Biological Trace Element Research (2020) 196:579–589
https://doi.org/10.1007/s12011-019-01955-5

Grape Seed Proanthocyanidin Extract Mitigates Titanium Dioxide Nanoparticle (TiO_2-NPs)–Induced Hepatotoxicity Through TLR-4/NF-κB Signaling Pathway

Eman T. Mohammed[1] · Ghada M. Safwat[1]

Received: 28 July 2019 / Accepted: 24 October 2019 / Published online: 7 November 2019

Abstract
With the progress of nanotechnology, the adverse effects of nanoscale materials are receiving much attention. Inhibition of toll-like receptor 4 (TLR-4)/nuclear factor kappa B (NF-κB) signaling is a hallmark for downregulating the expression of many inflammatory genes implicated in oxidative stress. Therefore, the present study aimed to demonstrate the influence of grape seed proanthocyanidin extract (GSE) on the hepatic TLR-4/ NF-κB signaling pathway in TiO_2-NP-induced liver damage in rats. Forty male Albino rats were divided into 4 groups (n = 10): G1 was used as a control, G2 received TiO_2-NPs (500 mg/kg/day orally) from the 17th to 30th day (acute toxicity), G3 received GSE (75 mg/kg/day orally) for 30 days, and G4 pre- and co-treated with GSE (for 30 days) and TiO_2-NPs (from the 17th to 30th day), with the aforementioned doses. TiO_2-NPs induced severe hepatic injury that was indicated by biochemical alterations in serum liver markers (acetylcholinesterase, ALT, ALP, total proteins, albumin, and direct bilirubin), oxidative stress indicators (MDA, GSH, and catalase), and histopathological alterations as well. Moreover, TiO_2-NPs triggered an inflammatory response via the upregulation of TLR-4, NF-κB, NIK, and TNF-α mRNA expressions. Pre- and co-treatments with GSE alleviated the detrimental effects of TiO_2-NPs which were enforced by the histopathological improvements. These results indicated that GSE effectively protected against TiO_2-NP-induced hepatotoxicity via the inhibition of TLR-4/NF-κB signaling and hence suppressed the production of pro inflammatory cytokines such as TNF-α and improved the antioxidant status of the rats.

Keywords Grape seed proanthocyanidin · TiO_2 nanoparticles · TLR-4/ NF-κB pathway · Liver · Antioxidants

Introduction

Nanomaterials have been widely applied to various fields including food, drug and textile industries, agriculture [1, 2], eco-friendly fabrication of biogenic nanoparticles [3–6], and cancer nanotherapy [7]. Recently, titanium dioxide nanoparticles (TiO_2-NPs) are used widely in industry and medicine because of their high stability and anticorrosive and photocatalytic properties [8]. Commercially, TiO_2-NPs are used in paints, coatings, plastics, papers, inks, pharmaceuticals, food products, cosmetics, toothpaste, tableted drugs, and sunscreens [9]. It can even be used as a pigment for whitening skim milk and brightening foods [10]. Therefore, TiO_2-NPs come into close contact with humans. According to the Federal Regulations of the US Government, the permissible limit of TiO_2-NPs in food products does not exceed 1% by weight [11]. Earlier studies have revealed that TiO_2-NPs are more toxic than fine particles [12]. Because of their smaller sizes and larger surface area, nanoparticles are easily taken up by cells and can induce pathological changes. Oral exposure mainly occurs through food products containing TiO_2-NP additives. TiO_2-NPs can be absorbed through the gastrointestinal tract into the systemic circulation and then accumulated in the liver, kidneys, spleen, and brain. The accumulation of TiO_2-NPs in the tissue could induce inflammatory injuries [13]. Acute exposure to TiO_2-NPs causes neurotoxicity [14], hepatotoxicity, nephrotoxicity, myocardial damage, spleen lesions, and inflammation in the lung and liver in mice and rats [11, 15, 16]. Signs of toxicity, including loss of appetite, passive behavior, and tremors exist after intraperitoneal injection of mice by TiO_2-NPs, [17]. Chronic exposure to TiO_2-NPs resulted in growth arrest, a decrease in the liver weight, and histopathological changes in the

✉ Eman T. Mohammed
eman.ibrahim@vet.bsu.edu.eg

Ghada M. Safwat
ghada.mohamed1@vet.bsu.edu.eg

[1] Department of Biochemistry, Faculty of Veterinary Medicine, Beni-Suef University, Beni Suef, Egypt

gills in zebrafish [18]. TiO_2-NPs may cause toxicity by several mechanisms including genotoxicity [15, 19] and oxidative stress and/or inflammatory responses [13, 20, 21] that induce inflammation and cell apoptosis [22].

Toll-like receptor 4 (TLR-4), a member of the toll-like receptor family, is a transmembrane protein encoded by the TLR4 gene. It is one of the key effectors in innate immunity. TLRs are produced by macrophage activation [23]. Its stimulation leads to activation of an intracellular nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) signaling pathway which facilitates the expression of inflammatory cytokines [24]. As previously indicated, TiO_2-NP induces the activation of NF-κB signaling in lung [25] and mouse liver [26] and kidney [27]. Known inducers of NF-κB activity are highly variable and include reactive oxygen species (ROS), tumor necrosis factor-alpha (TNF-α), interleukin 1-beta (IL-1β), bacterial lipopolysaccharides (LPS), isoproterenol, cocaine, and ionizing radiation [28]. However, another literature indicated that NF-κB is not involved in TiO_2-NP-induced inflammation [29].

Previous studies suggested the involvement of oxidative stress as one of the main mechanisms of TiO_2-NP -induced toxicity [30]; therefore, the oxidative stress must be neutralized by antioxidants. Antioxidants serve as potent scavengers for free radicals and prevent the occurrence of disease [31]. Recently, the use of medicinal plants for the treatment of toxicity has been widely reported due to their protective effects [32, 33].

Grape seed extract (GSE) (*Vitis vinifera*) is one of the most powerful antioxidants, which contains high levels of flavonoids, vitamin C, and vitamin E [34]. GSE protects cells by regulating cell oxidative damage, reducing organ injury, improving the balance between oxidants and antioxidants, and reducing the release of inflammatory mediators [35]. In addition, GSE has been reported to exert anti-carcinogenic effects [36]. About 60% to 70% of grape polyphenols are found in the seeds. These polyphenols are commonly known as proanthocyanidins. Other important polyphenols in grape seed include gallic acid, catechin, epicatechin, gallocatechin, and epigallocatechin [37].

Inhibition of TLR-4/ NF-κB signaling is a hallmark for downregulating the expression of many inflammatory genes implicated in oxidative stress. Despite the antioxidant activities of grape seed being well documented, the studies concerning its impact on TLR-4/ NF-κB signaling in TiO_2-NPs toxicity are limited. In this investigation, the pre- and co-treatments with the antioxidant GSE may cut down the liability to TiO_2-NPs toxicity. Therefore, we aimed to explore the hepatoprotective role of grape seed proanthocyanidins extract focusing on the hepatic TLR-4/NF-κB signaling pathway following TiO_2-NP-induced hepatotoxicity.

Materials and Methods

Chemicals

TiO_2 particles were purchased from Sigma-Aldrich, Egypt. Reduced glutathione (GSH), catalase, and malondialdehyde (MDA) commercial kits were purchased from Biodiagnostic Company for research kits, Egypt. Acetylcholinesterase, alanine aminotransferase (ALT), alkaline phosphatase (ALP), total proteins, albumin, and bilirubin kits were supplied from Greiner Diagnostic GmbH-Bahlingen, Germany. Other non-mentioned chemicals used in the present experiment were obtained from Sigma, USA.

