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Innovative perception on using Tiron to modulate the hepatotoxicity induced by titanium dioxide nanoparticles in male rats

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ABSTRACT

The extensive application of titanium dioxide nanoparticles (TiO_2 NPs) in the food industry arouses a debate regarding the probable risk associated with their use. Several recent studies reported that most nanoparticles (NPs) have adverse actions on the liver. The objective of this study is to examine whether Tiron plays a modulatory role against apoptotic damage induced by TiO_2 NPs in rat livers. Forty rats were randomly divided into 4 groups; a control group received phosphate-buffered saline, an intoxicated group received 100 mg/kg/day of TiO_2 NPs for 60 days, a treated group received 470 mg/kg/day of Tiron for the last 14 days after TiO_2 NPs administration, and a Tiron group received Tiron only as previously mentioned. Oral administration of TiO_2 NPs significantly increased serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). In the liver, TiO_2 NPs increased oxidative stress through increasing lipid peroxidation and decreasing GSH concentration and the levels of the SOD and GPx enzymes. TiO_2 NPs significantly upregulated the proapoptotic Bax gene and downregulated the antiapoptotic Bcl-2 gene. Histopathological examination of hepatic tissue reinforced the previous biochemical results. Apoptotic lesions were also obvious in this group. Treatment with Tiron as an antioxidant significantly decreased serum biochemistry, ameliorated oxidative stress in hepatic tissue, upregulated Bcl-2, decreased Bax expression and attenuated the histopathology of hepatic injury. These findings indicate that Tiron effectively diminishes the hazardous effects of TiO_2 NPs on rat liver.

1. Introduction

Industrial use of metal oxide nanoparticles (i.e., titanium oxide, iron oxide, silicon, etc.) has rapidly grown during the past decade. This has led to an increase in the occupational and environmental exposure of humans and other species to nanoparticles (NPs) [1]. The safety of nanomaterials has been a focus of worldwide concern because of the lack of information available regarding their potential risks for workers and the general population. Among the various commercial nanomaterials, titanium dioxide nanoparticles (TiO_2 NPs) are one of the most frequently used in industrial applications. Their potential toxicity to humans and the environmental impact of TiO_2 NPs have attracted considerable attention with their increased use in industrial applications. The food industry is starting to use various NPs as food additives or to improve food packaging in an attempt to optimize their product [2]. In the IARC [3] (2006), pigment-grade TiO_2 was classified as possibly carcinogenic to humans (Group 2B), depending on an appropriate demonstration of carcinogenicity in animals and insufficient evidence for human tumour promotion. Concern has been raised regarding the possible adverse health effects of ingested NPs. TiO_2 is well known to be a common food additive used for whitening and brightening foods, especially confectioneries, white sauces and dressings, and certain powdered foods. Recently, food-grade TiO_2 (referred to as E171) test results suggested that approximately 36% of the particles are less than 100 nm in at least one dimension [4]. Many in vivo studies suggested that nanoparticles can accumulate in several tissues such as the liver and kidneys and can generate various inflammatory responses. The liver is a main organ that executes a wide array of functions including biotransformation, metabolism and excretion of endogenous and exogenous compounds [5]. The toxic effects of TiO_2 NPs in liver

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tissue were reported previously. TiO_2 NPs have been reported to damage liver function and induce oxidative stress and lipid peroxidation in the rodent liver [6]. Tiron (4,5-dihydroxy-1,3-benzene disulfonic acid) is a water-soluble, cell-permeable analogue of vitamin E and functions as a direct hydroxyl radical and superoxide scavenger [7]. It is both a beneficial protective antioxidant and an efficient nontoxic metal chelator that has been commonly used in oxidative-stress-associated studies [8]. Tiron is a mitochondrial localized antioxidant that can permeabilize the organelle membrane and accumulate inside [9]. For example, it can reverse the reactive oxygen species (ROS)-induced cell apoptosis or act as a nontoxic chelating agent to alleviate acute toxic metal overload [10]. Tiron has been reported to be able to inhibit apoptosis in human lung cancer cells [11]. In this paper, we present an overview of the antioxidant role and the possible therapeutic effect of Tiron against TiO_2-NPs-induced hepatotoxicity in the rat liver.

2. Materials and methods

2.1. Materials

Titanium dioxide (TiO_2): Anatase form, white in colour, density of 3.9 g/ml, particle size of 10 nm, surface area > 150 m^2/g, and purity of 99.9% was purchased from Sigma Aldrich Chemical Co., Germany. Nanosized TiO_2 was suspended in a phosphate-buffered saline solution (PBS) at a concentration of 1 mg/ml, shaken and dispersed via sonication for 10 min before use. Tiron (a metal chelator and antioxidant), a white odourless powder, was purchased from Sigma Aldrich Chemical Co.

2.2. Animals and experimental design

Forty adult male albino rats weighing 180–200 g were housed in separate well-ventilated cages under standard conditions, with free access to a standard diet and water. The design and experiments were approved by the institutional committee, and the protocol conforms to the guidelines of the National Institutes of Health (NIH).

The rats were allowed a two-week adjustment period and were then divided into 4 groups (n = 10 rats for each group) as follows: control, intoxicated, treated and Tiron groups.

