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Potential Impact of Quercetin and Idebenone against Immuno- inflammatory and Oxidative Renal Damage Induced in Rats by Titanium Dioxide Nanoparticles Toxicity

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Abstract: The aim of this study was to investigate the toxic impacts of titanium dioxide nanoparticles (TiO_2-NPs) on rat kidneys and the possible prophylactic role of either quercetin or idebenone. TiO_2-NPs were administered orally at either 600 mg or 1 g/kg body weight for 5 consecutive days to evaluate dose-dependent toxicity referred to the OECD guidelines for testing of chemicals. The results showed that administration of either low or high repeated doses of TiO_2-NPs to rats significantly increases serum kideney function biomarkers (urea, creatinine and uric acid) as well as increases in serum glucose and serum immuno- inflammatory biomarkers including tumor necrosis factor- α (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP), immunoglobin g (IGg), vascular endothelial growth factor (VEGF, angiogenic factor) and nitric oxide (NO) with a concomitant decrease in renal GSH content versus normal control values. The increase in these biomarkers was more evident in rats intoxicated with high TiO_2-NPs repeated doses. Oral co- administration of either quercetin or idebenone (each 200mg/Kg body weight) daily for three weeks to rats intoxicated by either of the two doses markedly ameliorated TiO_2-NPs induced alteration in the above biomarkers. The prophylactic impacts of both agents on biochemical markers were more pronounced in rats received low TiO_2-NPs repeated doses. The biochemical investigation was supported by histological examination. In conclusion, The data showed the severity in renotoxicity of TiO_2-NPs was dose-dependent and the protective effect of quercetin and idebenone may be related to their antioxidant and anti-inflammatory properties.

Key words: quercetin, idebenone, renal, TiO_2, pro-inflammatory biomarkers

1 INTRODUCTION

With the increased application of nanomaterials, the discharge to environment through production, transportation, storage, and consumption process could be rapidly increased and it may exhibit adverse effects to human health. When inhaled, nanoparticles are efficiently deposited into lung cells. Translocation through epithelial and endothelial cells into the blood and lymph circulation may be occurred to reach potentially sensitive target sites including bone marrow, lymph nodes, spleen, heart, liver and, kidneys[1-3].

Titanium dioxide nanoparticles (TiO_2-NPs) have been widely used as a white pigment in paint, food colorant, ultraviolet blocker in cosmetics, welding rod-coating material, disinfectant in environment and wastewater, and photosensitizer for photodynamic therapy[4]. Experimental study showed that exposure to TiO_2 –NPs caused serious inflammatory damage to the liver, kidney, and myocardium of mice reflected by the alteration of serum biomarkers of these organ functions and disturbed the balance of blood sugar and lipid[3]. Also, some authors reported that TiO_2-NPs were accumulated in the kidney, resulting in nephric inflammation, cell necrosis and dysfunction. Nucleic

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factor-κB was activated by TiO_2- NPs exposure, promoting the expression levels of tumor necrosis factor-α, macrophage migration inhibitory factor, interleukin-2, interleukin-4, interleukin-6, interleukin-8, interleukin-10, interleukin-18, interleukin-1β, cross-reaction protein, transforming growth factor-β and interferon-γ[5]. Another experimental study revealed that the nephrotoxicity like pathology change of kidneys was observed after the exposure to TiO_2-NPs[2]. Also, exposure of the cultured BEAS-2B cells s to nanoparticles led to cell death, reactive oxygen species (ROS) increase, reduced glutathione (GSH) decrease, and the induction of oxidative stress-related genes such as heme oxygenase-1, thioredoxin reductase, glutathione-S-transferase, catalase[6].

The abnormalities in the antioxidant defense system and increased oxidative stress may lead to higher susceptibility to tissue damage. So, antioxidant may play an important role in protection against oxidative damage

The flavonol quercetin (3,3',4',5,7-pentahydroxyflavone) is one of the most abundant dietary flavonoids. Quercetin was reported to possess antioxidant, anti-hypertension, antimicrobial and antiprotozoan activities[7–10]. Quercetin, known as an anti-inflammatory/anti-allergy natural remedy, stabilizes mast cell membranes and prevents the release of histamine and other inflammatory agents in the body; it has been shown to reduce IL-6 and TNFα in lipopolysaccharide -challenged murine macrophages[11]. It has protective effect against renotoxicity, induced by the toxic effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin, via increased the levels of antioxidant defense system and preventing the histological damage in kidney[12].

