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Silver Nanoparticles Enhance Oxidative Stress, Inflammation, and Apoptosis in Liver and Kidney Tissues: Potential Protective Role of Thymoquinone

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Abstract

Silver nanoparticles (AgNPs) are the most common nanomaterials in consumer products. Therefore, it has been crucial to control AgNPs toxicological effects to improve their safety and increase the outcome of their applications. This work investigated the possible protective effect of thymoquinone (TQ) against AgNPs-induced hepatic and renal cytotoxicity in rats. Serum markers of liver and kidney functions as well as liver and kidney oxidative stress status, pro-inflammatory cytokines, apoptosis markers, and histopathology were assessed. TQ reversed AgNPs-induced elevation in serum liver and kidney function markers, including aspartate transaminase, alanine transaminase, urea, and creatinine. Moreover, TQ co-administration with AgNPs alleviates hepatic and renal oxidative insults by decreasing MDA and NO levels with a significant increase in the activity of antioxidant enzymes (superoxide dismutase, catalase, and glutathione recycling enzymes peroxidase and reductase) compared to AgNPs-treated rats. Besides, TQ upregulated hepatic and renal Nrf2 gene expression in AgNPs-intoxicated rats. Furthermore, TQ co-administration decreased the hepatic and renal pro-inflammatory mediators represented by IL-1β, TNF-α, TGF-β, and NF-κB levels. Besides, TQ co-administration decreased apoptotic protein (Bax) levels and increased the anti-apoptotic protein (Bcl-2) levels. These findings were confirmed by the histopathological examination of hepatic and renal tissues. Our data affirmed the protective effect of TQ against AgNPs cytotoxicity and proposed a possible mechanism of TQ antioxidant, anti-inflammatory, and anti-apoptotic effects. Consequently, we could conclude that using TQ might control AgNPs toxicological effects, improve their safety, and increase the outcome of their applications.

Keywords Silver Nanoparticles · ThyMoquinone · Oxidative Damage · Inflammation · Apoptosis · Hepatorenal Toxicity

Introduction

Recently, engineered nanomaterials have acquired immense attention in technological advancements. The unique nanoparticle (NPs) characteristics are based on their morphology, size, surface charge, and coating. These different characteristics are responsible for their effects on the biological systems [1]. Metallic NPs such as silver, gold, and iron are widely used in medicine and industry owing to their unique thermal, optical, catalytic, and electrical characteristics [2].

Silver nanoparticles (AgNPs) have been widely used in domestic utensils, food storage, the health care industry, environmental applications, and biological applications such as wound dressings, surgical instruments, and disinfectants due to their unique features. AgNPs have also been employed in catalysis, electronics, and biosensors due to their optical activity [3]. This widespread use has increased the potential for interactions of AgNPs with living organisms and potential exposure and toxicity to human health [4]. Humans are exposed to AgNPs directly or indirectly, which accumulate in different organs. Inhalation represents the main route of exposure through the extensive application of healthcare and hygiene sprays. AgNPs are used as coatings in surgical dressings, which may allow these nanoparticles to penetrate the skin barrier [5]. AgNPs are also commonly

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employed in food preservation and water disinfection [6]. As a result, AgNPs can enter the human body via the gastrointestinal system [5].

Previous in vitro and in vivo studies have reported the toxic effect of AgNPs [7, 8]. Carlson et al. [9] reported that murine alveolar macrophages exhibited cellular morphology alterations following the exposure to different sizes (15, 30, and 55 nm) of hydrocarbon-coated AgNPs. In another study, A549 cells treated with AgNPs (20 nm) at a dose of 0.6 nM for 2 days showed DNA damage associated with upregulated metallothioneins [10]. Additionally, mouse erythrocytes incubated with coated AgNPs with polyvinylpyrrolidone and citrate (10 nm) at different doses (2.5, 10, 40 µg/ml) exerted oxidative lesions through enhancing lipid peroxidation and decreasing glutathione and catalase in addition to increasing extracellular calcium [11]. Non-coated AgNPs showed cytotoxicity toward different cell lines such as macrophages, alveolar epithelial cells, hepatocytes, and embryonic kidney cells [12]. AgNPs were proven to enhance reactive oxygen species (ROS) [13], apoptosis [14], and inflammation [15]; authors reported that these effects depend on size, concentration, and route of administration [16, 17].

Assar et al. [18] studied the hepatotoxic effects of AgNPs (0.25, 0.5, and 1 mg/kg) for 15 and 30 consecutive days in rats. AgNPs enhanced lipid peroxidation and nitric oxide (NO) production and decreased glutathione (GSH) content. Additionally, AgNPs enhanced apoptotic events in the hepatocytes by enhancing pro-apoptotic proteins and inhibiting the anti-apoptotic protein. The authors also recorded hematological and histopathological changes. Moreover, oral administration of AgNPs at doses of 30, 125, 300, and 700 mg/kg induced histopathological alterations and enhanced apoptosis and inflammation in the renal tissue in rats [19]. Hence, it is mandatory to evaluate their toxicological effects to minimize and/or prevent side effects, improve their safety, and increase their application [20].

Thymoquinone (TQ) is the primary active ingredient in *Nigella sativa* oil with numerous biological and pharmaceutical activities [21–23]. Our previous studies revealed that oral administration of TQ at a dose of 10 mg/kg prevented oxidative insults, inflammatory and apoptotic reactions in the hepatorenal tissue following arsenic exposure [22, 24]. Abdel-Daim et al. [25] showed that TQ (10 and 20 mg/kg, orally gavaged) protected liver and kidney tissue in rats by inhibiting the development of oxidative damage and inflammation following acrylamide intoxication. Additionally, TQ reduced cyclosporine A and acute renal ischaemia/reperfusion-induced liver and kidney dysfunction. Authors attributed these results to the ability of TQ to scavenge free radicals and inhibit lipid peroxidation [26]. The antioxidant capacity of TQ is due to scavenging free radicals such as superoxide anion, hydroxyl, hydrogen peroxide, and peroxynitrite radicals, in addition to enhancing antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase [27]. Moreover, accumulative studies reported the ability of TQ to inhibit the production of pro-inflammatory cytokines and chemokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and cyclooxygenase 2 (COX-2) [28]. Furthermore, the anti-apoptotic activity of TQ has been attributed to the enhancement of anti-apoptotic protein (Bcl2) and inhibiting the pro-apoptotic proteins (Bax and caspase-3) [22, 24].