The control group received PBS orally, the intoxicated group received TiO_2 NPs at an oral dose of 100 mg/kg bw/day for 60 days [12], the treated group received TiO_2 NPs at 100 mg/kg bw/day for 60 days and 470 mg/kg bw/day of Tiron during the last 14 days of the experimental period [13], and the Tiron group received 470 mg/kg bw/day of Tiron during the last 14 days of the experimental period. The selected dose in this study was chosen after consulting a World Health Organization report from 1969. According to the report, the LD50 of TiO_2 for rats is over 12,000 mg/kg bw after oral administration. In addition, the quantity of TiO_2 NPs should not exceed 1% of the food weight according to the Code of Federal Regulations of the US Government. All symptoms and deaths were carefully recorded daily. At the end of the experiment, the rats were weighed and euthanized, and then, both blood and liver samples were collected for subsequent investigation. Blood samples were collected from the eye vein. Serum was collected by centrifuging blood at 3000 × *g* for 10 min.

2.3. Relative liver weight (RLW)

The relative weight of liver was calculated as the ratio of liver (wet weight, mg) to body weight (g) for each rat [14].

2.4. Serum parametric analysis

The liver functions were evaluated based on the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) according to the instructions provided by the manufacturers.

Table 1
Therapeutic influence of tiron on the liver function tests and relative liver weights inTiO2 NPs intoxicated rats.

Parameter	Control	Intoxicated group	Treated group	Tiron group
ALP	17.39 + 1.3[a]	68.2 + 3.2[b]	33.2 + 0.9[ac]	22.2 + 0.8[ad]
ALT	42.7 + 3.05[a]	182.6 + 2.4[b]	70.6 + 2.9[c]	33.6 + 0.5[d]
AST	92.7 + 0.9[a]	235.8 + 3.4[b]	156.8 + 2.3[c]	103.5 + 3.4[d]
RLW(g)	6.86 + 0.08	6.98 + 0.07	6.923 + 0.02	7.04 + 0.09

Data were expressed as means ± S.E.M (n = 10). Groups having different letters are significantly different from each other, RLW: relative liver weight, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase.

2.5. Estimation of lipid peroxidation and antioxidants in liver tissue

Liver specimens were homogenized in cold PBS (pH 7.4) using a Teflon homogenizer. The homogenates were centrifuged at 14,000 × *g* for 15 min at 4 °C. The supernatant was used to measure the MDA [15], superoxide dismutase (SOD) activity [16], reduced glutathione (GSH) concentration [17], and glutathione peroxidase enzyme (GPx) activity [18] and to estimate the protein content [19].

2.6. DNA laddering assay

To evaluate the degree of DNA damage, genomic DNA was extracted from liver tissue according to Ibrahim et al. [20]. DNA samples (10 µg) were separately loaded into 1.5% agarose gel electrophoresis for 45 min at 80 V. The migration of fragmented DNA on the agarose gel results in a characteristic laddering pattern, which is considered a distinctive feature of apoptotic DNA damage. The degree of fragmentation was determined by gel image analysis and quantitation software; Quantitiy one 4.6.6 BIO-RAD software.

2.7. Quantitative real-time PCR for Bax and Bcl-2 genes

Total RNA was isolated using an RNeasy Mini Kit (Qiagen). The Reverse transcription reaction was completed using a Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific) following the manufacturer's instructions.

The mRNA expression levels of the antiapoptotic gene Bcl-2 and preapoptotic gene Bax in the liver tissue were determined using real-time quantitative polymerase chain reaction (qRT-PCR). Sybr green master mix (Thermo Scientific) was used according to standard protocol, and the primers were designed using Primer 3 software; Bcl-2 forward primer: 5′-GAGGATTGTGGCCTTCTTTG-3′, reverse: 5′-CGTTA TCCTGGATCCAGGTG-3′ and Bax forward: 5′-ACCAAGAAGCTGAGCG AGTG-3′, reverse: 5′-CCAGTTGAAGTTGCCGTCTG-3′. The cDNA was amplified by 40 cycles of denaturation at 95 °C for 45 s, annealing at 57 °C for the Bcl-2 gene and 55 °C for the Bax gene for 45 s and extending at 72 °C for 45 s. The GAPDH gene was amplified during the same reactions to serve as a reference gene [21]. Duplicate plates were tested, and cycle threshold (Ct) values were used to calculate the gene/GAPDH ratio, with a value of 1.0 used as the control (calibrator). The normalized expression ratio was calculated using the ΔΔCt method.

2.8. Histopathological examination

The liver tissues from various groups were fixed in 10% neutral buffer formalin then managed to obtain 4 µm paraffin embedding sections. The tissue sections were stained with haematoxylin and eosin (H &E) [22].