TiO_2-NP Preparation and Characterization

TiO_2-NPs were prepared by high-energy ball mill (HEBM) technique according to the method that was described by Gusev and Kurlov [38]. The characterization of TiO_2-NPs was performed by a high-resolution TEM electron microscope (model JEM-2100, JEOL Ltd., Tokyo, Japan) to measure the shape and size of TiO_2-NPs. Size distribution and zeta potential of TiO_2-NPs in solution were measured by a Zetasizer Ver. 7.11 (serial number MAL1121994) (Malvern Instruments Ltd, Malvern, Worcestershire, UK).

Form and Preparation of Grape Seed Extract

Grape seed extract was provided in capsule form (Noxy life®) produced by The Arab Company for Gelatin and Pharmaceutical Products, under license of Nulife International USA. The grape seed extract formula provides a blend of standardized proanthocyanidins (95%) found in grape seed. Grape seed proanthocyanidins are a mixture of several polyphenols and flavones, as previously reported [39]. GSE powder was milled using a high-energy planetary ball mill (WBB-6 Gruendler Pulverizing Co., St. Louis, MO) and sieved using a 250-μm sieve to get ultra-fine powder (micronized form). It was administered as 75 mg/kg bw [40] after suspending in distilled water.

Experimental Animals

A total of 40 adult male Albino rats (*Rattus norvegicus*) weighing 150–180 g were obtained from Helwan farm of laboratory animals, Cairo, Egypt. Rats were kept under observation for 1 week before the onset of the experiment to be acclimatized and then housed individually in metal cages at room temperature (25 ± 2 °C), humidity (70%) under 12-h light–dark cycle. Water and diet were allowed to rats in a free manner. All experimental procedures were in accordance with the guidelines of local Animal Care and Use Committee established at the Beni-Suef University (BSU-IACUC). The

study was performed after obtaining an approval number (018-8) to conduct the animal experiments.

Experimental Design

The rats were randomly divided into four equal groups (10 rats each) and treated as follows:

Control group The rats were given distilled water for 30 days orally by gastric tube.

TiO_2-NPs group The rats received (500 mg/kg bw, 1/10 LD_{50}) of TiO_2-NPs [21] once daily for 14 days (17th–30th day) by gastric tube (acute toxicity). The nanopowder was suspended in distilled water at a concentration of 50 mg/ml/100 g rat, then dispersed by ultrasonic vibration for 15 min (LD_{50} is 5000 mg/kg bw in rats and mice after oral administration [41]).

GSE group Rats were given GSE (75 mg/kg bw) [40] for 30 days orally by gastric tube after suspending in distilled water.

GSE + TiO_2-NPs group Rats were pre- and co-treated with GSE (75 mg/kg bw) for 30 days interrupted by TiO_2-NPs administration (500 mg/kg bw) at the 17th–30th day orally by gastric tube.

The clinical signs and physical activity were observed during the period of the experiment.

Sampling and Tissue Preparation

Twenty-four hours after the last doses, blood samples were withdrawn via orbital sinus and allowed to clot for 30 min at room temperature, then centrifuged at 2000×*g* for 10 min at 4 °C. Serum was separated and stored at – 20 °C to be used for biochemical analysis. Rats were sacrificed and 1 g of liver tissue was used for the preparation of liver tissue homogenates using homogenizer (Teflon Homogenizer, India), for measurement of antioxidant and oxidative stress indices such as malondialdehyde (MDA), reduced glutathione (GSH), and catalase activity. Also specimens from the liver were fixed in 10% buffered formalin for histopathological examination. Another portion of liver was placed immediately in RNase inhibitor at − 80 °C for molecular biological investigations.

Biochemical Assays

Measurement of Liver Function Markers

Serum samples were used for kinetic determinations of the enzymatic activities of acetylcholinesterase, ALT, and ALP according to the methods described by Kovarik et al. [42], Zilva and Pannall [43], and Tietz et al. [44], respectively. Serum direct bilirubin was spectrophotometrically estimated by the direct diazo reaction [45], serum total proteins were estimated by following the Biuret method [46], and serum albumin was measured by following the bromocresol green method [47].

Measurement of Liver Oxidative/Antioxidant Indices

The liver tissue homogenates were used for the measuring of MDA, GSH concentrations, and catalase activity. MDA was determined as thiobarbituric acid reactive substances using the method described by Buege and Aust [48]. GSH was estimated according to the method of Beutler [49] based on the reduction of 5,5-dithiobis-2-nitrobenzoic acid (DTNB) by glutathione. Catalase activity was measured according to the UV assay method described by Aebi [50]. Hitachi spectrophotometry, model U – 2000 (Hitachi Ltd. Tokyo, Japan), was used for measuring all chemical reactions.

Preparation of Histological Sections

Liver samples were fixed in 10% buffered formalin solution for 48 h. Then they were processed (washed by water, dehydrated in graduated ethyl alcohol, cleared in xylene, and embedded in paraffin wax at 70 °C) according to the method described by Bancroft and Gamble [51]. Five-micron tissue thickness was mounted on clean glass slides and stained by hematoxylin and eosin. Each section was examined by a light microscope (B1 series, Motic, Xiamen, China).

Determination of TLR-4, NF-κB, NIK, and TNF-α mRNA Expressions by Real-Time Polymerase Chain Reaction

Total RNA was isolated from the liver tissue homogenates using RNeasy Purification Reagent (Qiagen, Valencia, CA) according to the manufacturer's instruction. The concentration of RNA was measured using a UV spectrophotometer. The extracted RNA was reverse transcribed into cDNA using high-capacity cDNA reverse transcription kit (#K1621, Fermentas, USA). Real-time qPCR amplification and analysis were performed using an Applied Biosystem with software version 3.1 (StepOne™, USA). The primers used in the amplification are shown in Table 1 and were designed by Gene Runner Software (Hasting Software, Inc., Hasting, NY) from RNA sequences from the gene bank based on published rat sequences. The reaction contained SYBR Green Master Mix (Applied Biosystems). Data from real-time assays were calculated using Applied Biosystem software. The results were expressed as a fold change of the relative expression levels of target genes from the control group using the $2^{-\Delta\Delta Ct}$ method [52].

Table 1 The primer sequence of the studied genes

Gene	Forward primer (5′————3′)	Reverse primer (′5————3′)
TLR-4	5′-GTTCTTCTCCTGCCTGACAC-3′	5′-TCCAGCCACTGAAGTTCTGA-3′
NF-κB	5′-CATTGAGGTGTATTTCACGG-3	5′-GGCAAGTGGCCATTGTGTTC-3
NIK	5′-TCACCAAAGACCCACCTCACCG-3	5′-GGACCGCATTCAAGTCATAGTCCC-3
TNF-α	5′-GCG ACG TGG AAC TGG CAG AAG-3′	5′-GGT ACA ACC CAT CGG CTG GCA-3′
B-actin	5′- GGTCGGTGTGAACGGATTTGG-3	5′-ATGTAGGCCATGAGGTCCACC-3

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison post-test. Graph Pad Instat software (version 3, ISS-Rome, Italy) was used. Significance was considered at $P \leq 0.05$.

Results

Characterization of TiO_2-NPs

TEM revealed spherical shaped particles with a homogeneous nanometric size distribution (range 63–142 nm) (Fig. 1a). Zeta potential of TiO_2NPs in a neutral solution was − 19.1 mV; *Z*-average diameter revealed a particle size of 338.7 nm (Fig. 1b) and poly dispersion index of 0.246. The obtained information indicates TiO_2 NPs suspension has a good dispersion stability.

Effect of TiO_2-NPs and GSE on Clinical Signs of Toxicity in Rats

No deaths were detected in the TiO_2-NP treated group. Loss of appetite and decreased physical activities were observed in the TiO_2-NP treated group. These symptoms were not clearly observed in the GSE + TiO_2-NP treated group.