Idebenone, a short-chain benzoquinone structurally related to coenzyme Q10, is a potent antioxidant and electron carrier[13]. The antioxidant properties of idebenone were determined by the inhibition of oxidative stress markers, including lipid peroxidation through its free radical scavenger activity[14, 15]. It has protective effect on cerebral energy metabolism[16, 17]. It also protects against mitochondrial damage and enhances respiration by improving the flux of electrons along the electron transport chain[18].

The objective of this work was to investigate the toxic effects of titanium dioxide nanoparticles (TiO_2-NPs) on rat kidneys and the possible prophylactic role of either quercetin or idebenone. The study was intended to address the toxicological activities of TiO_2-NPs at two doses (low and high doses) to determine the dose dependent renal pathological damages. The study was extended to investigate the possible renoprotective role of quercetin and idebenone, each alone, when co- administered with either of the two doses of TiO_2-NPs.

2 EXPERIMENTAL

2.1 Chemicals

The TiO_2-NPs (<100 nm) powders were purchased from Sigma Co. (USA). All other chemicals used in the study were of high analytical grade, product of Sigma and Merck companies.

2.2 Animals and treatments

Animal experiment was performed with compliance of the local ethics committee, 70 healthy male albino rats (120-150 g.) were supplied by the Experimental Animal Center, King Saud University. The animals were housed in clean polypropylene cages and maintained in an air-conditioned animal house at 20±2℃, 50–70% relative humidity and 12-h light/dark cycle. The animals were provided with commercial rat pellet diet and deionized water ad libitum. After one week acclimation, the rats were kept fasting over night before treatment.

TiO_2 was administered using two doses (600 mg/ Kg body or 1 g/ Kg body weight/day) according to the OECD[19] procedure. Rats were randomly divided into two classes according to the dose of TiO_2-NPs.

Class I comprised 5 groups of 10 rats each. In this class, ZnO-NPs were administered orally at 600 mg/kg body weight per day for 5 consecutive days. The groups were divided as follows;

Class I, groups of animals ingested low repeated doses (600 mg/ Kg body weight/day) and consists of four groups, each of ten rats

G1: Normal healthy animals

G2-G4 groups of animals administered orally 600 mg/ Kg body weight/day TiO_2-NPs for 5 consecutive days and divided as follow

G2: TiO_2- intoxicated animals

G3: TiO_2- intoxicated animals co-administered with quercetin (200 mg/Kg) (20).

G4: TiO_2- intoxicated animals co-administered with idebenone (200 mg/Kg) (21).

ClassII consists of 3 groups (G5-G7), of ten rats each. In this class TiO_2-NPs were administered orally at 1 g/ Kg body weight/ day for 5 consecutive days. The groups were divided as follow

G5: TiO_2- intoxicated animals for 5 consecutive days

G6: TiO_2- intoxicated animals with co-administration of quercetin (200 mg/Kg)

G7: TiO_2- intoxicated animals with co-administration of idebenone (200 mg/Kg)

TiO_2-NPs were suspended in 1% tween 80 and dispersed by ultrasonic vibration for 15 min. The control group was given 1% Tween solution instead. Quercetin and idebenone were orally administered daily for three weeks.

Three weeks later, the rats of all groups were kept fasting over night (12-14 h), then the blood samples were collected from each animal in all groups into sterilized

tubes for serum separation. Serum was separated by centrifugation at 3000 r.p.m. for 10 minutes and used for biochemical serum analysis. After blood collection, rats of each group were sacrificed under ether anesthesia and the renal samples were collected, weighed, and washed using chilled saline solution. The kidneys were minced and homogenized in ice-cold bi-distilled water to yield 10% homogenates using a glass homogenizer. The homogenates were centrifuged for 15 min at 4,000 rpm at 4℃, and the supernatants were used for biochemical tissue analysis.

2.2.1 Biochemical serum analysis

Serum samples were assayed for urea, creatinine, and uric acid as indicators of kidney function by using standard diagnostic kits. Glucose level was estimated using Diamond Diagnostic Kits[22]. TNF-α was quantified using a commercial ELISA kit (Endogen, Woburn, MA). IL-6 was measured by ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN) with an analytical CV of 6.3% and a detection level of 0.04 pg/mL[23]. IgG level was measured in serum using a sandwich enzyme-linked immunosorbent assay (ELISA) (Sigma Chemical Co., St. Louis, MO). CRP was measured with latex-enhanced immunonephelometry on a Behring BN II Nephelometer (Dade Behring). In this assay, polystyrene beads coated with rat monoclonal antibodies bind CRP present in the serum sample and form aggregates. The intensity of the scattered light is proportional to the size of the aggregates and thus reflects concentration of CRP present in the sample. The intra-assay and interassay coefficients of variation for CRP were 3.3% and 3.2%, respectively. The lower detection limit of the assay was 0.15 mg/L[24]. The level of VEGF in serum was determined at 492 nm by quantitative colorimetric sandwich enzyme linked immunosorbent assay (ELISA; R&D systems, UK) in accordance with the manufacturer's instructions[25]. Concentrations were calculated using a standard curve generated with specific standards provided by the manufacturer. Nitrite concentration (an indirect measurement of NO synthesis) was assayed spectrophotometrically using Griess reagent (sulfanilamide and N-1-naphthylethylenediamine dihydrochloride) in acidic medium[26].