Based on the previous studies and suggestions, the present work aim is to investigate the possible protective effect of TQ against AgNPs-induced hepatorenal damage by evaluating the redox hemostasis, apoptosis, and inflammatory response in the liver and kidney tissues of rats.

Materials and Methods

Nanoparticles

AgNPs (size less than 40 nm) were obtained from Sigma-Aldrich (730,793; St. Louis, MO, USA). According to the manufacturer's instructions, AgNPs characterization was performed using transmission electron microscopy (TEM), dynamic light scattering (DLS), and UV/Visible spectral analysis to ensure that the monodisperse AgNPs are free from agglomeration with a density of 0.986 g/mL at 25 °C; refractive index *n*20/D 1.333; and fluorescence—λ_{em} 401 nm (https://www.sigmaaldrich.com/EG/en/product/aldrich/730793).

Experimental Design

Twenty-eight adult male Wistar albino rats aged 3–4 months old and weighed 180–200 g (VACSERA, Cairo, Egypt) were kept on a standard diet (Teklad Global 19% Protein Extruded Rodent Diet, Envigo, USA) and tap water ad libitum for 1 week. The used rodent diet contains:

Ground wheat, ground corn, corn gluten meal, wheat middlings, soybean oil, calcium carbonate, dicalcium phosphate, brewers dried yeast, L-lysine, iodized salt, magnesium oxide, choline chloride, DL-methionine, calcium propionate, L-tryptophan, vitamin E acetate, menadione sodium bisulfite complex (source of vitamin K activity), manganous oxide, ferrous sulfate, zinc oxide, niacin, calcium pantothenate, copper sulfate, pyridoxine hydrochloride, riboflavin, thiamin mononitrate, vitamin A acetate, calcium iodate, vitamin B12 supplement, folic acid, biotin, vitamin D3 supplement, and cobalt carbonate.

After acclimatization, animals were randomly divided into four equal groups ($n = 7$):

1- Control group: received normal physiological saline 0.9% NaCl for 28 days.
2- TQ group: treated orally with TQ (10 mg/kg /day) [22]. This dose was selected based on our previous study, as TQ was found to protect hepatic tissue against oxidative stress, inflammation, and apoptosis induced by arsenic exposure.
3- AgNPs group: injected intraperitoneally by AgNPs (50 mg/kg/day) for 28 days [29]. The selected dose was found to enhance oxidative insults and triggered inflammation and apoptosis as well as histopathological changes in the kidney and liver tissue.
4- TQ-AgNPs group: treated orally with TQ (10 mg/kg/day), and then 2 h later, the rats were injected intraperitoneally with AgNPs (50 mg/kg/day) for 28 days.

The study protocol was reviewed and approved by the institutional animal care and use committee, Faculty of Science, Helwan University (HU/2020/Z/AEN0120-01), following the European Community Directive (86/609/EEC).

At the end of the experiment, rats were anesthetized by sodium pentobarbital intraperitoneal injection (200 mg/kg) and subjected to a complete autopsy. Blood samples were collected and incubated at room temperature for 10 min to clot, then centrifuged at 3000 × *g* for 10 min to collect the serum samples for further analysis. The liver and kidney were immediately dissected and divided into three parts. One part was homogenized with (10% w/v) ice-cold 50 mM Tris–HCl buffer (pH 7.4) and centrifuged at 3000 × *g* for 10 min at 4 °C then the supernatant was stored at − 20 °C for biochemical analysis. Another part was stored at − 80 °C for gene expression analysis. Finally, the third part was preserved in 10% of neutral-buffered formalin for histopathological examination.

Assessment of Liver and Kidney Functions

The levels of alanine transaminase (ALT), aspartate aminotransferase (AST), urea, and creatinine were assayed in serum samples using standard kits (Biodiagnostic, Giza, Egypt) according to the manufacturer's protocol.

Oxidative Stress Assays

GSH, which is considered a non-enzymatic antioxidant marker, was assessed with the colorimetric method following Elaman's protocol [30] in liver and kidney samples. NO levels were quantified by the Griess solution method [31]. Malondialdehyde (MDA), which is considered a lipid peroxidation marker, was measured following the method of Ohkawa et al. [32]. Superoxide dismutase (SOD) enzyme activity was assessed depending on the inhibition of the reduction of nitroblue tetrazolium dye by SOD and depending on the H_2O_2 decomposition rate following the method of Aebi [33]. Glutathione peroxidase (GPx) and reductase (GR) enzymes activities were assessed by measuring NADPH oxidation and reduction at 340 nm in the presence of glutathione, following the method of Paglia and Valentine [34] and Factor et al. [35], respectively.

Inflammatory Marker Assessment

TNF-α and IL-1β were measured using ELISA kits obtained from Thermo Fisher Scientific, USA, for TNF-α (Cat. no. BMS607-3) and IL-1β (Cat. no. BMS6002). Transforming growth factor-beta (TGF-β) was measured by ELISA kits obtained from R&D System, Minneapolis, MN, USA (Cat no: SB100C). Nuclear factor-kappa B (NF-κB, Novus Biologicals, Centennial, CO, USA; Cat. no. NB100-2176) was measured by ELISA kits following the manufacturer's instructions.

Apoptotic Biomarker Determination

Bax, which is a pro-apoptotic marker, and Bcl-2, which is an anti-apoptotic marker, were measured using ELISA kits from BioVision, Inc. for (rat Bax; Cat. No.: E4513) and Cusabio (rat Bcl-2; Cat. No.: CSB-E08854r).

PCR Analysis

After homogenizing the liver and kidney tissues, total RNA was extracted using RNeasy Plus Mini kits. The extracted RNA (100 ng) was then reverse transcribed into cDNA using a ScriptTM cDNA synthesis kit (Bio-Rad, CA). qRT-PCR was applied using an Applied Biosystems 7500 Instrument (Applied Biosystems, USA) to estimate the relative expression of the Nrf2 gene using Power SYBR® Green. B-actin was applied as a housekeeping gene.

Histopathological Examination

Fixation of liver and kidney specimens is done with 10% neutral buffered formalin. After that, samples were dehydrated, embedded in paraffin wax, and cut into 5 µm thick sections. In the next step, liver and kidney sections were deparaffinized, stained with hematoxylin and eosin, and examined under a Nikon Eclipse E200-LED microscope (Nikon Corporation, Tokyo, Japan) for histopathological changes.

