation showed that treatment of TCDD-toxified rats with CH or CH-NP resulted in a remarkable decrease in the content of collagen fibers. These results are supported by a previous study showing the antifibrotic properties of CH or CH-NP against TCDD that causes hepatic fibrosis [40]. Recently, Abdullah et al. [20] found that silymarin, CH-NP, and silymarin-CH-NP have antifibrotic properties by reducing the expression of key fibrosis mediators in rats with Ccl4-induced liver fibrosis.

Apoptosis, or programed cell death, is a normally occurring process to develop and maintain healthy tissues. [67]. Deregulated apoptosis is a potential cause of many diseases, including cancer, neurological disorders, and immunodeficiency. However, caspase-3, the primary effector of programed death of cells is one of the most common diagnostics for detecting apoptosis in many cell types, where several proteins connected to apoptosis are cleaved by stimulated caspase-3 [68]. Furthermore, caspase-3 activation is involved in many cell types' apoptosis [69]. In the present study, the TCDD-treated group exhibited much greater caspase-3 immunoreactivity than the control group. These outcomes are consistent with those reported by Eckle et al. [70], who found that dioxin-induced acute hepatotoxicity resulted in necrotic and

apoptotic hepatocellular damage with activated caspase-3. Abdu et al. [71] also reported that TCDD-induced cell death involves high levels of apoptosis in male rats.

In contrast, in this study, treatment of the TCDD-toxified group with CH and CH-NP reduced caspase-3 production. Our results suggested that CH and CH-NP might protect liver tissue by reducing the production of apoptotic proteins, these results are in line with those of Mondal et al. [72], who found that Morin CH-NP had more anti-inflammatory, anti-apoptotic, and antioxidant effects than free Morin against arsenic-induced hepatotoxicity. Furthermore, Eshak and Osman [52] found that liver apoptosis and necrosis had a high caspase-3 activity induced by Bisphenol A (BPA) in liver tissues that was significantly decreased by CH treatment. Many authors demonstrated the ability of CH-NP to inhibit caspase 3 expression in another cell than hepatic cells, Mahmoud et al. [73] demonstrated that caspase-3 immunoreactivity in acinar and ductal cells was enhanced by CH-NP treatment, Wardani et al. [32] found the anti-apoptosis effects of CH-NP by decreasing caspase-3 expression in a cardiac cell of diabetic rats, and Sudjarwo et al. [74] reported the protective effect of CH-Pinus merkusii nanoparticles through inhibiting caspase-3 expression caused by lead acetate in rat testes.

From the previous results in this study, it is important to note that the CH-NP appeared to have a better effect on some evaluated parameters than CH against TCDD toxicity, but statistically, there was no significant difference between CH and CH-NP, which is consistent with the results of previous study [59]. The exact cause of this result is unknown, but it could be due to the preparation method. The specific structural characteristics of CH and its derivatives, including its molecular weight, degree of deacetylation, site, and degree of substitution, all have a significant impact on the physiochemical properties of CH-NP [75].

Conclusion

The results of this study demonstrated that dioxin-induced hepatotoxicity resulted in altered histopathological and biochemical marker changes. The majority of evaluated biochemical and histological investigations improved slightly to clearly after the oral administration of CH or CH-NP to the TCDD-treated group, showing the potential for hepatoprotection, which may be attributed to the antioxidant properties of CH and CH-NP. However, biochemical and histological investigations were not completely neutralized by CH or CH-NP. Nevertheless, considering the discussed results, we suggest that a dose increase or longer treatment with CH or CH-NP might restore the normal levels. Therefore, additional investigations are recommended to determine the appropriate ameliorative concentration.

Acknowledgments

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No funding was received.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

The use of the animals was approved by the Sohag Institutional Animal care and use Committee (Sohag-IACUC) of Sohag University (number SOH-2-5-2022-1).