2.2.2 Biochemical assay of kidney tissue

The reduced glutathione (GSH) content of kidney was determined using the method of Bentler *et al.*[27] based on its reaction with 5,5'-dithiobis (2- nitrobenzoic acid) to yield the yellow chromophore, 5-thio-2-nitrobenzoic acid at 412 nm.

2.2.3 Histopathological techniques

Kidney samples were fixed in 4% buffered formaldehyde for 24 hours, followed by paraffin wax embedding. Sections (5-6 μm in thickness) were prepared and stained with hematoxylin and eosin (H&E) for histopathological examination of the kidney tissue. Staining with Masson's Trichrome (Sigma, USA) was used as a marker of fibrosis to assess the degree of fibrosis by identifying collagen fibers in kidney tissues. All the slides were examined under a light microscope.

Statistical analysis

Data were statistically analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean ± SD. Significant differences among values were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's test post-ANOVA. Value of $p < 0.05$ was considered statistically.

3 RESULTS

Table 1 and 2 respectively showed that the administration of TiO_2-NPs, either in low or in high repeated doses, significantly elevated serum kideney function biomarkers (urea, creatinine and uric acid) compared with normal control values ($p < 0.001$). The increase in these biomarkers was pronunced in rats intoxicated with high repested doses. Oral administration of quercetin or idebenone significantly reduced the levels of these markers compared with TiO_2-NPs intoxicated counterparts ($p < 0.001$).

The levels of serum glucose (Fig. 1), serum inflammatory markers, TNF- α (Fig 2), IL-6 (Fig. 3), CRP (Fig. 4), were markedly elevated in rats under the effect of either low or high repeated doses of TiO_2-NPs toxicity versus normal control values ($p < 0.001$). Also, TiO_2-NPs ingestion, using either low or high repeated doses, significantly increased serum IgG, VEGF and NO (Figs. 5, 6 and 7 respectively), with a concomitant decrease in renal GSH content compared with normal control values ($p < 0.001$) (Table 3). The toxic effect of the used metal oxide –NPs on these biomarkers was evident to some extent in rats received high repeated doses. Oral co-administration of quercetin or idebenone to TiO_2-NPs intoxicated rats significantly modulated the previously mentioned markers in relation to toxicated untrated animals ($p < 0.001$). The results of these biochemical markers were supported by the histopathological examination of renal tissues. This examination revealed that the animals which received a low dose of TiO_2-NPs showed atrophy of some glomeruli, degeneration of epithelial cells of some renal tubules and casts in lumina of a few tubules (Fig. 8B). Staining of renal tissue of intoxicated rats with Masson's trichrome, showed slight increase of collagen fibers in the interstitial tissue (Fig. 10B). Co-treatment of TiO_2- NPs –intoxicated rats with either quercetin or idebenone, showed normal feature of glomeruli and renal tubules (Fig. 8C and D respectively) as well as normal distribution of collagen fibers in the interstitial tissue (Fig. 10C and D respectively). Rats received high repeated doses of TiO_2-NPs (Fig. 9B) showed atrophy of most glomeruli, epithelial degeneration, necrosis and casts in

Table 1 Impact of either quercetin (Qur) or idebenone (Id) treatment on renal function biomarkers in rats intoxicated with low repeated doses of TiO_2 -NPs.

Groups	Urea (mg/dl)	Uric acid (mg/dl)	Creatinin (mg/dl)
Control	21.38 ± 2.70	2.19 ± 0.13	0.52 ± 0.01
TiO_2-NPs	30.68 ± 1.90^{a}	4.32 ± 0.35^{a}	0.87 ± 0.04^{a}
Qur	25.50 ± 1.10^{c**}	3.21 ± 0.23^{**b}	0.60 ± 0.03^{***b}
Id	26.40 ± 1.10^{c**}	2.94 ± 0.39^{**b}	0.59 ± 0.02^{***c}

Values are expressed as mean ± SD of 6 animals. $^{a}p \leq 0.001$, $^{b}p \leq 0.01$, $^{c}p \leq 0.05$ compared with the normal group, $^{***}p \leq 0.001$, $^{**}p \leq 0.05$ compared with TiO_2- NPs intoxicated group, using ANOVA followed by Bonferroni as a post-ANOVA test

Table 2 Impact of either quercetin or idebenone treatment on renal function biomarkers in rats intoxicated with high repeated doses of TiO_2 -NPs.