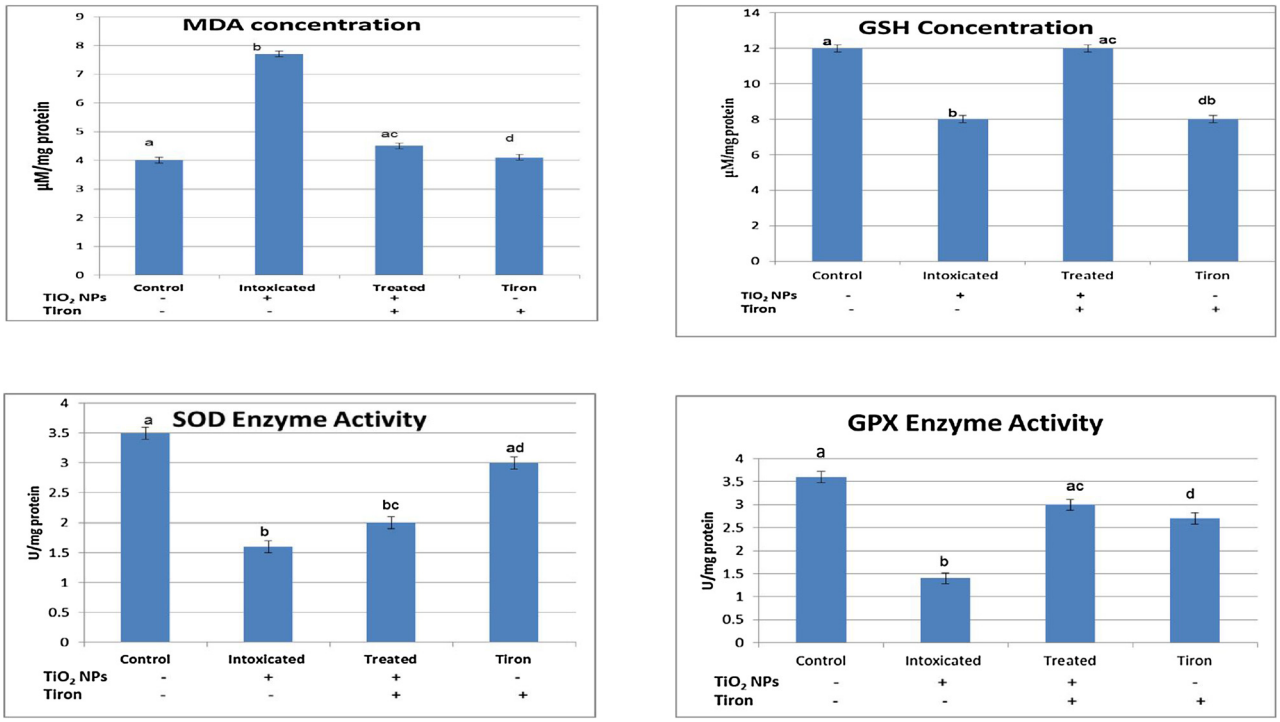

Fig. 1. The therapeutic influence of Tiron on lipid peroxidation and antioxidant parameters in liver intoxicated with TiO2 nanoparticles. Data were expressed as means ± S.E.M (n = 10). Groups having different letters are significantly different from each other.

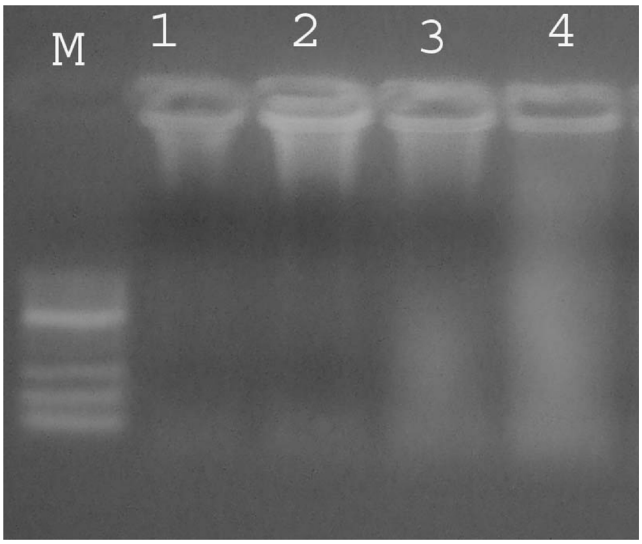

Fig. 2. The DNA laddering pattern in control and treated groups Protective influence of Tiron on DNA laddering in hepatic tissue intoxicated with TiO2 nanoparticles (M) 100bp DNA ladder (1) control group, (2) Tiron group, (3) treated group, (4) Intoxicated group (n = 10).

2.9. Immunohistochemistry for the Bax and Bcl-2 genes

Immunohistochemical analysis was performed following the methods described by Abdel-Rahman et al. [23] The tissue sections were deparaffinized in xylene and rehydrated in different grades of alcohol. The antigen retrieval was done by pretreating the sections with citrate buffer of pH 6 for 20 min. Sections were incubated with rabbit polyclonal anti-Bcl-2 antibody at a concentration of 1:50 (ab59348; Abcam, Cambridge, UK), and rabbit monoclonal anti-Bax antibody [E63] at a concentration of 1:250 (ab32503; Abcam, Cambridge, UK) for two hours in a humidified chamber. The sections were incubated with goat anti-rabbit IgG H&L (HRP) (ab205718; Abcam, Cambridge, UK), and 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma) was used as a chromogen. Finally, the slides were counterstained with haematoxylin and mounted with DPX. The negative control slides were prepared by replacing primary antibodies using PBS. The image analyses of the stained sections were performed using a Leica Qwin 500 Image Analyzer (Leica, Cambridge, England). In each group, five sections were examined. The percentage of immunopositive area (dark brown) (%) was calculated as the mean of 10 fields/slide.

2.10. Statistical analysis

The different analytical determinations in the biological samples were carried out in duplicate, and the results are expressed as the mean ± SE. Data for multiple variable comparisons were analysed by a one-way analysis of variance (ANOVA) test to analyse the significant differences ($P < 0.05$) between groups using SPSS version 16 package for Windows.

3. Results

3.1. Serum biochemistry and relative liver weights

The current results of this study revealed that the TiO_2-NPs-intoxicated group showed significantly higher ALT, AST and ALP enzyme activity than the control group, while the treated group showed significantly lower ALT by 61.3%, AST by 33.5%, and ALP by 51.3% than the intoxicated group, whereas non-significant changes were detected compared to the control group. Non-significant changes were detected for all parameters between the control and the Tiron group. We recorded no significant difference among the different experimental groups regarding the RLWs (Table 1).