Effect of TiO_2-NPs and GSE on Liver Function

Liver function biomarkers were presented in Tables 2 and 3 that showed a significant increase ($P \leq 0.05$) in the serum activity of ALT and ALP enzymes and direct bilirubin levels accompanied with a significant decrease in the serum acetylcholinesterase activity, total proteins, and albumin levels in the TiO_2-NP group in comparison with the control one. Additionally, pre- and co-treatments of TiO_2-NP-treated rats with GSE could markedly improve the liver function as indicated by a restoration of these biomarkers toward the normal.

Effect of TiO_2-NPs and GSE on the Antioxidant Status

Data presented in Table 4 showed a significant increase ($P \leq 0.05$) in MDA concentration and a significant decrease in GSH concentration and catalase activity in the TiO_2-NP group compared with those in the control group, indicating an enhanced lipid peroxidation in liver tissue following TiO_2-NP exposure. Pre- and co-treatments of TiO_2-NP-exposed rats with GSE could ameliorate these changes.

Effect of TiO_2-NPs and GSE on Histopathological Structure of Liver

Figure 2(B1–B3) showed the histopathological findings of the liver tissue slices from the TiO_2-NP-treated rats. It revealed a congested and dilated central vein, vacuolated and degenerated hepatocytes with a huge amount of Kupffer cells (Fig. 2B1). Another section in the same group showed severely congested blood vessels with perivascular leucocytes infiltration as well as endothelial hyperplasia (Fig. 2B2) and leucocytic scattered aggregates (Fig. 2B3). The liver section of the rats' liver pre- and co-treated with GSE and TiO_2-NPs showed an improvement of the liver structure indicated by normal blood sinusoids, normal central vein and few degenerated hepatocytes (Fig. 2D). GSE-treated rats showed normal structure of the liver (Fig. 2C) which indicates the harmless effect of GSE on hepatic tissue.

Effect of TiO_2-NPs and GSE on Hepatic Gene Expressions

Table 5 showed a significant ($P \leq 0.05$) upregulation of hepatic TLR-4, NF-κB, NIK, and TNF-α gene relative expression levels in the TiO_2-NP group in comparison with the control one. Meanwhile, the pre- and co-treatments with GSE succeeded to induce a marked improvement in these parameters.

Discussion

Several studies have demonstrated that TiO_2-NPs have an adverse effect on the liver because the liver is the major accumulation site for most nanoparticles including TiO_2 [15, 53]. Because of the high catalytic properties, TiO_2-NP exposure can generate ROS [54, 55] and oxidative stress which could in turn initiate lipid peroxidation and DNA damage [30]. The overproduction of ROS is thought to play a significant role in many of the observed

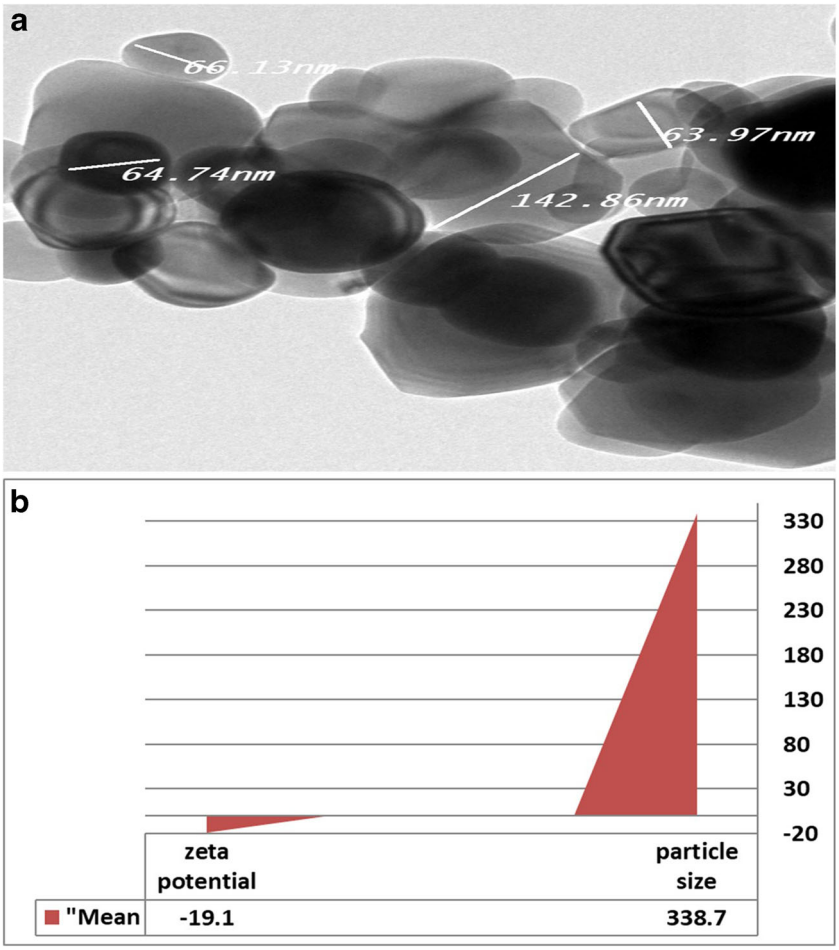

Fig. 1 **a** TEM images of TiO_2-NPs showing the shape and size of TiO_2-NPs, which appear spherical particles with a size range of 63–142 nm. **b** Zeta potential and size distribution of TiO_2-NPs revealing an apparent zeta potential of − 19.1 mV and a *Z*-average diameter of 338.7 nm in a neutral solution

biological responses to TiO_2-NPs [56]. The accumulation of these nanoparticles in liver causing oxidative damages and liver toxicity was demonstrated by disturbance of the liver function indices. In this study, TiO2-NPs induced liver damage, as confirmed by the increased serum ALT and ALP activities (Table 2), and direct bilirubin levels (Table 3). In accordance with Wang et al. [11] and Rizk et al. [57], TiO_2-NPs increased the activity of ALT. Furthermore, Liu et al. [58] and Morgan et al. [59] found that TiO_2-NPs could increase the activities of ALP and ALT. ALT is a cytosolic enzyme mainly located in the hepatocytes. The level of ALT in serum increases as a result of releasing this cellular enzyme into plasma by insult-induced hepatic damage. ALP is present in many tissues, including bone, intestine, kidney, liver, placenta, and white blood cells [60]**.** Damage to these tissues causes the release of ALP into the bloodstream. Bilirubin, a major breakdown product of hemoglobin, rises when there is liver damage [61]**.** Bilirubin synthesis is regulated by the heme oxygenase-1 which is rapidly induced by oxidative stress and inflammatory cytokines [62]**.** On the other hand, the active excretion of direct bilirubin occurs at the canalicular membrane, by means of cytoplasmic binding transporter proteins [63]**.** So, the damaged hepatocyte observed in the current study might be less able to produce the transporter proteins required for transporting of direct bilirubin to the gall bladder and therefore, it was returned

Table 2 Changes in serum acetyl cholinesterase, ALT, and ALP activities (U/L) in different groups

	Acetyl cholinesterase (U/L)	ALT (U/L)	ALP (U/L)
Control	4580 ± 105.2^{a}	42.19 ± 1.816^{a}	40.92 ± 2.25^{a}
TiO_2 –NPs	3647 ± 113.5^{b}	68.04 ± 4.496^{b}	59.45 ± 4.49^{b}
GSE	4563 ± 131.5^{a}	43.29 ± 2.009^{a}	41.88 ± 3.45^{a}
GSE+ TiO_2 –NPs	4174 ± 310.9^{ab}	46.74 ± 0.965^{a}	44.31 ± 2.18^{a}