Groups	Urea (mg/dl)	Uric acid (mg/dl)	Creatinin (mg/dl)
Control	21.38 ± 2.70	2.19 ± 0.13	0.52 ± 0.01
TiO_2-NPs	41.70 ± 3.30^{a}	5.93 ± 0.46^{a}	0.92 ± 0.03^{a}
Qur	26.78 ± 2.60^{***c}	3.40 ± 0.22^{***b}	0.71 ± 0.02^{***b}
Id	29.10 ± 1.80^{***c}	3.08 ± 0.54^{***b}	0.70 ± 0.03^{***b}

Values are expressed as mean ± SD of 6 animals. $^{a}p \leq 0.001$, $^{b}p \leq 0.01$, $^{c}p \leq 0.05$ compared with the normal group, $^{***}p \leq 0.001$ compared with TiO_2- NPs intoxicated group, using ANOVA followed by Bonferroni as a post-ANOVA test

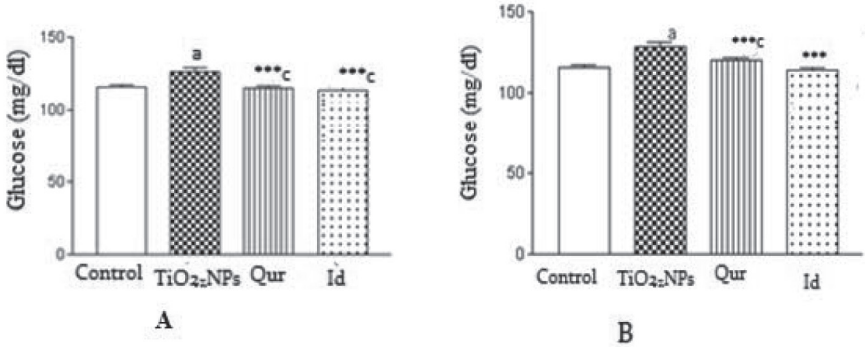

Fig. 1 Effect of either quercetin (Qur) or idebenone (Id) treatment on serum glucose in rats intoxicated with either low (A, 600 mg/kg body weight) or high (B, 1 g/kg body weight) repeated doses of TiO_2 -NPs. Values are expressed as mean±SD of 6 animals. $^{a}p \leq 0.001$, $^{c}p \leq 0.05$ compared with the normal group, $^{***}p \leq 0.001$, compared with TiO_2- NPs intoxicated group, using ANOVA followed by Bonferroni as a post-ANOVA test.

renal tubules (Fig. 9B). An increase of collagen fibers in the interstitial tissue was also observed (Fig. 11B) Co-ingestion of either quercetin or idebenone to rats along with TiO_2- NPs greatly improved the histomorphological pictures of rat kidneys as observed by normal feature of glomeruli and most of the renal tubules (Fig. 9C and D respectively) with a little increase of collagen fibers in the interstitial tissue (Fig. 11C and D respectively).

DISCUSSION

TiO_2-NPs induced renotoxicity has been previously documented by a number of studies[2, 3, 5]. Also, Kidney damage with morphological, pathological, and cellular changes leading to kidney dysfunction after exposure to NPs has been studied[28), 29)].

The present study demonstrated that administration of either low or high doses of TiO_2-NPs (600mg or 1 g/ Kg body weight/day) for five consecutive days induced neph-

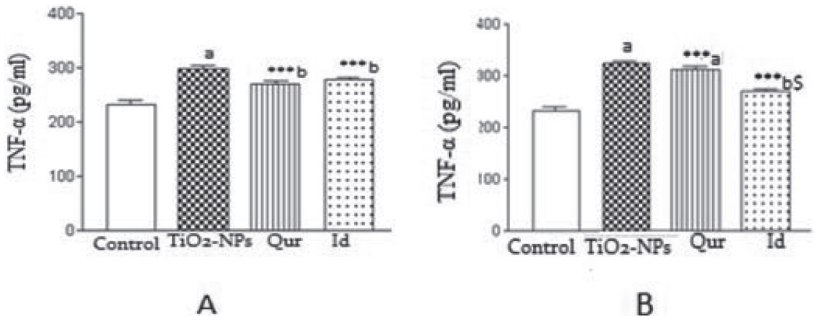

Fig. 2 Effect of either Qur or Id treatment on serum TNF-α in rats intoxicated with either low (A) or high (B) repeated doses of TiO2 -NPs. Values are expressed as mean±SD of 6 animals. $^{a}p \leq 0.001$, $^{b}p \leq 0.01$ compared with the normal group, $^{***}p \leq 0.001$, compared with TiO_2- NPs intoxicated group, $^{\$}p \leq 0.01$ compared with Qur group using ANOVA followed by Bonferroni as a post-ANOVA test.