3.2. Oxidative stress parameters

Alterations in ROS generation in liver can be considered markers of

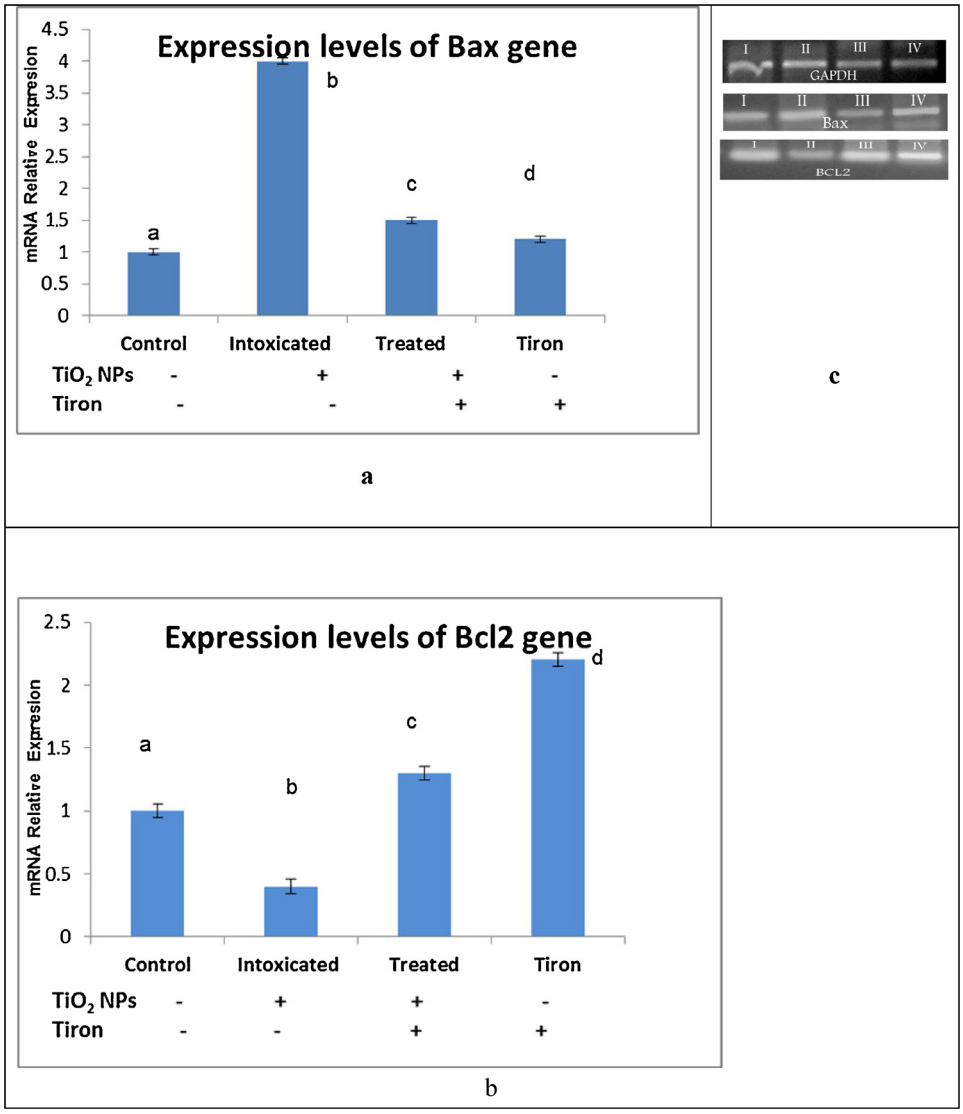

Fig. 3. The therapeutic influence of Tiron on Bax and BCL2 expression level in hepatic tissue exposed to TiO2 nanoparticles. (a) Relative fold change of mRNA expression of Bax. (b) Relative fold change of mRNA expression of Bcl − 2. Data are represented as mean ± SEM. Different superscripts are significantly different. (c) The results obtained by real − time PCR analysis were confirmed by agarose gel electrophoresis analysis of the PCR product. (n = 10). (I: control, II: intoxicated group, III: treated group, IV: Tiron group).

the adaptive response of the liver to oxidative damage. To prove the role of TiO_2 NPs as ROS generators, the levels of lipid peroxidation (MDA) in the liver were evaluated. The considerable increase in MDA in the TiO_2NPs-exposed liver suggested that ROS accumulation led to lipid peroxidation in the liver. Supplementation of Tiron to the treated group significantly reduced the elevated MDA by 37.6%. Under normal conditions, the overproduction of ROS was neutralized by antioxidant defence mechanisms, which included both enzymatic and non-enzymatic antioxidants. GSH is an important non-enzymatic antioxidant that plays a crucial role in the detoxification of ROS. GPx and SOD are important antioxidant enzymes for cellular defence against oxidative damage. The ANOVA results of the intoxicated group, demonstrated that TiO_2 NPs significantly reduced the GSH concentration and GPx and SOD activities compared to those of the control. Co-administration of Tiron as an antioxidant to the treated group significantly increased GSH by 31.7% and GPX enzyme activity by 76.04%, which nearly returned to its normal values when compared to the control, while a non-significant increase was detected for SOD enzyme activity (Fig. 1).

3.3. DNA laddering assay

As shown in Fig. 2, a marked DNA laddering pattern was detected in the intoxicated group with TiO_2 NPs. However, the group treated with Tiron showed a notable decrease in the DNA laddering. Intact high-molecular-weight genomic DNA was detected in both the control and Tiron groups.

3.4. The relative expression level of the proapoptotic Bax and antiapoptotic Bcl-2 mRNAs

In the intoxicated group, the mRNA expression level of the proapoptotic gene (Bax) was significantly elevated while the Bcl-2 gene was significantly downregulated. However, Tiron clearly played a protective role against TiO_2 NPs induced apoptosis, as it significantly downregulated the Bax gene and modulated the level of Bcl-2 expression. The Tiron and control groups showed non-significant differences in both mRNA levels (Fig. 3).