Statistical Analysis

Data were expressed as mean values ± SE (standard error), and one-way ANOVA statistically analyzed the significant differences among treatment groups. The criterion for

statistical significance was set at $p < 0.05$ for the biochemical data. All statistical analyses were performed using SPSS statistical version 21 software package (SPSS® Inc., USA).

Results

TQ Restored AgNPs-Induced Elevation in Serum Liver and Kidney Function Markers

To investigate AgNPs-induced liver and kidney injury and the potential antagonistic effect of TQ, serum ALT, AST, urea, and creatinine levels were assessed. As shown in Table 1, liver and kidney function markers were significantly ($p < 0.05$) increased in AgNPs-treated rats compared to the control group. On the other hand, TQ co-administration significantly reduced both liver and kidney function markers compared to AgNPs-treated group. However, TQ alone did not show any significant effect compared to the control group.

TQ Ameliorates AgNPs-Induced Oxidative Stress in Hepatic and Renal Tissues

To investigate AgNPs-induced oxidative stress and the potential antioxidant effect of TQ, the levels of MDA, NO, and GSH were evaluated in the liver and kidney (Fig. 1) tissues. AgNPs significantly ($p < 0.05$) increased MDA and NO levels and decreased GSH levels in liver and kidney tissues. However, TQ co-administration significantly attenuated AgNPs-induced oxidative stress by decreasing the elevated MDA and NO levels and increasing GSH levels in liver and kidney tissues.

Table 1 Effect of TQ on AgNPs-induced hepatorenal toxicity

	Control	TQ	AgNPs	TQ + AgNPs
ALT	48.21 ± 6.29	49.24 ± 5.72	103.81 ± 13.02$^{\#}$	71.19 ± 8.58$^{\#\$}$
AST	74.81 ± 12.23	78.78 ± 13.95	172.51 ± 22.42$^{\#}$	101.37 ± 20.06$^{\#\$}$
Urea	35.17 ± 17	32.68 ± 5.30	78.50 ± 13.29$^{\#}$	56.47 ± 8.15$^{\#\$}$
Creatinine	0.39 ± 0.06	0.37 ± 0.06	0.87 ± 0.15$^{\#}$	0.56 ± 0.09$^{\#\$}$

Data are presented as mean ± SD ($n = 7$). # and $ mean significant difference, $p < 0.05$, from control and AgNPs-treated group, respectively

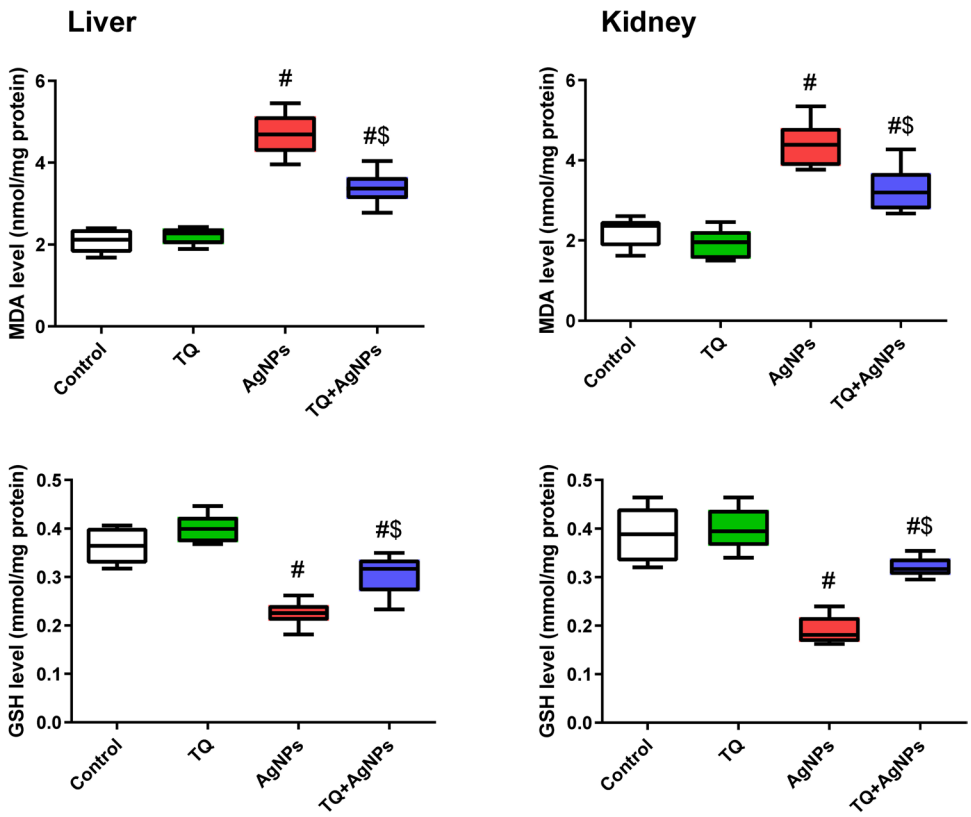


Fig. 1 The effect of TQ on AgNPs-induced oxidative stress in the liver and kidney tissues. Data are presented as mean ± SD ($n = 7$). # and $ mean significant difference, $p < 0.05$, from control and AgNPs-treated group, respectively

Moreover, the activity of the antioxidant enzymes, SOD, CAT, GPx, and GR, were evaluated in the liver and kidney (Fig. 2) tissues. AgNPs significantly ($p < 0.05$) suppressed the activity of the hepatic and renal antioxidant enzymes compared to the control group. On the other hand, TQ treatment for AgNPs-intoxicated rats significantly ($p < 0.05$) restored the activity of the hepatic and renal antioxidant enzymes compared to the AgNPs-treated group.

To elucidate the molecular mechanism underlying TQ antioxidant effect, mRNA expression of Nrf2 in the hepatic and renal tissue (Fig. 3) was determined. Nrf2 is a transcription factor that plays a vital role in the antioxidant and subsequent anti-inflammatory cellular response. AgNPs induced downregulation of hepatic and renal Nrf2 expression compared to the control group. TQ treatment significantly ($p < 0.05$) upregulated Nrf2 expression in AgNPs-intoxicated rats. Our gene expression analysis data demonstrated the role of the transcription factor, Nrf2, in the antioxidant and subsequent anti-inflammatory molecular mechanisms of TQ against AgNPs oxidative and inflammatory response.