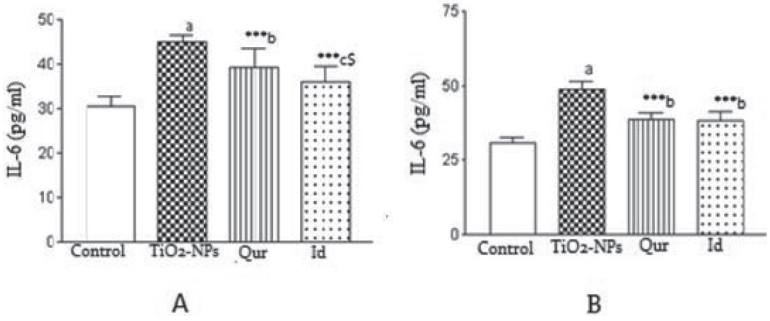

Fig. 3 Effect of either Qur or Id treatment on serum IL-6 in rats intoxicated with either low (A) or high (B) repeated doses of TiO_2 -NPs. Values are expressed as mean±SD of 6 animals. $^{a}p \leq 0.001$, $^{b}p \leq 0.01$, $^{c}p \leq 0.05$ compared with the normal group, $^{***}p \leq 0.001$, compared with TiO_2- NPs intoxicated group, $^{\$}p \leq 0.05$ compared with Qur group using ANOVA followed by Bonferroni as a post-ANOVA test.

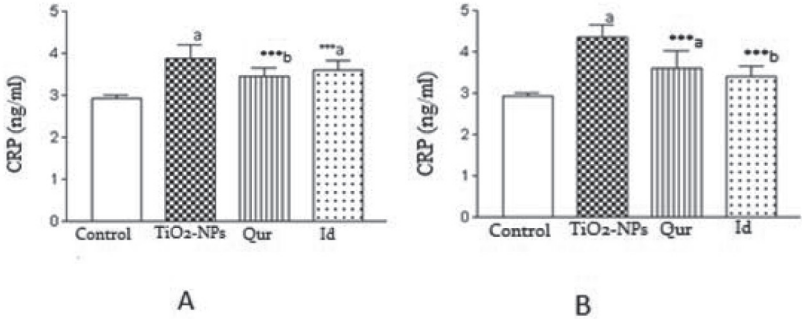

Fig. 4 Effect of either Qur or Id treatment on serum CRP in rats intoxicated with either low (A) or high (B) repeated doses of TiO_2 -NPs. Values are expressed as mean±SD of 6 animals. $^{a}p \leq 0.001$, $^{b}p \leq 0.01$ compared with the normal group, $^{***}p \leq 0.001$, compared with TiO_2- NPs intoxicated group, using ANOVA followed by Bonferroni as a post-ANOVA test.

rotoxicity in rats, as demonstrated by the significantly increased levels of serum urea, uric acid and creatinine. The alteration in these kidney function biomarkers was sever in rats exposed to high dose of TiO_2-NPs. The renotoxic effect of TiO_2-NPs was further confirmed by the destructed renal tissue which was severe in rats received high doses, as shown in the histological analysis. Our data are similar with many studies indicate that impairment of kidney functions and abnormal pathological changes with severe inflammatory response of kidney in animals exposed to different TiO_2-NPs doses[2, 5, 30]. Administration of either quercetin or idebenone with TiO_2-NPs exposure greatly alleviated TiO_2-NPs -induced kidney dysfunction. This protective effect was remarkable in rats ingested low TiO_2-NPs. Similar effect was found with quercetin which showed protective effect against the histological damage in kidney induced by