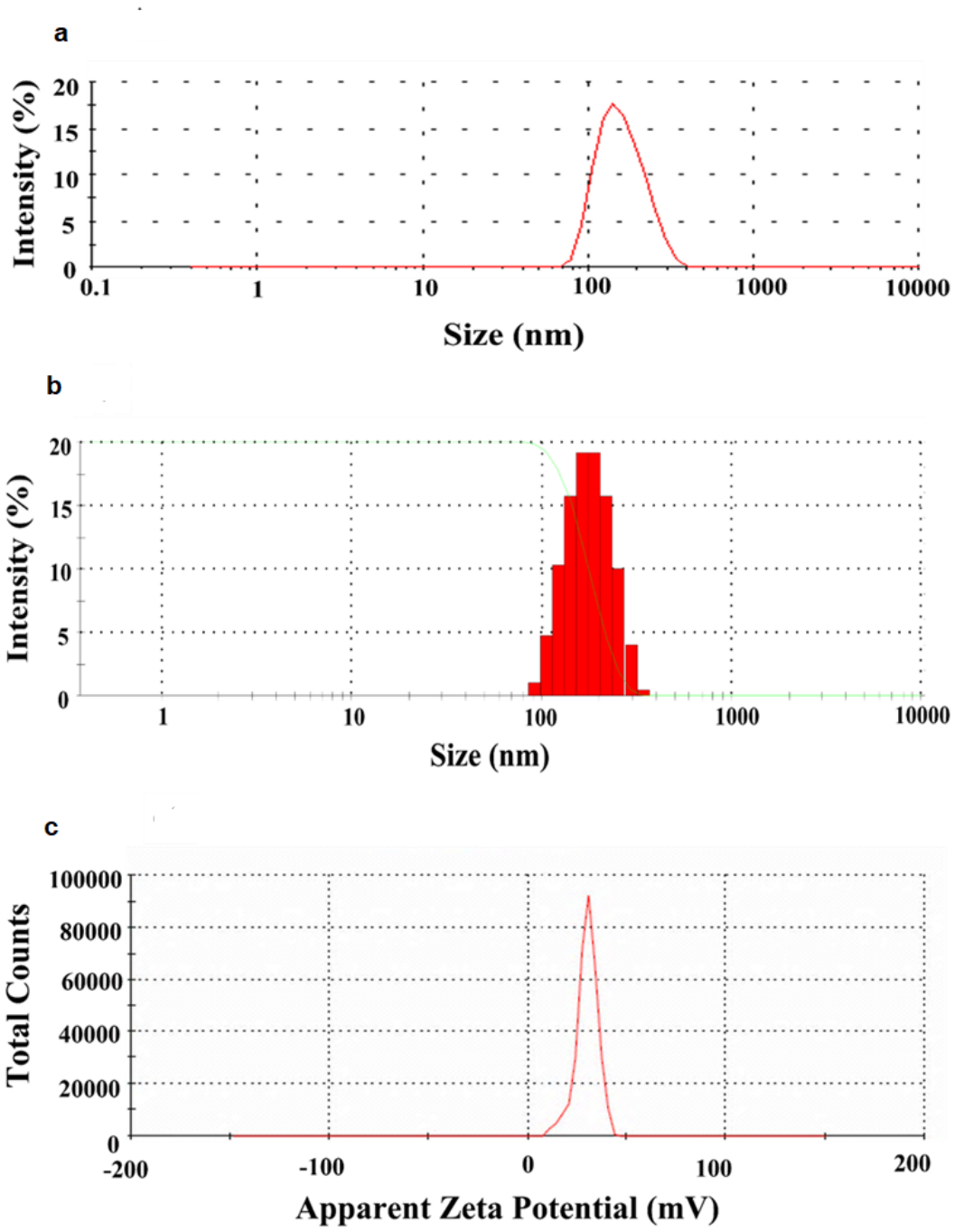


Fig. 1. Characterization of the prepared CH-NP. (a) Particle size by intensity peak at 216.9 nm, (b) size of the particle distribution, with a red column chart displaying a narrow size distribution range of 90 to 270 nm and a green disciplined Z-shaped peak displaying a homogeneous particle distribution, and (c) zeta potential peak at +29.8 mV. All data are presented as means ± SD (n = 3)