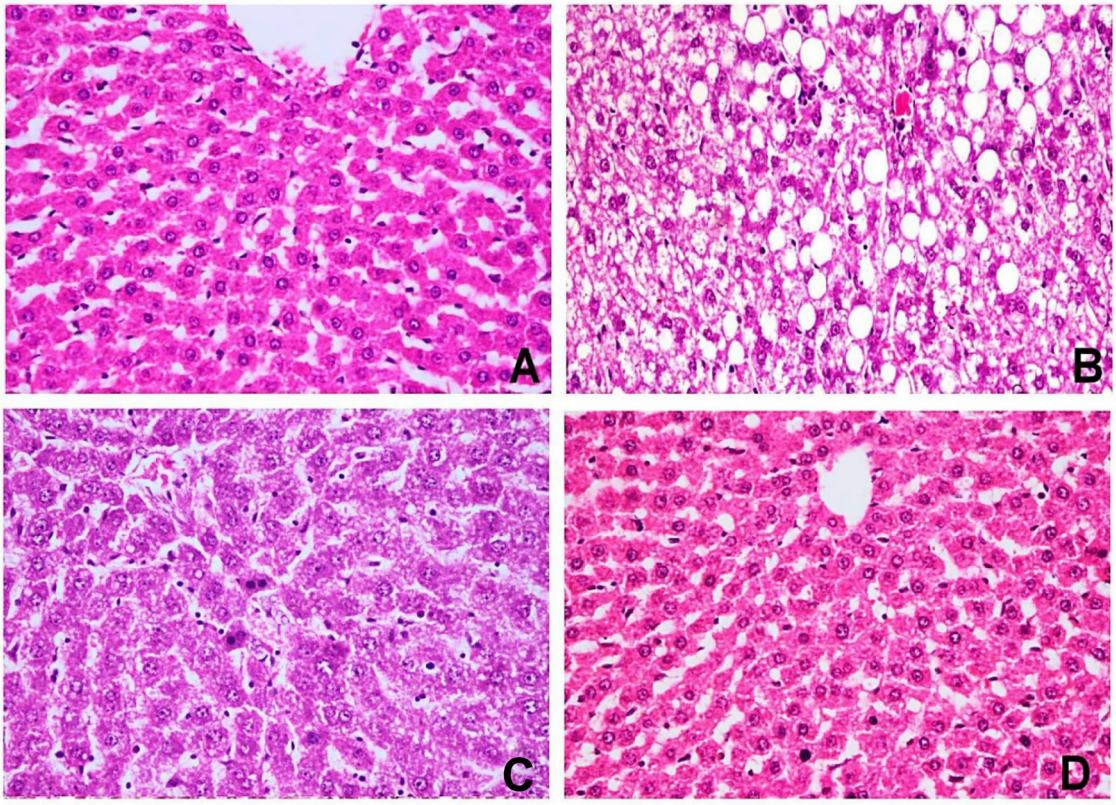

Fig. 4. Representative histopathological changes in the liver of the different experimental groups (H&E X400). A. Control group showing normal histological finding; **B**. TiO2 NPs treated group showing hepatocellular necrosis, macro vascular and micro vascular steatosis and disorganization of the hepatic cords; **C**. TiO2 NPs + Tiron showing sporadic hepatic cell necrosis, mild vacuolation of the hepatocytes and sinusoidal dilation with mild leukocytosis; **D**. Tiron treated group showing normal histological picture (n = 10).

3.5. Histopathological results

Livers from the control and Tiron-treated groups revealed a normal histological picture (Fig. 4A&D). The hepatic tissue of the TiO_2-NPs intoxicated group (Fig. 4B) showed marked histopathological changes in the form of hepatic apoptosis, hepatocellular necrosis, steatosis, sinusoidal dilation with leukocytosis, and distortion and disorganization of the hepatic cords. Meanwhile, the co-administration of Tiron to the intoxicated rats (Fig. 4C) markedly improved the histopathological picture of the liver compared to that of the TiO_2 NPs group. Apoptosis and hepatocellular necrosis was markedly reduced in the liver, with mild to moderate vacuolar degeneration of the hepatocytes.

3.6. Immunohistochemical analysis of Bcl-2 and Bax

Figs. 5 and 6 summarize the results of the immunohistochemical evaluation of Bcl-2 and Bax protein expression in the different experimental groups. Bcl-2 and Bax immunostaining was characteristically cytoplasmic and exhibited a brown colour. The immunopositive areas in the hepatocytes of intoxicated and treated groups were significantly higher than those of the control and the Tiron groups (Fig. 5E). The co-administration of Tiron to the treated group (Fig. 5C) showed a significantly larger Bcl-2 immunopositive area than the TiO_2-NPs-intoxicated group (Fig. 5B). In addition, the immunopositive area of Bax staining was significantly smaller in the treated group (Fig. 6C) than that in the TiO_2-NPs group (Fig. 6B). A non-significant difference in the immunopositive area was detected between the control and the Tiron groups (Fig. 6A&D).