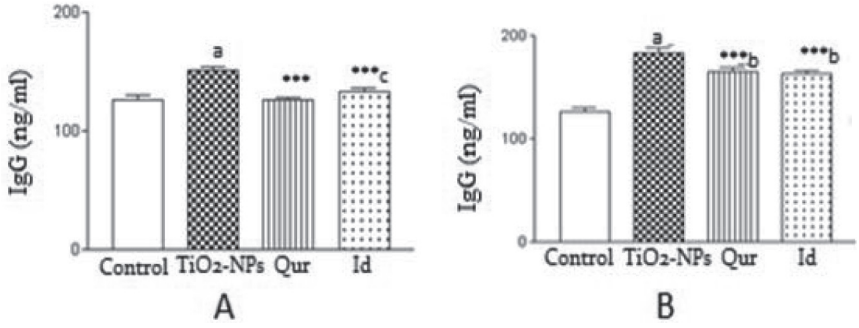

Fig. 5 Effect of either Qur or Id treatment on serum IgG in rats intoxicated with either low (A) or high (B) repeated doses of TiO_2 -NPs. Values are expressed as mean±SD of 6 animals. $^{a}p \leq 0.001$, $^{b}p \leq 0.01$, $^{c}p \leq 0.05$ compared with the normal group, $^{***}p \leq 0.001$, compared with TiO_2- NPs intoxicated group, using ANOVA followed by Bonferroni as a post-ANOVA test.

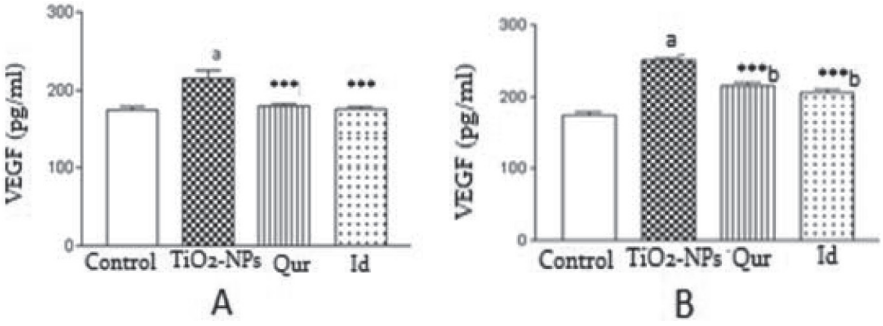

Fig. 6 Effect of either Qur or Id treatment on serum VEGF in rats intoxicated with either low (A) or high (B) repeated doses of TiO_2 -NPs. Values are expressed as mean±SD of 6 animals. $^{a}p \leq 0.001$, $^{b}p \leq 0.01$, compared with the normal group, $^{***}p \leq 0.001$ compared with TiO_2- NPs intoxicated group, using ANOVA followed by Bonferroni as a post-ANOVA test.

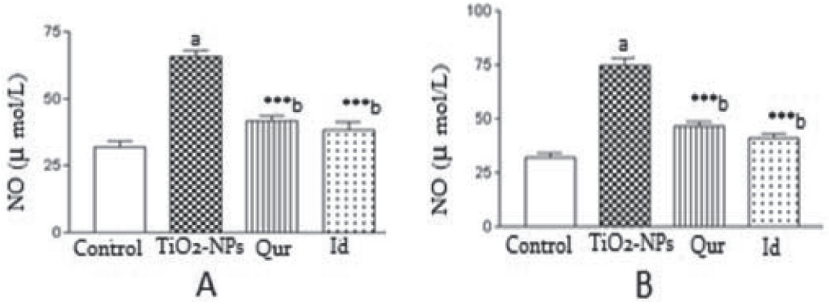

Fig. 7 Effect of either Qur or Id treatment on serum NO in rats intoxicated with either low (A) or high (B) repeated doses of TiO_2 -NPs. Values are expressed as mean±SD of 6 animals . $^{a}p \leq 0.001$, $^{b}p \leq 0.01$, compared with the normal group, $^{***}p \leq 0.001$ compared with TiO_2- NPs intoxicated group, using ANOVA followed by Bonferroni as a post-ANOVA test.

chemical toxins[12].

Previous study demonstrated that promotion of inflammatory cytokines expression by TiO_2-NPs is considered as mediators of TiO_2-NPs -induced renotoxicity[5]. This finding is coped with our result which revealed elevation in the levels of immunological pro-inflammatory biomarkers including, TNF-α, IL-6, and CRP, with concomitant increase in IGg in serum of rats intoxicated with TiO_2- NPs in relation to normal group. The inflammatory toxic effect of this metal oxide nanoparticles was more pronounced in rats intoxicated with high dose. Similarly, studies had showed that TiO_2 NPs promoted the expression of inflammatory cytokines in the lung, liver, spleen and brain of rats and mice[31-34]. The stimulation of the inflammatory mediators presented in this study may be due to the alteration in gene expression levels of these mediators by TiO_2-NPs tox-

Table 3 Impact of either quercetin or idebenone treatment on renal GSH (mg/g) content in rats intoxicated with either low or high repeated doses of TiO_2 -NPs.