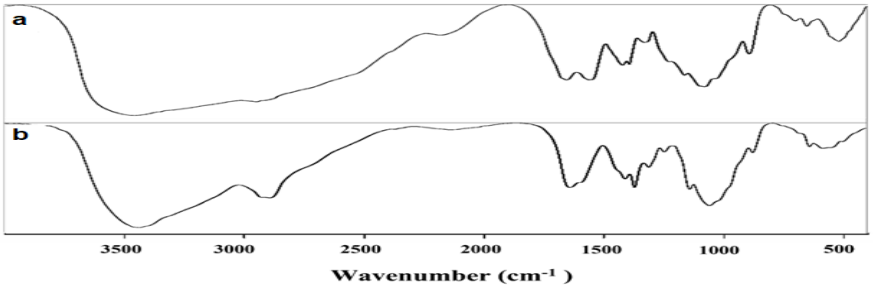


Fig. 2. FTIR spectra of CH-NP (a) and CH (b).

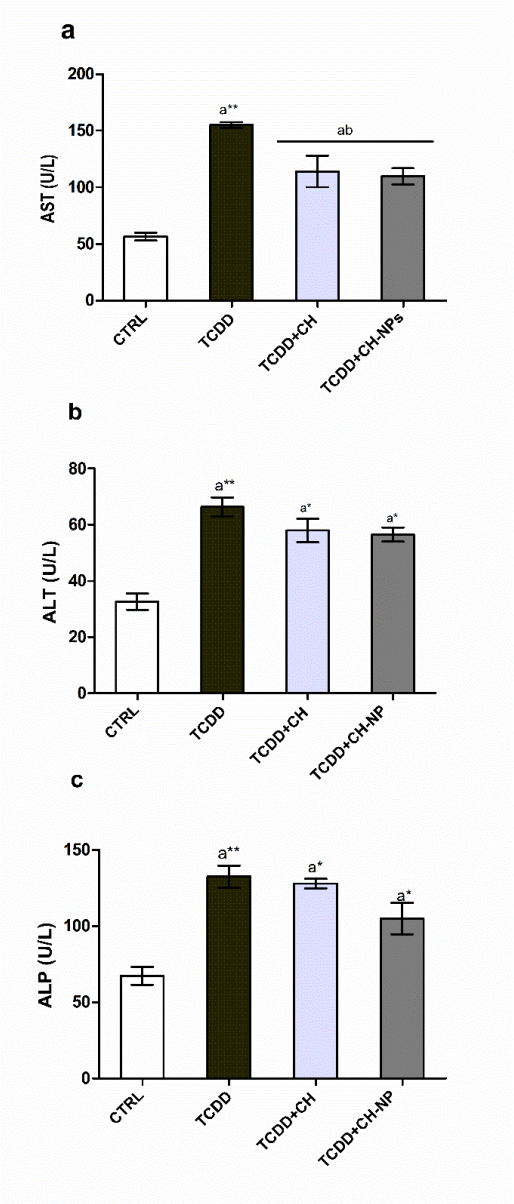


Fig. 3. Effect of CH or CH-NP administration on the serum levels of (a) aspartate aminotransferase (AST), (b) alanine aminotransferase (ALT), and (c) alkaline phosphatase in rats treated with TCDD.