4. Discussion

The present work was accomplished to evaluate the potential therapeutic role of Tiron against hepatic toxicity induced by TiO_2 NPs in male rats. The nano-anatase TiO_2 NPs were administered at an oral dose of 100 mg/kg/day for 60 days, which induced significant increases in serum levels of ALT, AST, ALP as well as a hepatic oxidative stress biomarker (MDA). Meanwhile, administration of TiO_2 NPs significantly reduced GSH concentration and SOD and Gpx enzyme activities. Similarly, TiO_2 NPs induced significant oxidative DNA damage detected by the DNA laddering assay. Moreover, our findings verified the role of TiO_2 NPs in inducing apoptosis by increasing the mRNA expression level of Bax and decreasing that of Bcl-2. Histopathological examination of hepatic tissue reinforced these biochemical and molecular results. Reductions in the internal organ weights are considered sensitive indices of intoxication. The current study reported non-significant changes in the liver weights among different experimental groups. These results may suggest that administration of TiO_2 NPs and/ or Tiron produced non-significant effects on the normal liver weights. The increased level of hepatic enzymes indicated liver injury, as reported by Attia et al. [24]. Serum ALT and AST activities were significantly elevated, which is inferred to be an impairment of hepatic integrity and loss of hepatocyte functions, as they are the main indicators of the efficiency of liver function and are very sensitive to any pathological process of the liver [25]. Meanwhile, the significantly high elevation in serum ALP levels is indicative of the disruption of the liver's excretory functions [26]. A high level of such enzymes indicates destruction of liver cells, which was distinctly recorded in the histopathological results of the intoxicated group. The alterations in liver transaminases levels and serum ALP are consistent with previously reported patterns of TiO_2NPs induced hepatotoxicity [27]. Most of the NPs tend to

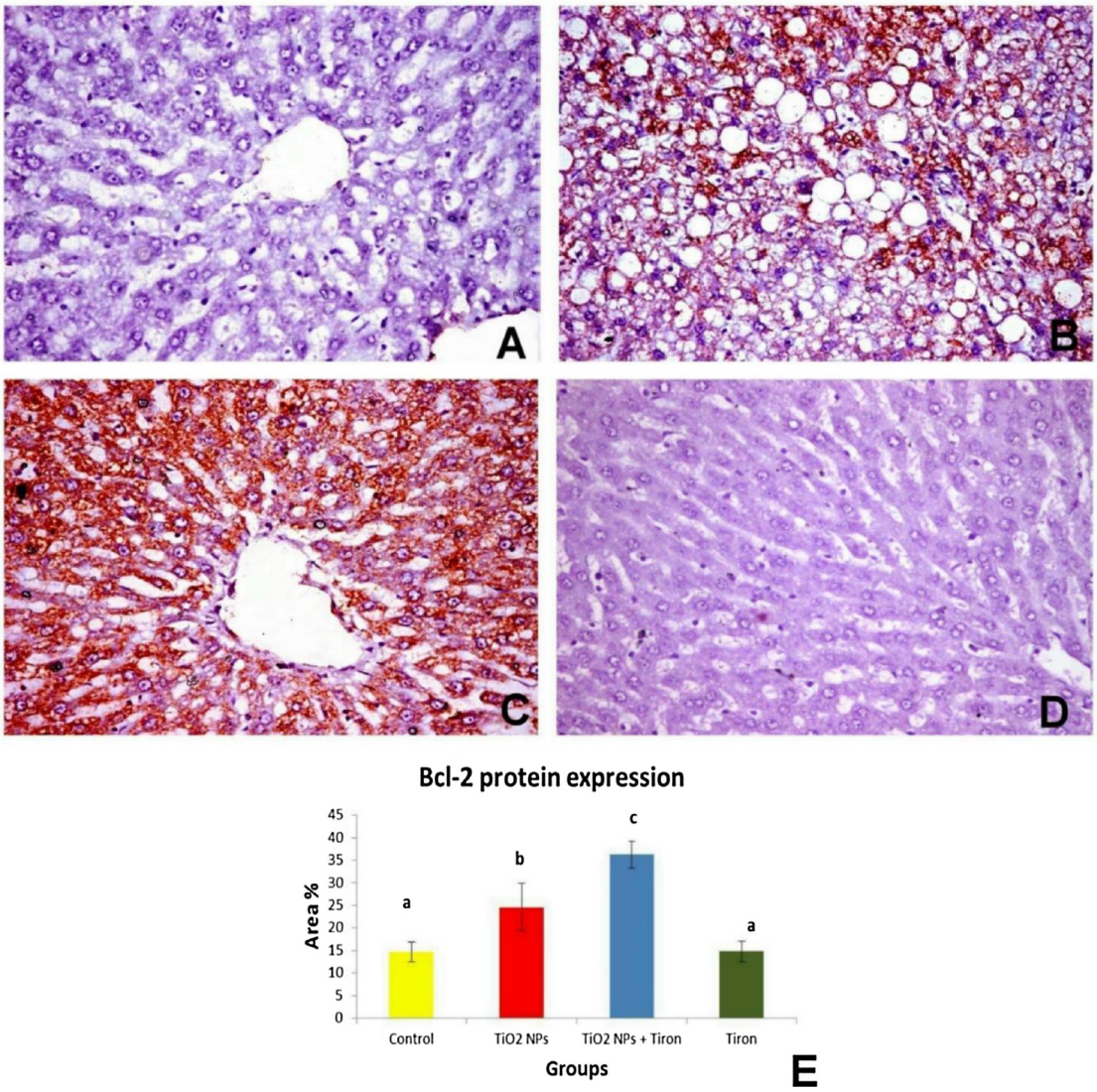

Fig. 5. Representative Bcl-2 immunostaining in the liver of different groups (X400). A. control group; **B**.TiO2 NPs treated group showing moderate immunoreactivity ; **C**.TiO2 NPs + Tiron showing intense immunostaining reaction; **D**. Tiron group; **E**. The bar chart represents positive Bcl − 2 immunostaining reaction expressed as area%. Values with different superscripts are significantly different ($p < 0.05$) (n = 10).