Groups	Low dose	high dose
Control	1.30 ± 0.04	1.30 ± 0.04
TiO_2-NPs	0.66 ± 0.04^{***a}	0.59 ± 0.03^{***a}
Qur	0.98 ± 0.08^{***c}	0.90 ± 0.06^{***b}
Id	0.90 ± 0.01^{***b}	0.91 ± 0.05^{***b}

Values are expressed as mean ± SD of of 6 animals. $^{a}p \leq 0.001$, $^{b}p \leq 0.01$, $^{b\,c}p \leq 0.05$ compared with the normal group, $^{***}p \leq 0.001$ compared with TiO_2- NPs intoxicated group, using ANOVA followed by Bonferroni as a post-ANOVA test

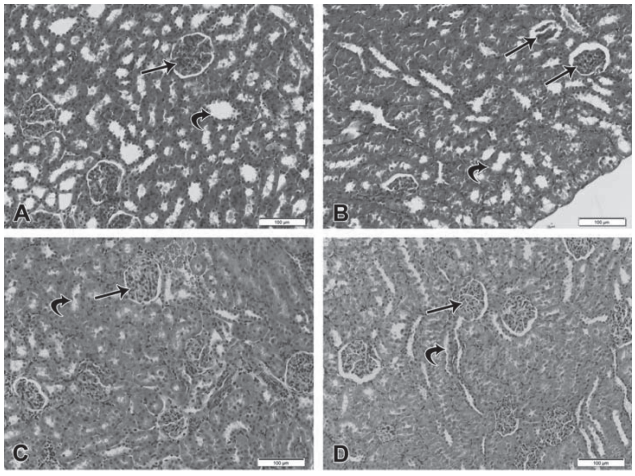

Fig. 8 Photomicrographs of kidney sections stained with H and E from animals received small dose of TiO_2- NPs. Scale bar= 100 μM. (A) Section of kidney from control animal showing normal renal corpuscle (arrow) and renal tubules (curved arrow). (B) Section of kidney from animal received low repeated doses of TiO_2- NPs showing atrophy of some glomeruli (arrows) and epithelial degeneration of most of renal tubules (curved arrows). Few renal tubules showing casts in their lumina. (C) Section of kidney of rat received small repeated doses of TiO_2- NPs and treated with Qur, showing normal feature of glomeruli (arrow) and renal tubules (curved arrow). (D) Section of kidney of rat received small repeated doses of TiO_2- NPs and treated with Id showing normal feature of glomeruli (arrow) and renal tubules (curved -arrow).

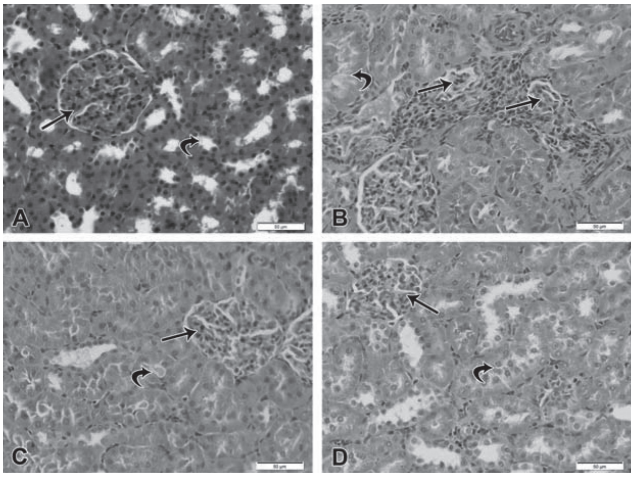

Fig. 9 Photomicrograph of kidney sections, stained with Hematoxylin and Eosin, scale bare = 50 μm. (A) Section of kidney of control rat showing normal architecture of renal corpuscles (arrow) and tubules (curved arrow). (B) Section of kidney from rat received high repeated doses of TiO_2- NPs showing atrophy of most glomeruli (arrows) and epithelial degeneration, necrosis and casts in renal tubules (curved arrow). (C) Section of kidney of rat received high dose of TiO_2- NPs and treated with Qur, showing segmentation of few glomeruli (arrow) and casts in the lumen of renal tubules with intact epithelial lining (curved arrow). (D) Section of kidney of rat received high dose of TiO_2- NPs and treated with Id showing normal feature of glomeruli (arrow) and most of the renal tubules (curved arrow).

icity. Previous studies suggested that that TiO_2-NPs exposure could significantly up-regulate mRNA expression levels of several inflammatory mediator genes, including TNF-α, IL-6 and CRP[5].

The marked increase in the circulating IgG in rat sera intoxicated with either doses of TiO_2-NPs is another immune disorder induced by this NPs toxicity. It was suggested that the increase in the circulating antibody production is the result of production of different inflammatory cytokines including TNF-α with potential impact on immunoglobulin production during inflammation[35]. Co-ingestion of either quercetin or idebenone to TiO_2-NPs markedly was effective in down- modulating the elevation in serum inflammatory markers implying their anti-inflammatory beneficial action. This anti-inflammatory effect of the two agents was also previously documented[10, 36].