Values are expressed as mean ± SEM (n = 10). Means with different letters (a, b) in a column are significantly different at level $P < 0.05$. *TiO_2-NPs*, titanium dioxide nanoparticles; *GSE*, grape seed proanthocyanidin extract; *ALT*, alanine aminotransferase; *ALP*, alkaline phosphatase

Table 3 Changes in serum total proteins, albumin, and direct bilirubin concentrations in different groups

	Total proteins (mg/dl)	Albumin (mg/dl)	Direct bilirubin (mg/dl)
Control	8.33 ± 0.22^{a}	3.60 ± 0.16^{a}	0.77 ± 0.09^{a}
TiO_2 -NPs	6.86 ± 0.03^{b}	2.77 ± 0.13^{b}	2.60 ± 0.29^{b}
GSE	8.22 ± 0.32^{a}	3.55 ± 0.14^{a}	0.75 ± 0.13^{a}
GSE+ TiO_2 -NPs	7.87 ± 0.21^{a}	3.16 ± 0.11^{ab}	1.08 ± 0.18^{a}

Values are expressed as mean ± SEM (n = 10). Means with different letters (a, b) in a column are significantly different at level $P < 0.05$. *TiO_2-NPs*, titanium dioxide nanoparticles; *GSE*, grape seed proanthocyanidin extract

back to blood elevating its level in serum. Accordingly, the present study showed that the serum direct bilirubin level was elevated in TiO_2-NP-treated rats. Meanwhile, the elevated levels of direct or conjugated bilirubin might be due to the decreased secretion from the liver or obstruction of the bile ducts evidenced by the increased ALP activity. Our findings indicated that hepatocyte damage altered their transport function and membrane permeability as well as leakage of ALT and ALP enzymes from the injured cells. Tests of the biosynthetic function of the liver include serum total proteins, albumin, and acetylcholinesterase which are synthesized in the liver and transported into the circulation [64, 65]. Acetylcholinesterase is present in serum and liver, which hydrolyzes blood-circulating acetylcholine [66] and regulates cell growth and cell adhesion [67]. Serum acetylcholinesterase activity is reduced in liver dysfunction due to reduced synthesis [68]. The impaired activity of the enzyme following TiO_2-NPs exposure indicates organ dysfunction [69]. In accordance with the results of Liu et al. [58], the serum acetylcholinesterase activity, total proteins, and albumin concentrations significantly decreased after exposure to TiO_2-NPs (Tables 2 and 3). These findings may be explained as a reduction in the synthetic function of the damaged liver induced by TiO_2-NP exposure.

The increased level of ROS triggers a cascade of reactions including lipid peroxidation, development of a series of inflammatory responses and apoptosis [70, 71]. In the current study, TiO_2-NPs successfully enhanced lipid peroxidation in liver tissue which is indicated by the increased MDA and the decreased GSH concentrations. These findings were in accordance with those of Morgan et al. [59] and Abdou et al. [72]. GSH is the major tripeptide non-enzymatic antioxidant present in the liver. Rizk et al. [57] reported that a decrease in GSH level might have been due to increased scavenging of ROS that were produced as a result of hepatotoxicity. Catalase is one of the important enzymes in the supportive team of defense against ROS. The inhibition of hepatic catalase activity reported in this study may be attributed to the increased generation of free radicals. Durairaj et al. [73] reported that the reduction in the activity of catalase may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (H_2O_2).

In the current study, TiO2-NP-induced hepatotoxicity is indicated by the disrupted tissue function as well as the observed pathological changes. The histopathological examination of the representative sections of the liver showed that treatment with TiO_2-NPs caused liver damage including dilation and congestion of central vein, which was accompanied by vacuolated and degenerated hepatocytes, more Kupffer cells, perivascular leucocytic infiltration, and endothelial hyperplasia (Fig. 2B1–B3). These pathological findings come in agreement with the findings of Wang et al. [11].

Oxidative stress triggered the pro-inflammatory signaling cascades in the liver as indicated in the current study by the significant upregulation of mRNA expression levels of TLR-4, NF-κB, NIK, and the subsequent increased TNF-α expression levels (Table 5) following TiO_2-NPs exposure. TLRs receptors are expected to contribute to the molecular interactions between NPs and cells. TLRs are transmembrane proteins that include both an extracellular domain (responsible for ligand recognition) and a cytoplasmic domain (required for initiating signaling) [74]. TLRs recognize a wide range of foreign materials including NPs, lipopolysaccharide as well as ROS, and reactive nitrogen species [75]. TiO_2 -NPs induced ROS production and simultaneous activation of TLR-4 [76]. On the other hand, the activation of TLR-4 enhances ROS overproduction. The binding of NPs to TLR-4

Table 4 Changes in MDA, GSH concentrations, and catalase activity in the liver of rats in different groups

	MDA (n mol/g tissue)	GSH (mg/g tissue)	Catalase (U/g tissue)
Control	30.75 ± 1.59^{a}	26.73 ± 0.82^{a}	0.47 ± 0.03^{a}
TiO_2 –NPs	47.22 ± 2.78^{b}	21.69 ± 1.34^{b}	0.19 ± 0.02^{b}
GSE	31.29 ± 2.84^{a}	24.81 ± 0.09^{a}	0.47 ± 0.04^{a}
GSE+ TiO_2 –NPs	40.38 ± 1.75^{b}	25.00 ± 0.18^{a}	0.36 ± 0.03^{a}

Values are expressed as mean ± SEM (n = 10). Means with different letters (a, b) in a column are significantly different at level $P < 0.05$. *TiO_2-NPs*, titanium dioxide nanoparticles; *GSE*, grape seed proanthocyanidin extract; *MDA*, malondialdehyde; *GSH*, reduced glutathione