accumulate in liver tissue [28]. Several bi-distribution studies indicated that TiO_2 NPs mainly accumulated in liver tissue and induced a certain oxidative impairment [29,30]. The catalytic properties of TiO_2 NPs as one of the transition metal oxides are well documented to generate ROS [31]. This possible mechanism contributing to a TiO_2-NPs-induced oxidative stress could be described as "ROS-induced ROS release," which includes mitochondrial permeability transition pores in the inner membrane anion channel, inducing an excessive oxidative stress that results in an increase in ROS generation [32]. These results agree with those of El-Ghor et al., [33] who determined that TiO_2 NPs could damage DNA in a mouse brain. The current study evaluated both the constitutive mRNA expression and its corresponding protein outcome, of the studied genes, to figure out the possible difference between both levels [34].The overexpression of Bax and depletion of Bcl-2 at the mRNA and protein levels could be due to increased p53 levels, which resulted in the modulation of the Bax/Bcl-2 ratio[30]. Our findings are in accordance with previous studies wherein TiO_2 has been shown to provoke an apoptotic effect on liver tissue. At the molecular level, TiO_2 NPs significantly activated p38, c-Jun N-terminal kinase, nuclear factor kappa B, Nrf-2 and heme oxygenase-1 expression, which in turn led to an increased production of ROS, as well as lipid, protein and DNA peroxidation [35]. The role of oxidative stress in the mechanism of TiO_2-NP-induced hepatotoxicity has also been reported by Sha et al. [36]. The overproduction of ROS breaks down the balance of the oxidative/antioxidative system, resulting in lipid peroxidation and a reduction in antioxidative enzymes, as detected in our study. Importantly, note that all the cellular alterations induced by TiO_2 NPs observed in this work, including a decrease in antioxidant enzymatic defence and an elevation in MDA, could activate apoptotic pathways. Excessive generation of ROS induces mitochondrial membrane permeability and damages the respiratory chain to trigger the apoptotic process [37]. TiO_2 NPs induce mitochondrial depolarization [38]. In addition, mitochondrial depolarization leads to a decrease in oxygen consumption, NADH levels in mitochondria [39] and depletion of cellular adenosine triphosphate (ATP) [40]. Bcl-2 prevents cell death, whereas Bax appears to accelerate the cell death signal [41]. Bcl-2 is in the outer mitochondrial membrane and promotes cell survival, whereas Bax shows proapoptotic effects, either by interacting with Bcl-2 or by directly interacting with mitochondria [42]. Our results confirmed that inducing apoptosis in the TiO_2NPsintoxicated liver could be related to genomic instability and mitochondrial mediated pathways induced by cellular oxidative stress [43]. Compared with in the control group, there was a