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

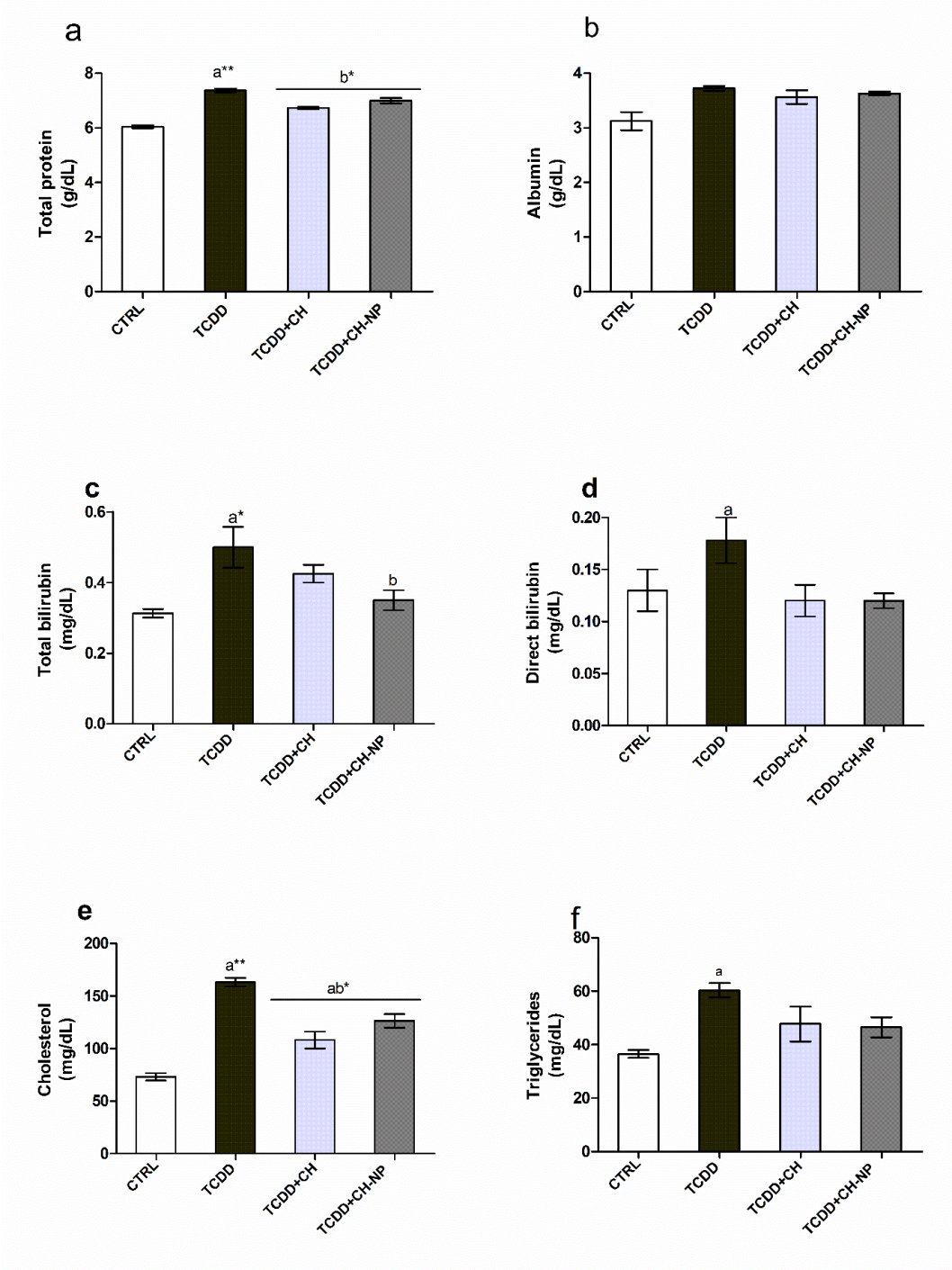
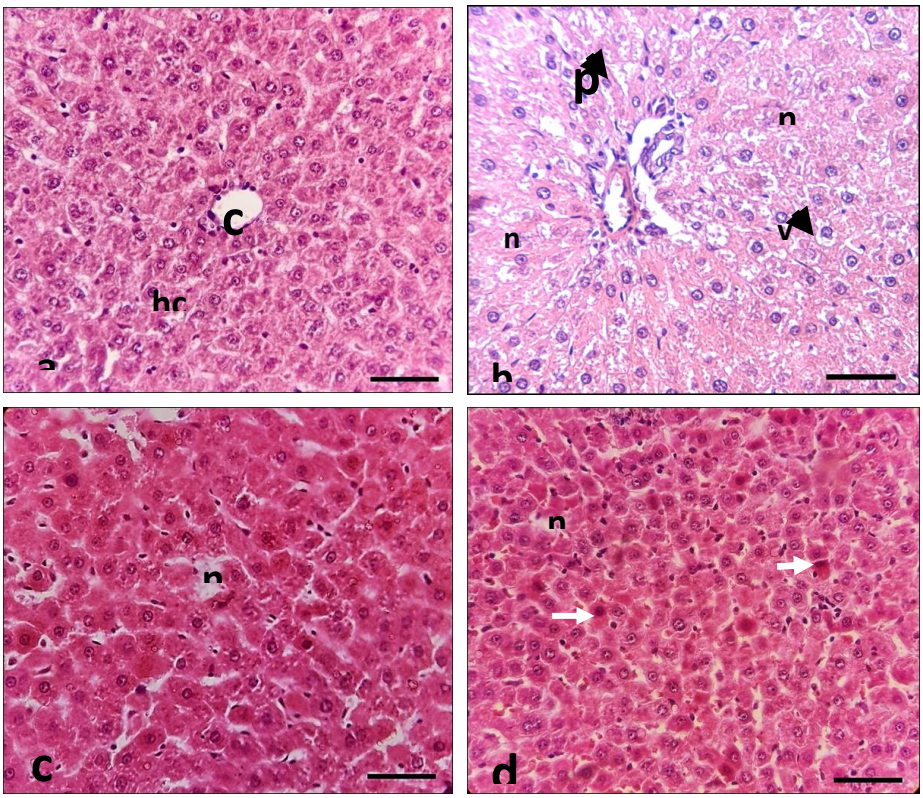