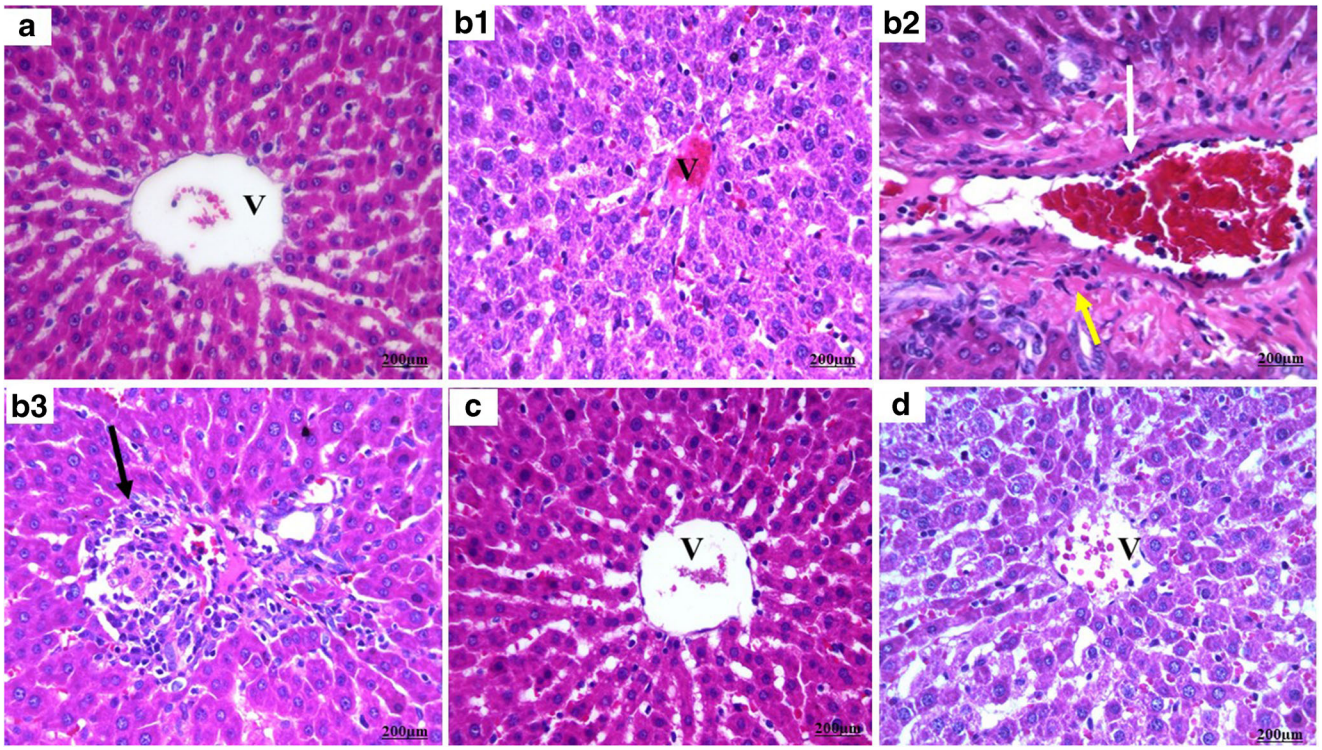

Fig. 2 Photomicrograph of liver in adult albino rats (H&E stain). A: Control group shows normal liver architecture with central vein (v), normal hepatocytes, blood sinusoids, and Kupffer cell × 100. B1–B3: TiO_2NP group: B1 shows dilated and congested central vein, vacuolated and degenerated hepatocytes. Note the huge amount of Kupffer cells × 100. B2 shows severely congested blood vessels with perivascular leucocytic infiltration (yellow arrow) as well as endothelial hyperplasia (white arrow). B3 shows leucocytic scattered aggregates (black arrow) × 100. C: GSE group shows normal hepatocytes, blood sinusoids, and central vein (V) × 100. D: GSE + TiO_2NPs group shows improved liver architecture, normal blood sinusoids, and central vein (V). Note the few degenerated hepatocytes and numerous Kupffer cells × 100

at the cell membrane would result in their uptake and the subsequent activation of NF-κB [77]. NF-κB is a nuclear transcription factor that controls the gene expression of a large number of inflammatory cytokines that are critical for the regulation of apoptosis, cancer, inflammation, and various tissue injuries [78]. NF-κB also regulates many aspects of innate and adaptive immune responses [79]. The various stimuli like stress, cytokines, and ROS can activate NF-κB through two major signaling pathways, the canonical and non-canonical (or alternative) pathways, both being important for regulating immune and inflammatory responses [80]. A NF-κB-inducing kinase (NIK) is a kinase that activates the canonical and non-canonical NF-κB pathways [81]. NIK phosphorylates and activates IκB kinase complex (IKKα) homodimers [82]. IKKα phosphorylates IkB inhibitory proteins called inhibitor of kappa B (IκBs), leading to their degradation in the proteasome and the subsequent nuclear translocation of canonical [83] and non-canonical [84] NF-κB. In the nucleus, NF-κB attaches to a specific DNA response element and thus triggers the transcription of pro-inflammatory cytokines such as TNF-α and IL-1β [85]. TNF-α plays a central role in the development of acute hepatic failure after severe trauma and sepsis by directly or indirectly inducing hepatocyte necrosis rather than apoptosis [86]. It is well known that ROS stimulate TNF-α which is a NF-κB-dependent gene; on the other hand, TNF-α is also a strong inducer for NF-κB [87]. In this issue, results of our study coincide the results of the previous studies that

Table 5 Changes in liver TLR-4, NF-κB, NIK, and TNF-α gene relative expression levels in different groups

	TLR-4	NF-κB	NIK	TNF-α
Control	1.03 ± 0.020^a	1.01 ± 0.007^a	1.01 ± 0.013^a	1.03 ± 0.015^a
TiO_2-NPs	18.4 ± 1.53^b	13.9 ± 0.513^b	14.9 ± 0.644^b	10.9 ± 0.754^b
GSE	1.07 ± 0.052^a	1.03 ± 0.020^a	1.12 ± 0.092^a	1.08 ± 0.060^a
GSE + TiO_2-NPs	8.10 ± 0.361^c	2.63 ± 0.237^c	5.42 ± 0.296^c	3.53 ± 0.240^c

Values are expressed as mean ± SEM ($n = 10$). Means with different letters (a, b, c) in a column are significantly different at level $P < 0.05$. *TiO_2-NPs*, titanium dioxide nanoparticles; *GSE*, grape seed proanthocyanidin extract; *TLR-4*, toll-like receptor 4; *NF-κB*, nuclear factor kappa-light-chain enhancer of activated B cells; *NIK*, NF-κB-inducing kinase; *TNF-α*, tumor necrosis factor alpha

suggested the signaling pathway of liver inflammation and damage after exposure to TiO_2-NPs might occur via activation of TLR-4, NIK, NF-κB, and TNF-α in hepatic tissues which might directly lead to a series of inflammatory responses and hepatic damage.

Many natural products that have antioxidant and anti-inflammatory activities can inhibit NF-κB activation [88]. So the current study aimed to investigate the mechanistic pathway of GSE as an antioxidant agent on TiO_2-NP-induced hepatotoxicity. Our results have demonstrated the efficacy of GSE in maintaining the normal function of liver by restoring the altered liver markers (serum acetyl cholinesterase, ALT, ALP, total proteins, albumin, and direct bilirubin) (Tables 2 and 3). These findings were previously reported by Shin and Moon [89] in dimethyl nitrosamine-induced liver fibrosis in rats. This improvement in liver function may be attributed to the antioxidant properties of polyphenols in GSE that can reduce oxidative stress, maintain cell membrane integrity and restore the hepatocytes function.

Pre- and co-treatments with GSE displayed good antioxidant effects against TiO_2-NPs -induced oxidative damage in liver as indicated by the decreased MDA and the increased GSH concentrations and catalase activity (Table 4). These findings were in agreement with the results of Sharma et al. [90] who reported increased levels of GSH and catalase activity following GSE treatment in UV-exposed mice and the results of Li et al. [91] who found that GSE administration markedly suppressed lipid peroxidation in thioacetamide-induced hepatic fibrosis in mice. GSE showed good antioxidant effects. Phenolic compounds and flavonoids are the major phytochemicals present in the GSE [34, 92]. The phenolic group of polyphenols accepts an electron and forming a stable phenoxyl structure that intersects the continuous oxidation in the cell and prevents the formation of free radicals. So, GSE protects cells by reducing the oxidative damage and the release of inflammatory mediators [35]. Therefore, pre- and co-treatments with GSE restored the normal hepatic architecture with only mild pathological alterations (Fig. 2D) and these findings confirmed the protective effect of GSE against TiO_2-NP-induced hepatic damage.

TLR-4 and NF-κB are critical signaling mediators in inflammatory response; therefore, inhibition of TLR-4/NF-κB signaling with antioxidants will alleviate the inflammatory response and prevent cell death [93].

The novel results of this study are the inhibition of TLR-4/NF-κB signaling pathway by GSE indicated by the significant reductions in the mRNA expression levels of TLR-4, NF-κB, NIK, and TNF-α in the hepatic tissues obtained from GSE+ TiO_2-NP-treated rats (Table 5). In this respect, Sharma et al. [90] and Mantena and Katiyar [94] reported the inhibitory effect of GSE proanthocyanidins on UV-induced oxidative stress and NF-κB signaling pathway in normal human epidermal keratinocytes as well as in SKH-1 hairless mice. Chiefly, the efficacy of flavonoid extractions is extensively studied. Flavonoids contribute to the regulation of LPS-induced inflammatory response in RAW264.7 cells through TLR-4 mediated NF-κB and JNK pathways [95]. Additionally, Yang et al. [96] reported that inhibition of TNF-α is able to suppress the activation of TLR-4 and NF-κB signaling pathway that consequently inhibits cytokine production and protect hepatic tissues from being injured by excessive immune reactions.