TQ Ameliorates AgNPs-Induced Inflammation in Hepatic and Renal Tissues

To investigate the anti-inflammatory effect of TQ in response to AgNPs exposure, the pro-inflammatory cytokines levels, IL-1β, TNF- α, and TGF-β were evaluated in the liver and kidney (Fig. 4) tissues. AgNPs significantly ($p < 0.05$) increased hepatic and renal IL-1β, TNF-α, and TGF-β levels compared to the control group. On the other hand, TQ co-administration significantly ($p < 0.05$) reversed the elevated hepatic and renal pro-inflammatory cytokine levels compared to AgNPs-treated group.

To get more information about the mechanism underlying TQ anti-inflammatory effect, NF-κB level was estimated in the liver and kidney (Fig. 5) tissues. NF-κB plays a role in the expression of pro-inflammatory cytokines genes. AgNPs significantly ($p < 0.05$) increased hepatic and renal NF-κB levels compared to the control group. In contrast, TQ co-administration significantly decreased hepatic and renal NF-κB levels in AgNPs-treated rats. These data elucidate the role of NF-κB in TQ anti-inflammatory effect.

TQ Ameliorates AgNPs-Induced Apoptosis in Hepatic and Renal Tissues

To investigate the anti-apoptotic activity of TQ against cell loss induced by AgNPs, Bax and Bcl2 levels were estimated in liver and kidney tissue (Fig. 6). Bax regulates apoptosis as a pro-apoptotic protein and Bcl-2 as an anti-apoptotic protein. AgNPs significantly ($p < 0.05$) increased hepatic and renal Bax levels with decreased Bcl2 levels. Conversely, TQ co-administration ameliorated the apoptotic effect of AgNPs by restoring Bax level toward control value and significantly increasing Bcl2 level compared to AgNPs-treated group.

TQ Prevents Histopathological Changes in Hepatic and Renal Tissues Following the Exposure to AgNPs

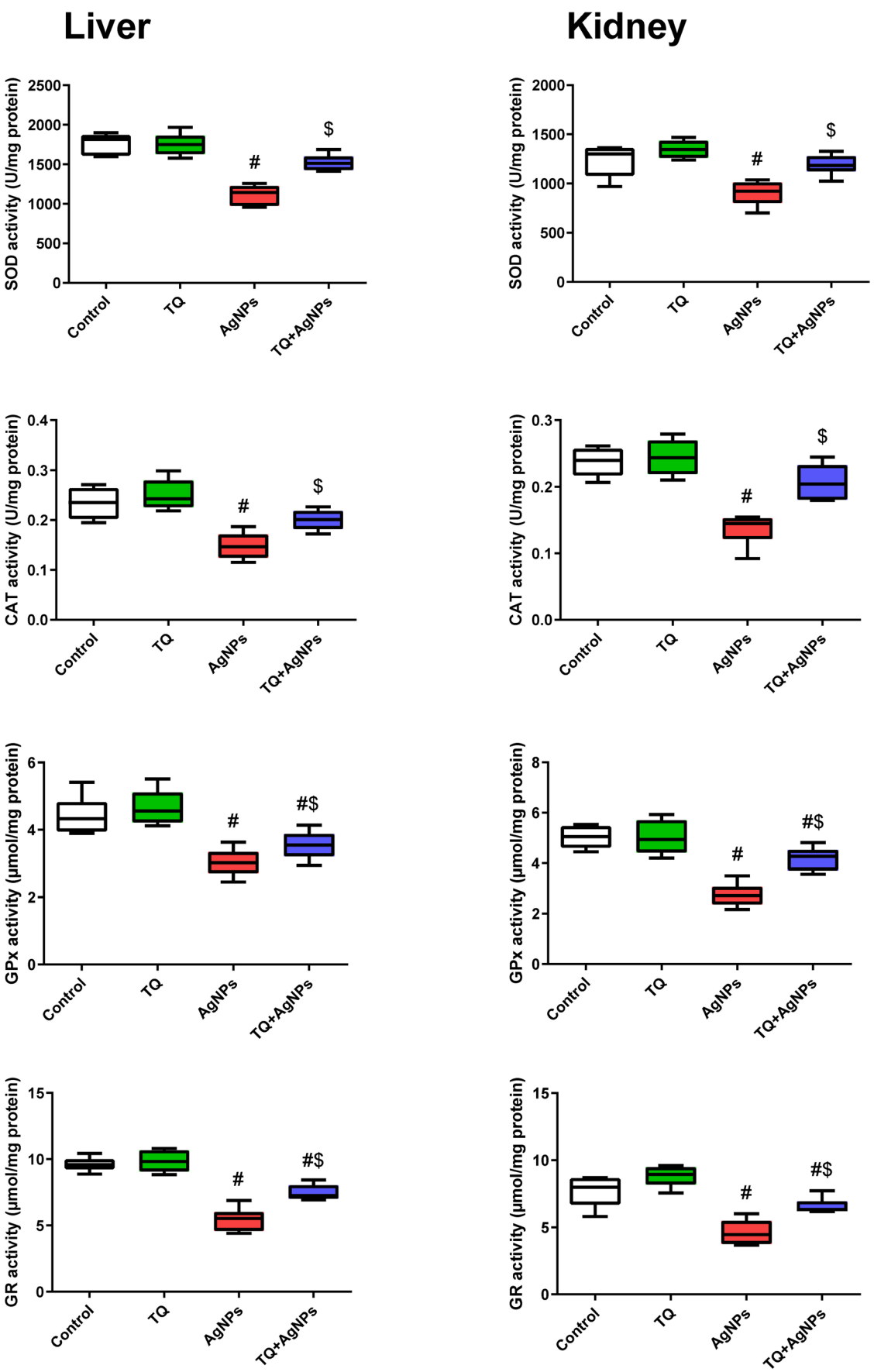
The control and TQ groups showed normal hepatocytes with central vein structure. However, AgNPs-exposed rats exhibited inflamed, degenerated, and apoptotic hepatocytes associated with prominent Kupffer cells (Fig. 7). These histological changes are obviously mitigated following TQ pretreatment (Fig. 7). Moreover, the control and TQ groups showed normal glomeruli with renal tubule structure. Nevertheless, AgNPs-challenged rats exhibited congested glomeruli, degenerated renal tubules accompanied by severe apoptosis, inflammatory cells infiltration into the intratubular spaces, and debris in the lumen of renal tubules (Fig. 7). These pathological alterations were prominently improved following TQ pretreatment (Fig. 7).