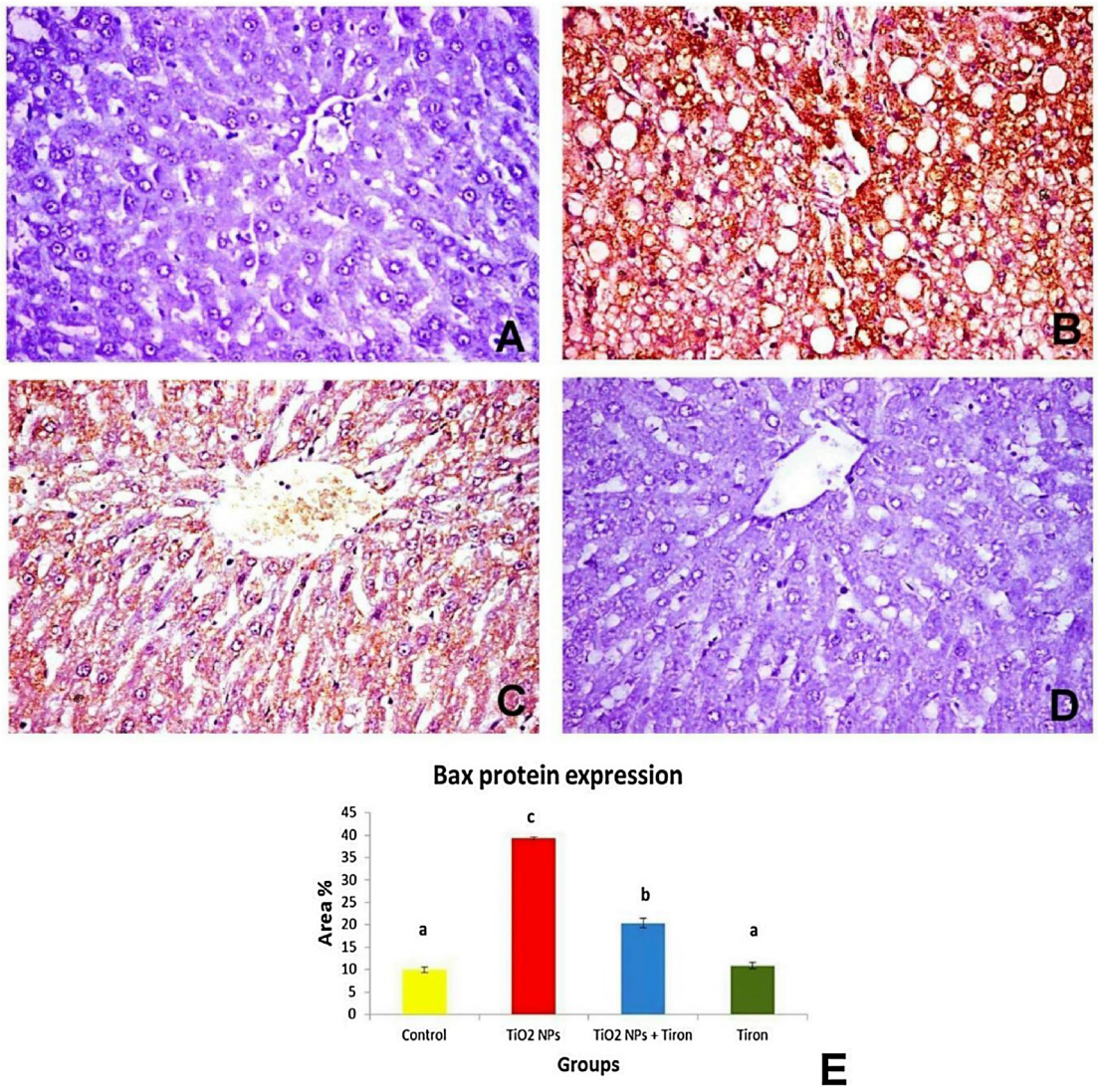

Fig. 6. Representative Bax immunostaining in the liver of different groups (X400). **A**. control group showing very weak immunoreactivity; **B**.TiO2 NPs treated group showing strong cytoplasmic immunoreactivity; **C**.TiO2 NPs + Tiron showing mild immunostaining reaction; **D**. Tiron group treated group showing very weak immunoreactivity; **E**. The bar chart represents Bax immunostaining reaction expressed as area%. Values with different superscripts are significantly different ($p < 0.05$) ($n = 10$).

significant decrease in Bcl-2 content accompanied by an increase in Bax in the TiO_2 NPs group. TiO_2 NPs could be incorporated into cellular membranes and/or endocytosed from the extracellular fluid, fusing with lysosomes and then damaging organelles [44]. The results of our study also indicated that the intense histopathological changes in liver tissues by 10-nm TiO_2 NPs were in line with results reported by Doudi and Setorki [45]. Generally, the impacts of antioxidants have been widely tested for the prevention and treatment of several hepatic injuries. Some studies have revealed that free radical scavengers are extensively beneficial in preventing and treating chronic liver injury [46]. The efficacy of the exogenous therapy of Tiron in accelerating TiO_2 NPs elimination and in reversing hepatic intoxication was discussed in the present study. Tiron at the investigated dose (470 mg/kg/rat) significantly recovered the hepatocyte distortion and improved the hepatic antioxidant content. The stabilization of AST, ALT and ALP levels by therapy with a chelating agent is a clear indication of improvement in the functional status of the liver cells. The ability of Tiron to reduce the serum levels of transaminases and ALP was reported previously [46]. Tiron was reported to be a potent antioxidant that could ameliorate the elevation of superoxide anions and GSH depletion and maintain the activity of SOD [47]. Improvements in hepatic antioxidant content and histopathological examination following Tiron treatment have also been reported [46]. Tiron may have demonstrated such hepatoprotective efficacy either through its antioxidant competence by reducing the generation of free radicals as previously reported [48] or by reducing inflammatory cell infiltration [49]. Tiron is a beneficial protective agent against the pathological effects of oxidative stress, as it can scavenge various radicals. It is an effective antioxidant that can also prevent the inactivation of antioxidant enzymes as SOD and the inhibition of the metal-catalysed peroxidation of DNA or lipids [48–50]. The small size of Tiron facilitates its entrance inside cells and therefore modifies intracellular electron transfer reactions by antioxidant mechanisms via scavenging free radicals [51]. The effective medicinal role of Tiron may be attributed to its diphenolic nature, which forms water-soluble complexes with many metals [50]. Oyewole and Birch-Machin [9] revealed that Tiron drastically decreases mitochondrial and nuclear DNA damage. NF-κB is a central transcription factor that controls transcription of DNA and cytokine production. Overexpression of these factors is responsible for tissue damage. NF-κB is significantly inhibited with Tiron, suggesting the protective influence of Tiron may be through the

signalling pathway of NF-Kb [50], which may be the main reason for the significant reduction in the gene and protein expression of Bax. Interestingly, Tiron chemically has been reported to enhance NF-κB-dependent gene transcription with antiapoptotic effects such as expression of Bcl-2 genes [49]. The antioxidative action of Tiron plays an important role in its hepatoprotective effects against TiO_2-NPs induced hepatotoxicity. Oxidative stress is well known to directly affect various types of cell death including apoptosis and necrosis. Therefore, Tiron may reduce apoptosis and necrosis of hepatocytes induced by TiO_2 NPs by diminishing oxidative stress.

5. Conclusion

In conclusion, the curative effect of Tiron against hepatic damage induced by TiO_2 NPs was assessed through a novel insight. The outcomes of this study plainly confirmed that Tiron attenuated the progression of liver apoptosis through down regulation of Bax gene expression and decreased its corresponding protein synthesis with up-regulation of Bcl-2 gene expression and protein, decreasing the DNA laddering percentage, and restoring pathological damage in the treated group compared to that in the TiO_2-NPs-intoxicated rats. Moreover, it is reasonable to assume that Tiron potency is potentially mediated through radical scavenging and a metal-chelating antioxidant effect.

Disclosure

The authors report no conflicts of interest in this work.

Authors' contributions

Prof Dr. Ashraf Morgan managed the study design. Dr. Marwa Ibrahim performed the molecular biology studies and drafted the manuscript. Dr. Hanan Abdelsalam performed the biochemical parameters and helped to draft the manuscript. Dr Mona Khamees evaluated the molecular and biochemical parameters and drafted the manuscript. Dr Reham Mohamad performed the pathological and immunohistochemical analysis. All authors read, revised and approved the final manuscript.

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