The present results indicated that targeted inhibition of the TLR-4/NF-κB signaling pathway might be a possible underlying mechanism of antioxidative and anti-inflammatory responses achieved by GSE proanthocyanidins for alleviating TiO_2-NP hepatotoxicity.

Conclusion

In conclusion, the signaling cascade in TiO2 NP-induced hepatotoxicity might occur via ROS production and activation of TLR-4 after binding with TiO_2-NPs → excess ROS → NIK → NF-κB → TNF-α → inflammation and tissue injury. The current data suggest that GSE proanthocyanidins with their antioxidant activities could modulate the oxidative damage and inflammatory response via inhibiting TLR-4/NF-κB signaling pathway in the liver following TiO_2-NPs toxicity which is closely related to oxidative stress. Therefore, the inhibition of TLR-4/NF-κB signaling pathway is expected to become a novel strategy for the prevention of hepatotoxicity. Also, GSE proanthocyanidins may be a potential choice for the prevention and alleviation of nanotoxicities.

Acknowledgments Authors thank all the staff members of the Biochemistry and Pathology Departments, Faculty of Veterinary Medicine, Beni-Suef University, Egypt, for their help and advices.

Compliance with ethical standards All experimental procedures were in accordance with the guidelines of local Animal Care and Use Committee established at the Beni-Suef University (BSU-IACUC). The study was performed after obtaining an approval number (018-8) to conduct the animal experiments.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Sathiyavimal S, Vasantharaj S, Bharathi D, Saravanan M, Manikandan E, Kumar SS, Pugazhendhi A (2018) Biogenesis of copper oxide nanoparticles (CuONPs) using Sida acuta and their incorporation over cotton fabrics to prevent the pathogenicity of Gram negative and Gram positive bacteria. J Photochem Photobiol B 188:126–134
2. Shanmuganathan R, MubarakAli D, Prabakar D, Muthukumar H, Thajuddin N, Kumar SS, Pugazhendhi A (2018) An enhancement of antimicrobial efficacy of biogenic and ceftriaxone-conjugated

silver nanoparticles: green approach. Environ Sci Pollut Res 25(11): 10362–10370
3. Oves M, Aslam M, Rauf MA, Qayyum S, Qari HA, Khan MS, Alam MZ, Tabrez S, Pugazhendhi A, Ismail IMI (2018) Antimicrobial and anticancer activities of silver nanoparticles synthesized from the root hair extract of Phoenix dactylifera. Mater Sci Eng C Mater Biol Appl 1(89):429–443
4. Suganthy N, Ramkumar VS, Pugazhendhi A, Benelli G, Archunan G (2018) Biogenic synthesis of gold nanoparticles from Terminalia arjuna bark extract: assessment of safety aspects and neuroprotective potential via antioxidant, anticholinesterase, and antiamyloidogenic effects. Environ Sci Pollut Res 25(11):10418–10433
5. Pugazhendhi A, Prabhu R, Muruganantham K, Shanmuganathan R, Natarajan (2019) Anticancer, antimicrobial and photocatalytic activities of green synthesized magnesium oxide nanoparticles (MgONPs) using aqueous extract of Sargassum wightii. J Photochem Photobiol B 190:86–97
6. Srinivasan M, Venkatesan M, Arumugam V, Natesan G, Saravanan N, Murugesan S et al (2019) Green synthesis and characterization of titanium dioxide nanoparticles (TiO_2 NPs) using Sesbania grandiflora and evaluation of toxicity in zebrafish embryos. Process Biochem 80:197–202
7. Pugazhendhi A, Edison TNJI, Karuppusamy I, Kathirvel B (2018) Inorganic nanoparticles: a potential cancer therapy for human welfare. Int J Pharm 539(1-2):104–111
8. Riu J, Maroto A, Rius FX (2006) Nanosensors in environmental analysis. Talanta 69(2):288–301
9. Sadrieh N, Wokovich AM, Gopee NV, Zheng J, Haines D, Parmiter D et al (2010) Lack of significant dermal penetration of titanium dioxide from sunscreen formulations containing nano and submicron-size TiO2 particles. J Toxicol Sci 115:156–166
10. Trouiller B, Reliene R, Westbrook A, Solaimani P, Schiestl RH (2009) Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice. Cancer Res 69:8784–8789
11. Wang J, Zhou G, Chen C, Yu H, Wang T (2007) Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. Toxicol Lett 168:176–185
12. Zhao J, Bowman L, Zhang X, Vallyathan V, Young SH, Castranova V, Ding M (2009) Titanium dioxide (TiO2) nanoparticles induce JB6 cell apoptosis through activation of the caspase-8/Bid and mitochondrial pathways. J Toxicol Environ Health A 72:1141–1149
13. Shi H, Magaye R, Castranova V, Zhao J (2013) Titanium dioxide nanoparticles: a review of current toxicological data. Part Fibre Toxicology 10:15
14. Kandeil MA, Mohammed ET, Hashem KS, Aleya L, Abdel Daim MM (2019) Moringa seed extract alleviates titanium oxide nanoparticles (TiO2-NPs)-induced cerebral oxidative damage, and increases cerebral mitochondrial viability. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-019-05514-2
15. Ma L, Zhao J, Wang J, Liu J, Duan Y, Liu H et al (2009) The acute liver injury in mice caused by nano-anatase TiO2. Nanoscale Res Lett 4:1275–1285
16. Tang M, Zhang T, Xue Y, Wang S, Huang M et al (2010) Dose dependent in vivo metabolic characteristics of titanium dioxide nanoparticles. J Nanosci Nanotechnol 10:8575–8583
17. Chen JY, Dong X, Zhao J, Tang GP (2009) In vivo acute toxicity of titanium dioxide nanoparticles to mice after intraperitoneal injection. J Appl Toxicol 29:330–337
18. Chen J, Dong X, Xin Y, Zhao M (2011) Effects of titanium dioxide nanoparticles on growth and some histological parameters of zebrafish (Danio retio) after long-term exposure. Aquat Toxicol 101:493–499
19. Fadoju O, Ogunsuyi O, Akanni O, Alabi O, Alimba C, Adaramoye O, Cambier S, Eswara S, Gutleb AC, Bakare A (2019) Evaluation of cytogenotoxicity and oxidative stress parameters in male Swiss mice co-exposed to titanium dioxide and zinc oxide nanoparticles. Environ Toxicol Pharmacol 70:103204
20. Hirakawa K, Mori M, Yoshida M, Oikawa S, Kawanishi S (2004) Photo-irradiated titanium dioxide catalyzes site specific DNA damage via generation of hydrogen peroxide. Free Radic Res 38:439–447
21. Shrivastava R, Raza S, Yadav A, Kushwaha P, Flora SJ (2014) Effects of sub-acute exposure to TiO_2, ZnO and Al_2O_3 nanoparticles on oxidative stress and histological changes in mouse liver and brain. Drug Chem Toxicol 37(3):336–347
22. Kang SJ, Kim BM, Lee YJ, Hong SH, Chung HW (2009) Titanium dioxide nanoparticles induce apoptosis through the JNK/p38-caspase-8-bid pathway in phytohemagglutinin-stimulated human lymphocytes. Biochem Biophys Res Commun 386:682–687
23. Vives-Pi M, Somoza N, Fernandez-Alvarez J, Vargas F, Caro P, Alba A, Gomis R, Labeta MO, Pujol-Borrell R (2003) Evidence of expression of endotoxin receptors CD14. Toll-like receptors TLR4 and TLR2 and associated molecule MD-2 and of sensitivity to endotoxin (LPS) in islet beta cells. Clin Exp Immunol 133:208–218
24. Vaure C, Liu Y (2014) A comparative review of toll-like receptor 4 expression and functionality in different animal species. Front Immunol 5:316
25. Sun Q, Tan D, Ze Y, Sang X, Liu X, Gui S, Cheng Z, Cheng J, Hu R, Gao G et al (2012) Pulmotoxicological effects caused by long-term titanium dioxide nanoparticles exposure in mice. J Hazard Mater 235–236:47–53
26. Cui Y, Liu H, Zhou M, Duan Y, Li N, Gong X, Hu R, Hong M, Hong F (2011) Signaling pathway of inflammatory responses in the mouse liver caused by TiO2 nanoparticles. J Biomed Mater Res A 96:221–229
27. Gui S, Zhang Z, Zheng L, Cui Y, Liu X, Li N, Sang X, Sun Q, Gao G, Cheng Z et al (2011) Molecular mechanism of kidney injury of mice caused by exposure to titanium dioxide nanoparticles. J Hazard Mater 195:365–370
28. Chandel NS, Trzyna WC, McClintock DS, Schumacker PT (2000) Role of oxidants in NF-kappa B activation and TNF-alpha gene transcription induced by hypoxia and endotoxin. J Immunol 165(2):1013
29. Wilson D, Zaqout M, Heo JH, Park EK, Oak CH, Ueno S (2012) Nuclear factor-kappa B is not involved in titanium dioxide-induced inflammation. J UOEH 34(2):183–191
30. Song B, Zhou T, Yang W, Liu J, Shao L (2016) Contribution of oxidative stress to TiO2 nanoparticle-induced toxicity. Environ Toxicol Pharmacol 48:130–140
31. Islam MR, Shahnaj MP, Raihan MO, Hasan SMR, Islam ME (2011) In vitro and in vivo antioxidant potential of ethanolic extract of Syzigium jambos (L.) bark. IJRAP 2:810–815
32. Mohamed ET, Safwat GM (2016) Evaluation of cardioprotective activity of Lepidium sativum seed powder in albino rats treated with 5-fluorouracil. Beni-Suef University Journal of Basic and Applied Sciences 5:208–215
33. Kandeil MAM, Hassanin KMA, Mohammed ET, Safwat GM, Mohamed DS (2018) Wheat germ and vitamin E decrease BAX/BCL-2 ratio in rat kidney treated with gentamicin. Beni-Suef University Journal of Basic and Applied Sciences 7(3):257–262
34. Sachs A (1997) A natural alternative for treating colds, infections, herpes, candida and many other ailments. The Authoritative Guide to Grapefruit Extract. Stay Healthy Naturally. Life rhythm; Mendocino, California, pp775-795
35. Alkhedaide AQ (2015) The anti-inflammatory effect of grape seed extract in rats exposed to cadmium chloride toxicity. Int J Adv Res 3:298–305
36. Cetin A, Kaynar L, Koçyiğit I, Hacioğlu SK, Saraymen R, Oztürk A, Orhan O, Sağdiç O (2008) The effect of grape seed extract on radiation-induced oxidative stress in the rat liver. Turk J Gatroenterol 19:92–98