Discussion

AgNPs are widely used in numerous industrial and medicinal products such as cosmetics, paints, biosensors, food packaging, wound dressings, and antimicrobial agents [36]. Despite these promising advantages, assessment and decreasing AgNPs toxicity are crucial. In this study, we investigated the protective effect of TQ against AgNPs toxicity by observing the molecular, biochemical, and histopathological changes in liver and kidney tissues.

The small size of the NPs beside the route of administration defines AgNPs biodistribution and target organs [37]. NPs size is one of the essential factors that play a role on the NPs uptake and cellular distribution. Previous research papers confirmed the accumulation of Ag^+ in specific tissues, including lungs, spleen, liver, and kidneys of rats dosed with AgNPs, suggesting that the small "Nano-size" of AgNPs facilitates its accumulation into specific target organs where they may further generate Ag^+ [38]. AgNPs enter the body through inhalation, ingestion, transdermal, and parenteral injection. Some studies reported that liver and kidney tissues are the main targets of AgNPs accumulation in rats after oral administration and intravenous injection [39–41]. AgNPs accumulation leads to tissue injury, oxidative stress, inflammation, and apoptosis [42].

In our study, liver tissue injury induced by AgNPs treatment is evidenced by elevated serum levels of liver function biomarkers, AST and ALT, indicating cell membrane leakage, membrane permeability disturbance, and structural integrity loss. AgNPs-induced kidney tissue injury is evidenced by increased serum urea and creatinine levels, indicating a disruption in kidney clearance function. Moreover,


Liver
Kidney
SOD activity (U/mg protein)
CAT activity (U/mg protein)
GPx activity (µmol/mg protein)
GR activity (µmol/mg protein)
Control
TQ
AgNPs
TQ+AgNPs
#
$
#$

◂**Fig. 2** The effect of TQ on the suppressed activity of antioxidant enzymes induced by AgNPs in the liver and kidney tissues. Data are presented as mean ± SD ($n = 7$). # and $ mean significant difference, $p < 0.05$, from control and AgNPs-treated group, respectively

histopathological examination of liver and kidney tissues confirms that AgNPs induced hepatorenal injury.

Oxidative stress could result from an imbalance between pro-oxidants and antioxidants [43]. In this study, AgNPs-induced oxidative stress is confirmed by increased generation of NO and MDA and the exhaustion of antioxidant enzymes, namely, SOD, CAT, GPx, and GR, along with the decreased level of GSH. Several reports support our findings as they reported the potency of AgNPs in the induction of oxidative stress by increasing ROS and reducing the activity of antioxidant enzymes [44–46]. Li et al. [47] suggested an explanation for enhanced ROS generation by confirming the accumulation of AgNPs in the mitochondria, leading to reduced mitochondrial membrane potential and mitochondrial impotence that may increase ROS production.

Recently, it has been well accepted that AgNPs induce oxidative stress either by targeting the gene expression of antioxidant enzymes or directly interacting with enzyme surfaces, especially SOD and CAT [48]. Wei et al. [49] proved the surface interaction between AgNPs and SOD and CAT, forming an NPs-protein enzyme complex. Subsequently, NPs-enzyme interaction leads to conformational changes and enzyme activity inhibition. Moreover, AgNPs exhibit a strong affinity for GSH thiol groups; this could explain the decreased GSH levels in liver and kidney cells [50].

Our data showed similar results of oxidative stress status in kidney tissues. These results are in accordance with previous reports [40, 51]. These studies suggested that AgNPs accumulate in kidney tissues and other target organs such as liver tissues, then dissociation into Ag^+ might lead to oxidative stress and inflammation.

TQ, the main constituent of *Nigella sativa*, has been investigated for its immunomodulatory, antioxidant, anti-inflammatory, and anticarcinogenic effects [52]. These advantageous effects prompted us to assume that TQ could control AgNPs toxicity. Therefore, in this work, we investigated the possible hepatic and renal protective effect of TQ against AgNPs-induced oxidative, inflammatory, and apoptotic effects.

TQ co-administration significantly improved the levels of serum liver and kidney function markers in AgNPs-intoxicated rats. These results confirmed the protective effect of TQ. The antioxidant property is the main effect of TQ. Previous studies attributed the capability of TQ to protect against several conditions such as hepatic cancer and ischemia-induced liver injury to oxidative stress suppression as TQ could decrease NO synthesis through direct suppression of nitric oxide synthases (iNOS and eNOS) besides TQ's ability to normalize the depleted GSH level and antioxidant enzymes [53]. Moreover, previous studies confirm the potency of TQ to protect against oxidative stress induced by several hepatotoxic agents such as CB 1954 [54], cisplatin [55], cyclophosphamide [56], Aflatoxins [57], and carbon tetrachloride [58] through decreasing lipid peroxidation and NO synthesis along with increased expression of antioxidant enzymes. Our data revealed that TQ could relieve the oxidative stress induced by AgNPs in hepatic and renal tissues. TQ decreased the levels of MDA and NO and increased the levels of GSH and antioxidant enzyme activities in AgNPs-intoxicated rats.

To get more information about the ability of TQ to ameliorate AgNPs-induced oxidative stress, the expression level of Nrf2 was estimated using qRT-PCR in hepatic and renal tissues. Nrf2 is a key transcription factor that regulates the transcription of the antioxidant enzymes by binding to the antioxidant response element (ARE) in the DNA [59]. Our data revealed that AgNPs intoxication leads to downregulation of Nrf2 expression in both hepatic and renal tissues. These findings may clarify the reason behind the decreased activity of antioxidant enzymes in liver and kidney tissues. On the other hand, TQ treatment normalized the expression of hepatic and renal Nrf2 in AgNPs-intoxicated rats. Our findings reveal the ability of TQ to mitigate the oxidative stress induced by AgNPs in hepatic and renal tissues.

Once the oxidative balance is impaired, the inflammatory response and mitochondria-related cell death will follow. Increased ROS production promotes inflammatory signals that lead to increased production of pro-inflammatory cytokines such as IL-1β and TNF-α and TGF-β. These pro-inflammatory cytokines are commonly used as a marker for toxicant-induced inflammation [60]. Herein, we evaluated the inflammatory effect of AgNPs and the possible anti-inflammatory effect of TQ. Our data confirmed the inflammatory impact of AgNPs represented by increased hepatic and renal pro-inflammatory cytokines, IL-1β, TNF- α, and TGF-β, and increased NF-κB level. The activation of NF-κB regulates the expression of pro-inflammatory cytokines genes [61].