Fig. 4. Effect of CH or CH-NP administration on the serum levels of (a) total protein and (b) albumin, (c) total bilirubin (d) direct bilirubin, (e) cholesterol and (f) triglycerides in rats treated with TCDD.

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



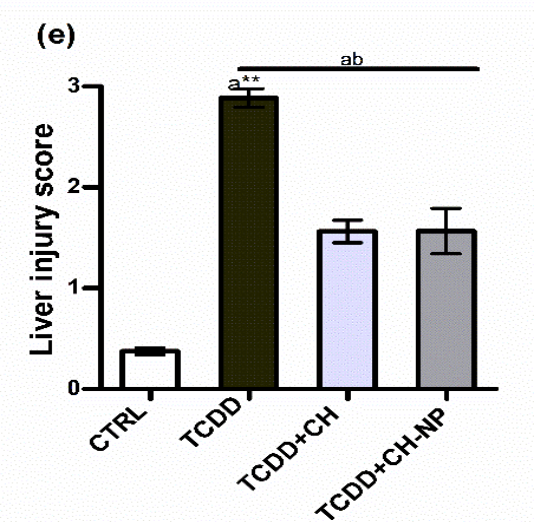


Fig. 5. Staining with H&E (a) The CTRL group showed normal hepatocytes arranged in hepatic cords with the presence of blood vessels (cv). (b) TCDD-treated groups showing vacuolation (v), disarrangement of hepatocytes, a large necrotic area of cells (N), pyknosis of cell nuclei (white arrow), and the disorganization of cytoplasmic structures (arrow) (c) TCDD + CH-treated group and (d) TCDD + CH-NP group showing an improvement in liver tissue where the liver tissue had less cellular degeneration and an undamaged structure that included some normal nuclei than the TCDD-treated group, but some apoptotic (white arrow) and necrotic (n) hepatocytes were still observed. Scale bar: 50 µm. Analysis of live injury score (e). Data presented as mean ± SEM of five animals per group. Significant (a) relative to the control group and (b) relative to the TCCD group. Significance: P < 0.05, highly significant (*): P < 0.01, very significant (**): P < 0.001.