37. Shi J, Yu J, Pohorly JE, Kakuda Y (2003) Polyphenolics in grape seeds-biochemistry and functionality. J Med Food 6(4):291–299
38. Gusev AI, Kurlov AS (2008) Production of nanocrystalline powders by high-energy ball milling: model and experiment. Nanotechnology 19(26):265–302
39. Mittal A, Elmets CA, Katiyar SK (2003) Dietary feeding of proanthocyanidins from grape seeds prevents photocarcinogenesis in SKH-1 hairless mice: relationship to decreased fat and lipid peroxidation. Carcinogenesis 24:1379–1388
40. Devi A, Jolitha A, Ishii N (2006) Grape seed proanthocyanidin extract (GSPE) and antioxidant defense in the brain of adult rats. Med Sci Monit 12(4):124–129
41. Warheit RA, Hoke DB, Finlay C, Donner EM, Reed KL, Sayes CM (2007) Development of a base set of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle risk management. Toxicol Lett 171:99–110
42. Kovarik Z, Radić Z, Berman HA, Simeon-Rudolf V, Reiner E, Taylor P (2003) Acetyl cholinesterase active centre and gorge conformations analyzed by combinatorial mutations and enantiomeric phosphonates. Biochem J 1(73):33–40
43. Zilva JF, Pannall PR (1979) Plasma enzymes in diagnosis in clinical chemistry in diagnosis and treatment. Lioyd – Luke London. Chap 17:338
44. Tietz NW, Burtis CA, Duncan P, Ervin K, Petitclerc CJ et al (1983) A reference method for measurement of alkaline phosphatase activity in human serum. Clin Chem 29:751–761
45. Tolman KG, Rej R (1999) Liver function. In: Burtis CA, Ashwood ER (eds) Tietz Textbook of clinical Chemistry. Third ed. W.B. Saunders company, Philadelphia, pp 1125–1177
46. Henry RJ (1964) Clinical Chemistry. Harper & Row Publishers, New York, p 181
47. Doumas BT, Watson WA, Biggs HG (1971) Albumin standards and the measurement of serum albumin with bromocresol green. Clinica Chimica Acta 31(1):87–96
48. Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302–310
49. Beutler E, Duron O, Kelly BM (1963) Improved methods for the determination of glutathione. J Lab Clin Med 61:882–888
50. Aebi H (1984) Methods Enzymol 105:121–126
51. Bancroft JD, Gamble M (2002) Theory and practice of histological techniques, 5th ed. Churchill Livingstone, New York, pp 377–694
52. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the2 (-Delta Delta C(T)) Method. Methods 25(4):402–408
53. Meena R, Paulraj R (2012) Oxidative stress mediated cytotoxicity of TiO2 nano anatase in liver and kidney of Wistar rat. Toxicol Environ Chem 94:146–163
54. An H, Ling C, Xu M, Hu M, Wang H, Liu J, Song G, Liu J (2019) Oxidative damage induced by nano-titanium dioxide in rats and mice: a systematic review and meta-analysis. Biol Trace Elem Res 24
55. Gheshlaghi ZN, Riazi GH, Ahmadian S, Ghafari M, Mahinpour R (2008) Toxicity and interaction of titanium dioxide nanoparticles with microtubule protein. Acta Biochim Biophys Sin 40(9):777–782
56. Fu Y, Zhang Y, Chang X, Zhang Y, Ma S, Sui J, Yin L, Pu Y, Liang G (2014) Systemic immune effects of titanium dioxide nanoparticles after repeated intratracheal instillation in rat. Int J Mol Sci 15: 6961–6973
57. Rizk MZ, Ali SA, Hamed MA, El-Rigal NS, Aly HF, Salah HH (2017) Toxicity of titanium dioxide nanoparticles: Effect of dose and time on biochemical disturbance, oxidative stress and genotoxicity in mice. Biomed Pharmacother 90:466–472
58. Liu HT, Ma LL, Zhao JF, Liu J, Yan JY, Ruan J, Hong FS (2009) Biochemical toxicity of nano-anatase TiO2 particles in mice. Biol Trace Elem Res 129:170–180
59. Morgan A, Ibrahim MA, Galal MK, Ogaly HA, Abd-Elsalam RM (2018) Innovative perception on using Tiron to modulate the hepatotoxicity induced by titanium dioxide nanoparticles in male rats. Biomed Pharmacother 103:553–561
60. Kaplan MM (*1972*) Alkaline phosphatase. N Engl J Med 286(4): 200–202
61. Sanjiv C (2002) The liver book: a comprehensive guide to diagnosis, treatment and recovery. Atria Jimcafe Company, New York
62. Yamamoto M, Maeda H, Hirose N, Radhakrishnan G, Katare RG, Hayashi Y et al (2007) Bilirubin oxidation provoked by nitric oxide radicals predicts the progression of acute cardiac allograft rejection. Am J Transplant 7:1897–1906
63. Bohme M, Muller M, Leier I, Jedlitschky G, Keppler D (1994) Cholestasis caused by inhibition of the adenosine triphosphate-dependent bile salt transport in rat liver. Gastroenterology 107(1): 255–265
64. McQueen MJ (1995) Clinical and analytical considerations in the utilization of cholinesterase measurements. Clin Chim Acta 237: 91–105
65. García-Ayllón MS, Silveyra MX, Candela A, Compañ A, Clària J, Jover R et al (2006) Changes in liver and plasma acetylcholinesterase in rats with cirrhosis induced by bile duct ligation. Hepatology 43:444–453
66. Gómez JL, García-Ayllon MS, Campoy FJ, Vidal CJ (2000) Muscular dystrophy alters the processing of light acetylcholinesterase but not butyrylcholinesterase forms in liver of Lama2(dy) mice. J Neurosci Res 62:134–145
67. Soreq H, Seidman S (2001) Acetylcholinesterase-new roles for an old actor. Nat Rev Neurosci 2:294–302
68. Zhou X, Tu ZG (eds) (2003) Clinical Biological Chemical and Biological Chemical Inspection. 3rd edition. People's Medical Publishing House, Beijing, pp 325–328
69. Ma L, Liu J, Li N, Wang J, DuanY YJ, Liu H, Wang H, Hong F (2010) Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO2 delivered to the abdominal cavity. Biomaterials 31:99–105
70. Inoue K, Takano H (2011) Aggravating impact of nanoparticles on immune-mediated pulmonary inflammation. Sci World J 11:382e90
71. Rim KT, Song SW, Kim HY (2013) Oxidative DNA damage from nanoparticle exposure and its application to workers' health: a literature review. Saf Health Work 4:177e186
72. Abdou KH, Moselhy WA, Mohamed HM, El-Nahass ES, Khalifa AG (2019) Moringa oleifera leaves extract protects titanium dioxide nanoparticles-induced nephrotoxicity via Nrf2/HO-1 signaling and amelioration of oxidative stress. Biol Trace Elem Res 187(1): 181–191
73. Durairaj A, Vaiyapuri TS, Kanti MU, Malaya G (2008) Protective activity and antioxidant potential of Lippia nodiflora extract in paracetamol induced hepatotoxicity in rats. Iran J Pharmacol Ther 7:83–89
74. Huyton T, Rossjohn J, Wilce M (2007) Toll-like receptors: structural pieces of a curve-shaped puzzle. Immunol Cell Biol 85:406–410
75. Kawasaki T, Kawai T (2014) Toll-like receptor signaling pathways. Front Immunol 5:461
76. Dhupal M, Oh JM, Tripathy DR, Kim SK, Koh SB, Park KS (2018) Immunotoxicity of titanium dioxide nanoparticles via simultaneous induction of apoptosis and multiple toll-like receptors signaling through ROS-dependent SAPK/JNK and p38 MAPK activation. Int J Nanomedicine 13(13):6735–6750
77. Fan H, Peck OM, Tempel GE, Halushka PV, Cook JA (2004) Toll-like receptor 4 coupled GI protein signaling pathways regulate extracellular signal-regulated kinase phosphorylation and AP-1 activation independent of NF-kappa B activation. Shock 22:57–62
78. Li Q, Verma IM (2002) NF-jB regulation in the immune system. Nat Rev Immunol 2:725–734
79. Vallabhapurapu S, Karin M (2009) Regulation and function of NF-kappaB transcription factors in the immune system. Annu Rev Immunol 27:693–733