In line with our results, previous reports concluded the inflammatory effect of different sizes of AgNPs on various organs. Shehata et al. [29] demonstrated that oral administration of AgNPs resulted in increased inflammatory cytokines in liver and kidney tissues of rats. Choia et al. [62] confirmed the hepatotoxic and inflammatory effect of AgNPs in adult zebrafish. Other research groups revealed the toxic and inflammatory effect of AgNPs manifested by increased inflammatory cytokines such as IL-1β and TNF-α and TGF-β in mice [63], guinea pig [64], and in vitro studies on hepatic cells [65].

On the other hand, our data showed that TQ pre-treatment prevented AgNPs-induced hepatic and renal

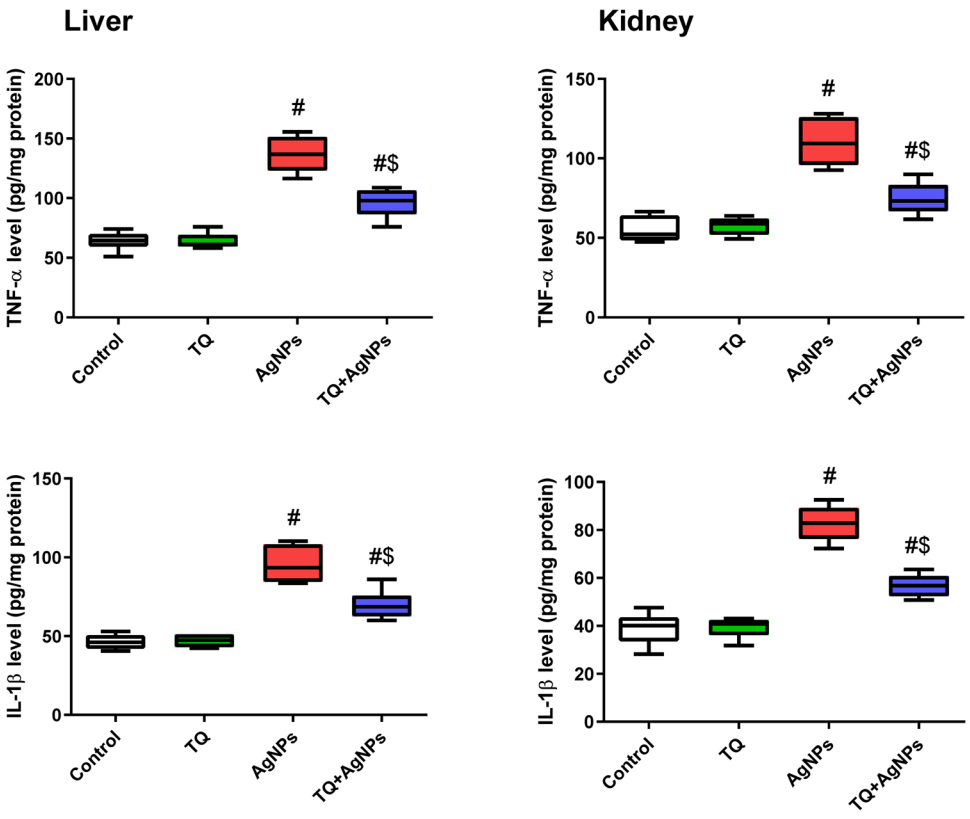


Fig. 3 The effect of TQ on the mRNA expression of Nrf2 in the liver and kidney tissues following the exposure to AgNPs. Data are presented as mean ± SD ($n = 7$). # and $ mean significant difference, $p < 0.05$, from control and AgNPs-treated group, respectively

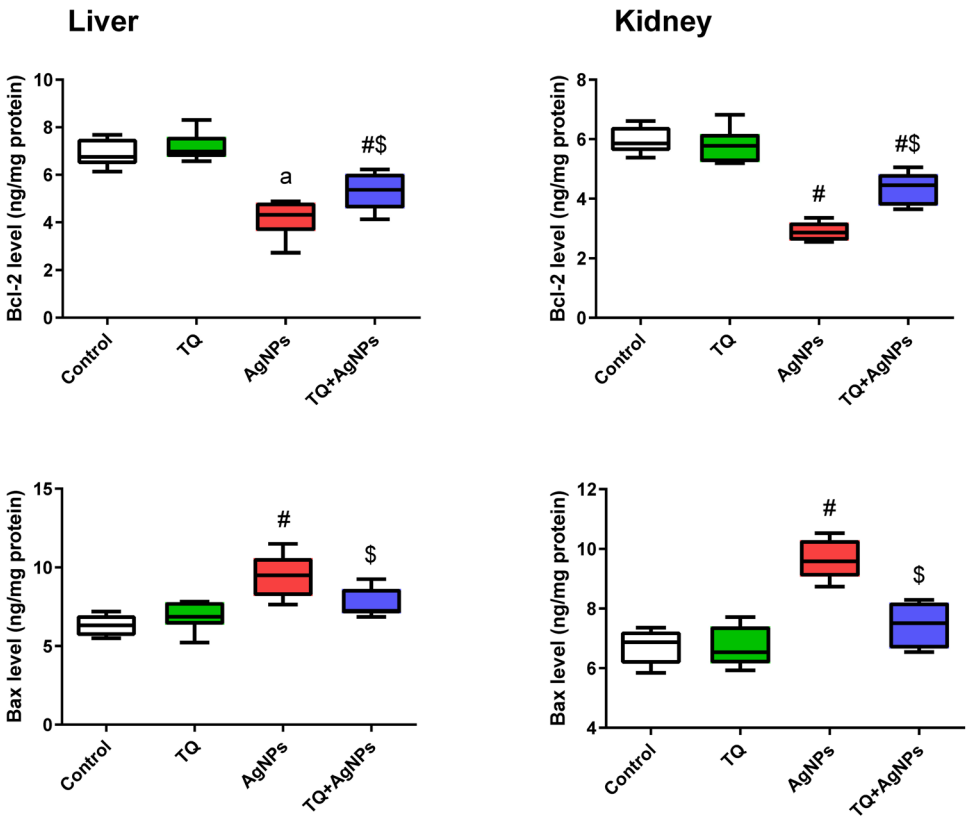


Fig. 4 The protective effect of TQ against AgNPs-induced inflammation response (IL-1β, TNF- α, and TGF-β) in the liver and kidney tissues. Data are presented as mean ± SD ($n = 7$). # and $ mean significant difference, $p < 0.05$, from control and AgNPs-treated group, respectively