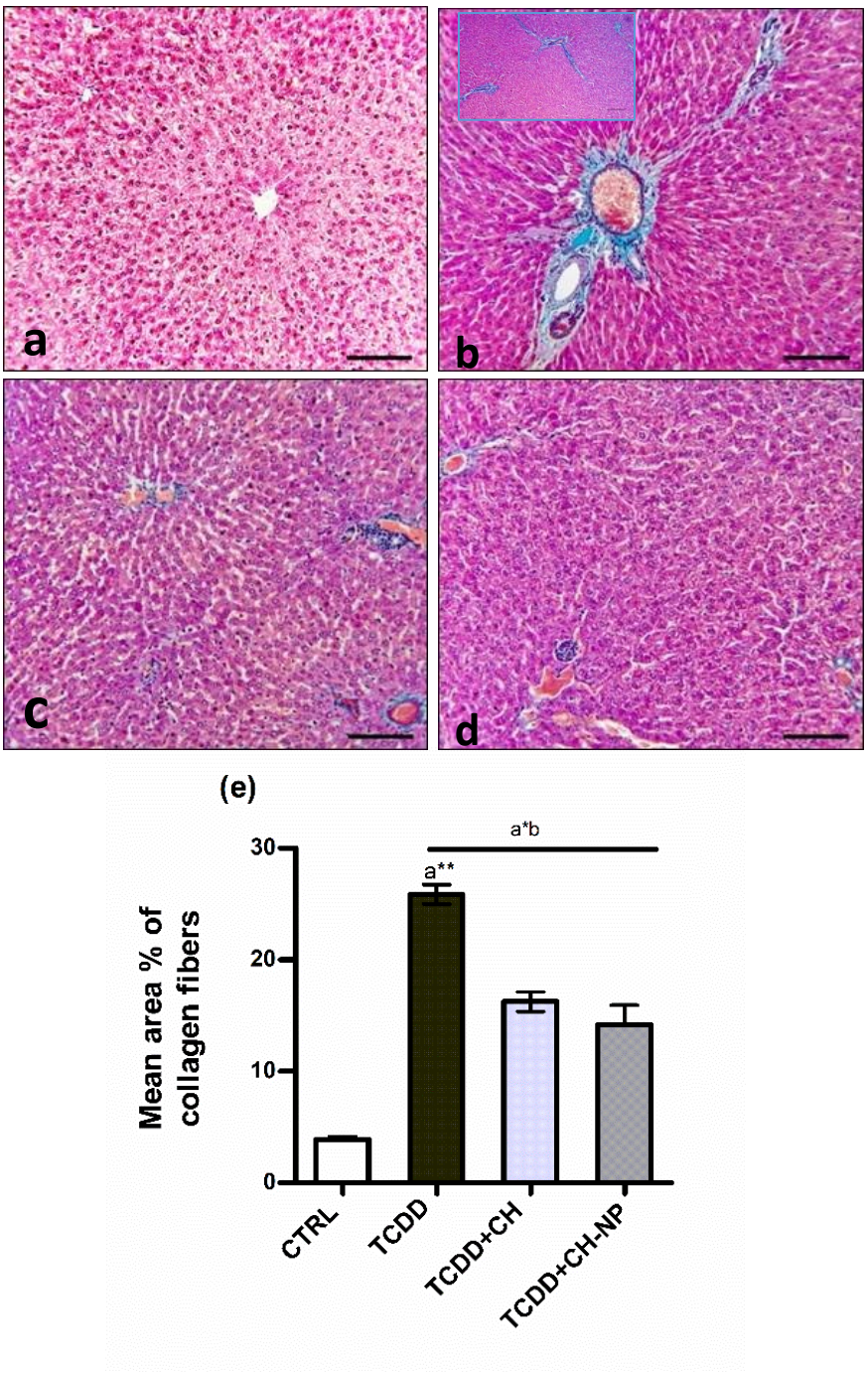
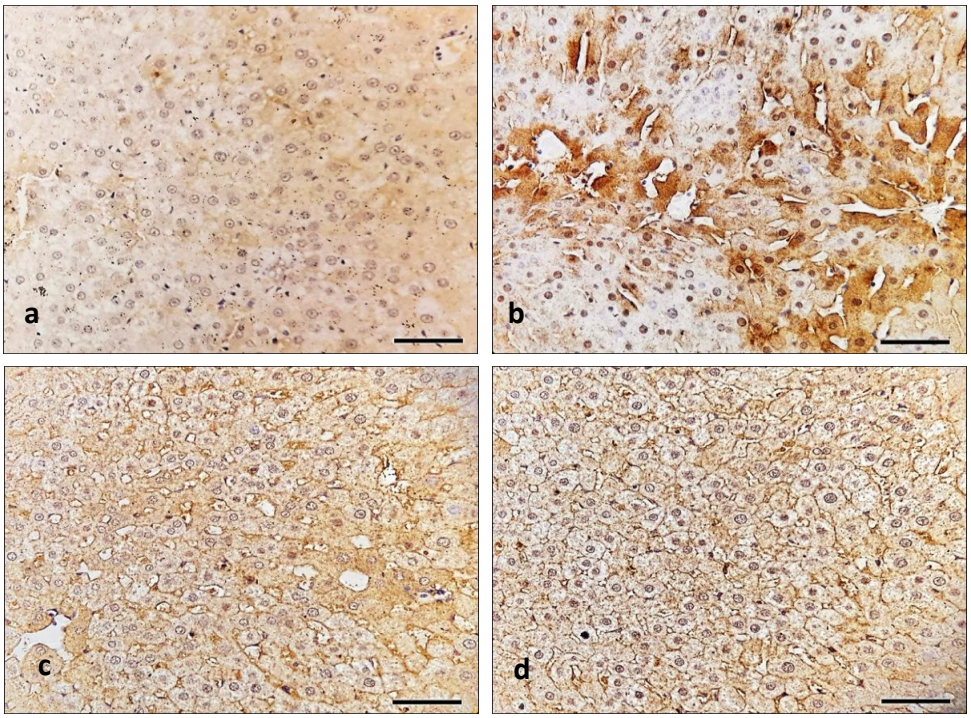