80. Sun SC (2011) Non-canonical NF-kappaB signaling pathway. Cell Res 21:71–85
81. Thu YM, Richmond A (2010) NF-κB inducing kinase: a key regulator in the immune system and in cancer. Cytokine Growth Factor Rev 21(4):213–226
82. Senftleben U, Cao Y, Xiao G, Greten FR, Kra G, Bonizzi Y et al (2001) Activation by IKK alpha of a second, evolutionary conserved, NF-kappa B signaling pathway. Science 293:1495–1499
83. Hayden MS, Ghosh S (2008) Shared principles in NF-kappaB signaling. Cell 132:344–362
84. Sun SC (2012) The noncanonical NF-κB pathway. Immunol Rev 246:125–140
85. Huang X, Tang J, Cai H, Pan Y, He Y, Dai C et al (2015) Anti-Inflammatory Effects of Monoammonium glycyrrhizinate on lipopolysaccharide-induced acute lung injury in mice through regulating nuclear factor-kappa B signaling pathway. Evid Based Complement Alternat Med. https://doi.org/10.1155/2015/272474
86. Wang JH, Redmond HP, Watson RW, Bouchier-Hayes D (1995) Role of lipopolysaccharide and tumor necrosis factor-alpha in induction of hepatocyte necrosis. Am J Phys 269:G297–G304
87. Zhang K, Kaufman RJ (2008) From endoplasmic-reticulum stress to the inflammatory response. Nature 454:455–462
88. Paur I, Balstad TR, Kolberg M, Pedersen MK, Austenaa LM, Jacobs DR, Blomhoff R (2010) Extract of oregano, coffee, thyme, clove, and walnuts inhibits NF-kappaB in monocytes and in transgenic reporter mice. Cancer Prev Res 3(5):653–663
89. Shin MO, Moon JO (2010) Effect of dietary supplementation of grape skin and seeds on liver fibrosis induced by dimethylnitrosamine in rats. Nutr Res Pract 4(5):369–374
90. Sharma SD, Meeran SM, Katiyar SK (2007) Dietary grape seed proantho-cyanidins inhibit UVB induced oxidative stress and activation of mitogen-activated protein kinases and nuclear factor-KB signaling in in vivo SKH-1hairless mice. Mol Cancer Ther 6:995–1005
91. Li J, Li J, Li S, Heb B, Mi Y, Caoa H, Zhanga C, Li L (2012) Ameliorative effect of grape seed proanthocyanidin extract on thioacetamide-induced mouse hepatic fibrosis. Toxicol Lett 213: 353–360
92. Bogs J, Ebadi A, McDavid D, Robinson S (2006) Identification of the flavonoid hydroxylases from grape-vine and their regulation during fruit development. Plant Physiol 140(1):279–291
93. Liang B, Chen R, Wang T, Cao L, Liu Y, Yin F, Zhu M, Fan X, Liang Y, Zhang L, Guo Y, Zhao J (2013) Myeloid differentiation factor 88 promotes growth and metastasis of human hepatocellular carcinoma. Clin Cancer Res 19:2905–2916
94. Mantena SK, Katiyar SK (2006) Grape seed proanthocyanidins inhibit UV radiation-induced oxidative stress and activation of MAPK and NF-KB signaling in human epidermal keratinocytes. Free Radic Biol Med 40:1603–1614
95. Liu D, Cao G, Han L, Ye Y, SiMa Y, Ge W (2016) Flavonoids from Radix Tetrastigmae inhibit TLR4/MD-2 mediated JNK and NF-κB pathway with anti-inflammatory properties. Cytokine 84:29–36
96. Yang F, Li X, Wang LK, Wang LW, Han XQ, Zhang H, Gong ZJ (2014) Inhibitions of NF-kappaB and TNF-alpha result in differential effects in rats with acute on chronic liver failure induced by d-Gal and LPS. Inflammation 37:848–857