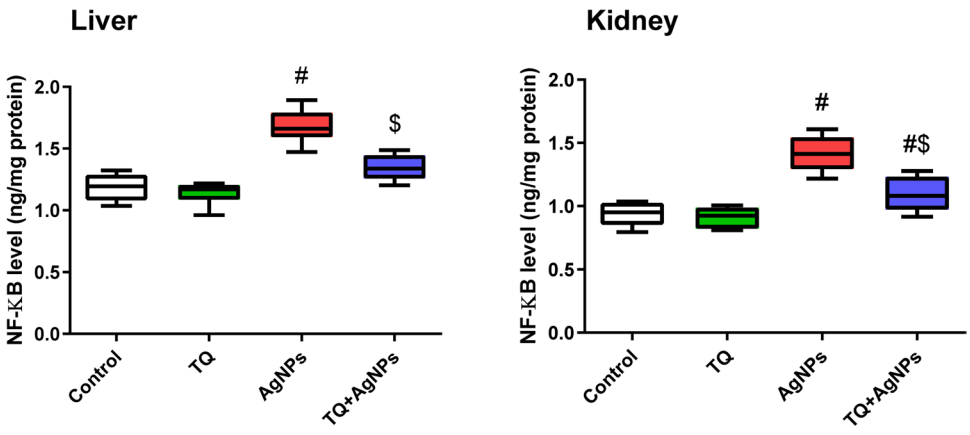


Fig. 5 The effect of TQ co-administration on NF-κB level in the liver and kidney tissues. Data are presented as mean ± SD ($n = 7$). # and $ mean significant difference, $p < 0.05$, from control and AgNPs-treated group, respectively

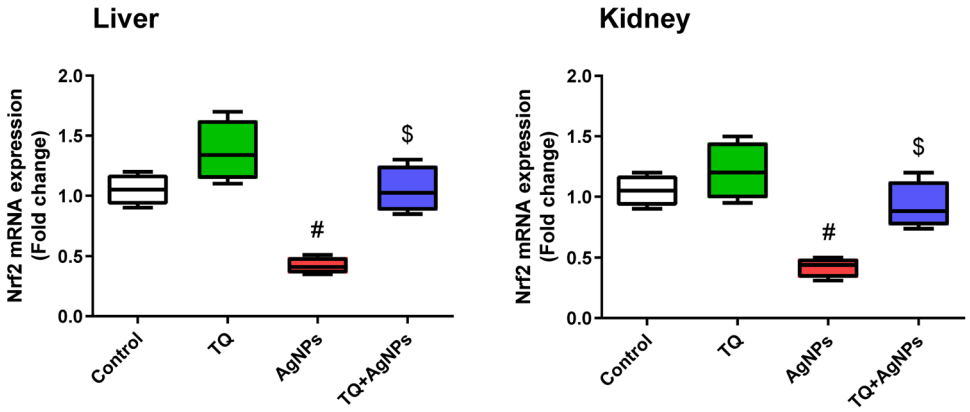


Fig. 6 The protective effect of TQ on the apoptotic markers (Bax and Bcl-2) in response to AgNPs exposure in the liver and kidney tissues. Data are presented as mean ± SD ($n = 7$). # and $ mean significant difference, $p < 0.05$, from control and AgNPs-treated group, respectively

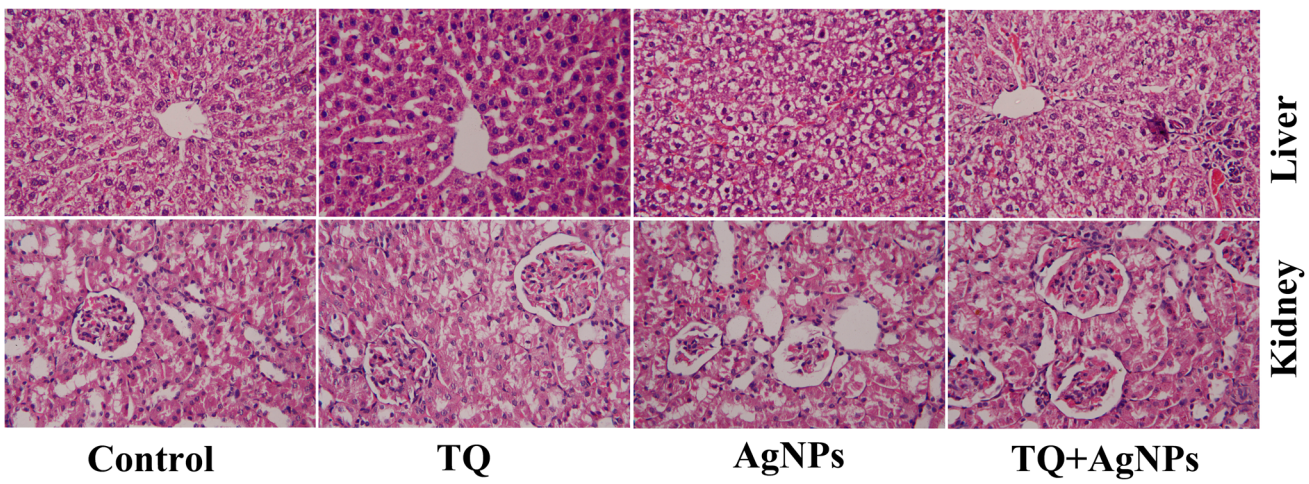


Fig. 7 TQ's protective impacts on the histological alterations in liver and kidney tissues following exposure to AgNPs (×400)

inflammatory responses, as evidenced by the decreased pro-inflammatory cytokines and NF-κB levels. It is well documented that TQ possesses an anti-inflammatory effect that protects several disorders such as asthma, arthritis, diabetes, and neuroinflammatory diseases. The noted anti-inflammatory effect of TQ is proved by decreased pro-inflammatory cytokines and suppression of the NF-κB pathway [66].