Fig. 6: Photomicrographs of liver tissue sections stained with Masson's trichrome stain (a) The CTRL group showing blue-stained collagen fibers is organized between hepatic portal vein branches (b) The TCDD-treated group showed more collagenous bundles throughout the tissue in the portal portal vein branches, which displayed an extremely thick epithelium surrounding the central veins and irregularly random fibers found between the hepatic cells TCDD + CH (c) and TCDD + CH-NP (d) treated group showing, a few collagenous fibers surrounding the central veins group. Scale bar: 100 µm. Mean area percentage of collagen fiber (e). Data presented as mean ± SEM of five animals per group. Significant (a) relative to the control group and (b) relative to the TCCD group. Significance: P < 0.05, highly significant (*): P < 0.01, very significant (**): P < 0.001.



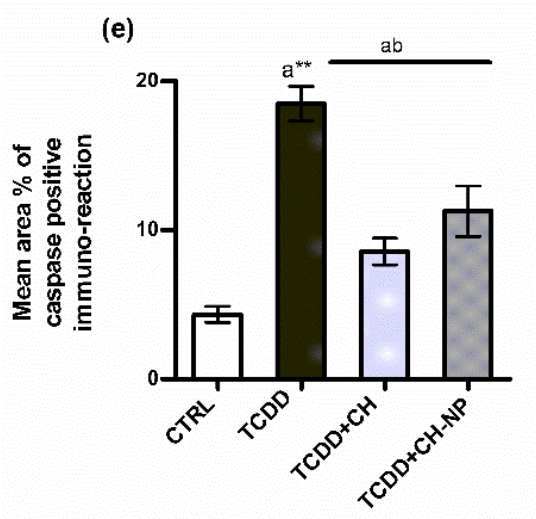


Fig. 7. Photomicrographs of liver tissue sections stained with Caspase-3 immunostaining. (a) CTRL group showing (b) TCDD-treated group showed deep brown staining in the hepatic tissue(c) TCDD + CH-treated group and (d) TCDD + CH-NP group showing a marked decrease in caspase-3 amount faint brown Caspase-3-immunostaining in the hepatic tissue. with compared to dioxin group alone. Scale bar: 50 µm. The mean area percentage of Caspase-3 positive reactions (e). Data presented as mean ± SEM of five animals per group. Significant (a) relative to the control group and (b) relative to the TCCD group. Significance: P < 0.05, highly significant (*): P < 0.01, very significant (**): P < 0.001.