Bax regulates apoptosis as a pro-apoptotic protein and Bcl-2 as an anti-apoptotic protein; therefore, we investigated the effect of AgNPs and TQ on these protein levels. Our data support previous reports [7, 67] about the apoptotic effect of

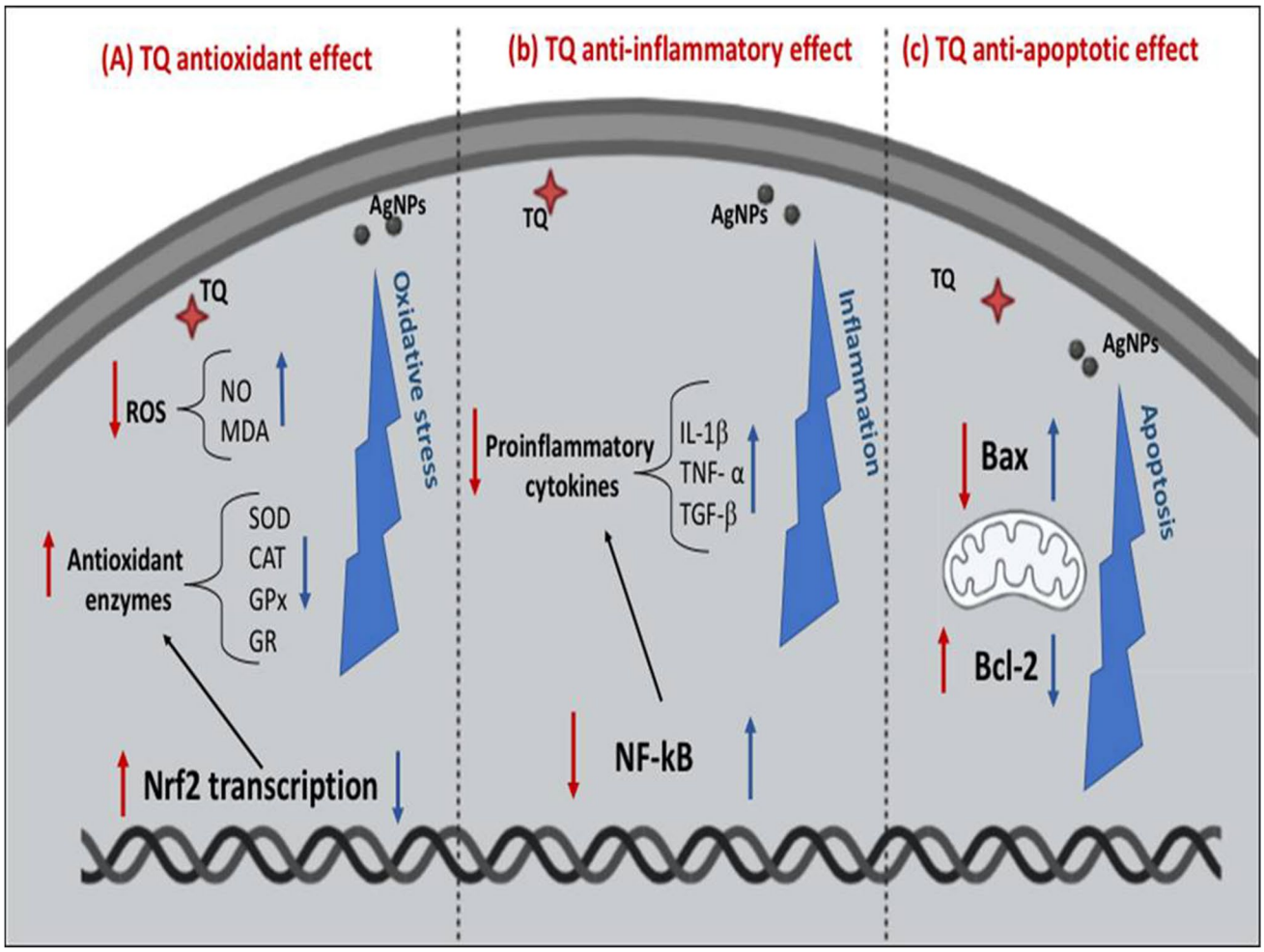


Fig. 8 Schematic diagram shows the protective effect of TQ against AgNPs-induced hepatorenal toxicity. **A** illustrates the possible mechanism of TQ antioxidant effect. **B** illustrates the possible mechanism of TQ's anti-inflammatory effect. **C** illustrates the possible anti-apoptotic effect of TQ anti-apoptotic effect. Blue arrows represent the effect of AgNPs, and red arrows represent the effect of TQ. Up-directed arrows (↑) mean significant increase, and down-directed arrows (↓) mean significant decrease

AgNPs in both hepatic and renal tissues manifested by elevated Bax and decreased Bcl-2 levels. Besides, immunohistochemistry revealed increased hepatic and renal caspase-3 levels. These data demonstrate the mechanism of AgNPs-induced apoptosis. Our results showed that TQ significantly reversed the changes in Bax and Bcl-2, confirming the protective anti-apoptotic effect of TQ against AgNPs toxicity. These results agreed with previous studies that show the anti-apoptotic effect of TQ [68, 69].

Conclusion

Our study concluded the protective effect of TQ against AgNPs-induced hepatorenal toxicity in rats. Moreover, as shown in Fig. 8, we proposed the possible mechanism underlying TQ antioxidant, anti-inflammatory, and anti-apoptotic effects. Based on the recorded results, we recommend the usage of TQ to control AgNPs toxicity, improve AgNPs safety, and increase the outcomes of AgNPs application. However, further investigation is required to understand the molecular mechanisms implicated in the antioxidative activity of TQ against the exposure to AgNPs.

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Author Contribution Conceptualization and supervision: A.E.A., R.B.K., and A.A.; animal treatments and molecular and biochemical methodologies were performed by B.S., K.J.A., K.S.A., O.A., K.E.H., and M.A.E.; histological methodology and investigation were conducted by F.A., H.A.A., H.A., and A.S.F.; data analysis, software, data curation, and visualization were performed by H.K.A., M.S.L., K.F.A., and A.A.; writing-reviewing and editing manuscript was performed

by B.S., A.E.A., and R.B.K. All authors participated in the design and interpretation of the study and approved the final manuscript.

Data Availability All relevant data are within the paper.

Declarations

Ethics Approval The study protocol was reviewed and approved by the institutional animal care and use committee, Faculty of Science, Helwan University (HU/2020/Z/AEN0120-01), following the European Community Directive (86/609/EEC).

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

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