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التأثير المحسن للكيتوزان وجسيمات الكيتوزان النانوية على السمية الكبدية الناجمة عن الديوكسين في ذكور الجرذان البيضاء

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الملخص

الكيتوزان منتج طبيعي ينتج من قشور القشريات. في الآونة الأخيرة، تم استخدام كل من الكيتوزان وجسيمات الكيتوزان النانوية في العديد من التطبيقات الصيدلانية والطبية الحيوية لخصائصها الفريدة.

صممت الدراسة الحالية لتقييم الأدوار الوقائية المحتملة للكيتوزان وجسيمات الكيتوزان النانوية كمضادات للأكسدة ضد السمية التي يسببها الديوكسين (TCDD) 2,3,7,8-Tetrachlorodibenzo-p-dioxin) على بعض. معايير الكيمياء الحيوية وكذلك الأضرار النسيجية المرضية في أنسجة الكبد لدى ذكور الجرذان البيضاء. في هذه الدراسة، تم تقسيم عشرين فأرًا بالغًا من ذكور الجرذان البيضاء إلى أربع مجموعات تحتوي كلا منها على خمسة جرذان، لجميع المجموعات، استمرت فترة التجربة لمدة 4 أسابيع متتالية. المجموعة الأولى: الضابطة. المجموعة الثانية: تم حقنها بمادة الديوكسين (TCDD) (10 ميكروجرام/كجم محقونة داخل الغشاء البريتوني). المجموعة الثالثة: تم حقن الجرذان بمادة الديوكسين (10 ميكروجرام/كجم محقونة داخل الغشاء البريتوني) وتم إعطاؤهم كيتوزان بجرعة 200 مجم/كجم عن طريق الفم. المجموعة الرابعة: تم حقن الجرذان بمادة الديوكسين (TCDD) (10 ميكروجرام/كجم محقونة داخل الغشاء البريتوني) وتم إعطاؤهم جسيمات الكيتوزان النانوية بجرعة 200 مجم/كجم عن طريق الفم. اظهرت المجموعة المعاملة بمادة الديوكسين (TCDD) زيادة معنوية في مستويات الأنزيمات الناقلة للأمين (الأمينوتراتسفيريز)، وإنزيم الفوسفاتيز القاعدي و وكذلك البروتين الكلي، والبيليروبين الكلي، والبيليروبين المباشر، والكوليسترول، والدهون الثلاثية، بينما لا يوجد تغيير كبير في مستوى الألبيومين في الدم و ذلك بالمقارنة بمثيلاتها في المجموعة القياسية. كذلك وجود تغيرات نسيجية مرضية واضحة في أنسجة الكبد مع زيادة في الياف الكولاجين وفي إنتاج كاسباس -3.

بعد التجريع الفموي للكيتوزان أو جسيمات الكيتوزان النانوية إلى المجموعة المعالجة بالديوكسين (TCDD)تحسنت غالبية الفحوصات البيوكيميائية والنسيجية بعضها بشكل طفيف والآخر تحسنا معنويا مقارنة الي المجموعة المعاملة بمادة الديوكسين ، مما يشير إلى إمكانية حماية الكبد ، والتي قد تُعزى إلى الخصائص المضادة للأكسدة التي يتميز بها الكيتوزان وجسيمات الكيتوزان النانوية. ومع ذلك، فإن المعالجة بالكيتوزان أو جسيمات الكيتوزان النانوية لم تحسن بالكامل التغيرات الكيميائية الحيوية والنسيجية. في ضوء هذه النتائج، تقترح الدراسة الحالية أن زيادة الجرعة أو الفترة العلاجية باستخدام الكيتوزان أو جسيمات الكيتوزان النانوية قد يستعيد المستويات الطبيعية. يوصى بإجراء أبحاث إضافية في هذا الصدد لتحديد التركيز المحسن الأفضل.