In line with some authors, the present study also demonstrated that TiO_2-NPs induced elevation in serum glucose level in rats administered TiO_2-NPs which was evident in rats ingested high dose[3]. It was reported that up-regulation of CRP is closely associated with metabolic disturbances including, insulin resistance and related complications

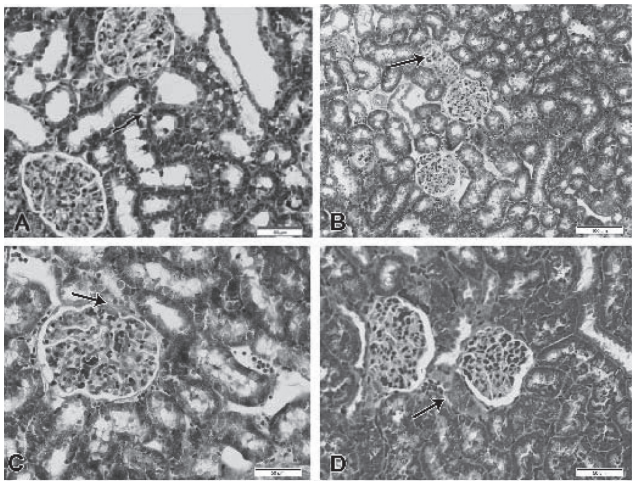

Fig. 10 Photomicrograph of kidney sections, stained with Masson's trichrome, scale bare = 50 (A) Section of kidney of control rat showing normal distribution of collagen fibers in the interstitial tissue (arrow). (B) Section of kidney from rat received low repeated doses of TiO_2-NPs showing slight increase of collagen fibers in the interstitial tissue (arrow). (C& D) Sections of kidneys of rats received small dose of TiO_2- NPs and treated with and treated with either Qur (C) or Id (D) showing normal distribution of collagen fibers in the interstitial tissue (arrow).

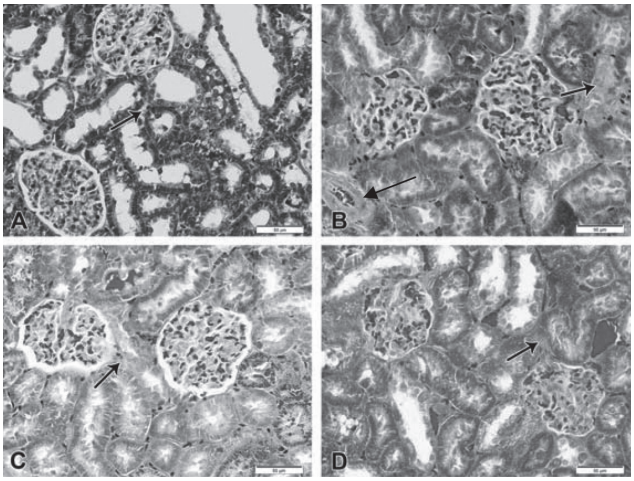

Fig. 11 Photomicrograph of kidney sections, stained with Masson's trichrome, scale bare = 50 (A) Section of kidney of control rat showing normal distribution of collagen fibers in the interstitial tissue (arrow). (B) Section of kidney from rat received high repeated doses of TiO_2-NPs showing increase of collagen fibers in the interstitial tissue (arrows). (C& D) Sections of kidneys of rats received large dose of TiO_2-NPs and treated with either quercetin, (C) or idebenone (D) showing a little increase of collagen fibers in the interstitial tissue (arrow).

such as fatty liver disease and hyperglycemia[37]. It also has a principle role in the activation of proinflammatory pathways in various cell types[38, 39]. The intake of quercetin or idebenone immediately with TiO_2-NPs ingestion presented in this study successfully down- regulated the elevation in serum glucose. The regulation in serum glucose by treatment with either agents may attributed to their ability to inhibit the production of CRP which has the major role in metabolic disturbances. Beside, quercetin was found to have hypoglycemic effect[40], however, idebenone was reported to have a regulatory effect on energy metabolism[41].

The current study showed also renal injury related to TiO_2-NPs toxicity stimulate the production of NO and the expression of VEGF in serum of TiO_2-NPs intoxicated rats versus normal ones. Clinical studies demonstrated that renal injury was found associated with renal peritubular capillaries (PTCs) loss which are essential to maintain the normal structure and function of renal tubules. The integrity of PTCs seems to be regulated by growth factors. Loss of PTCs may result in ischemia and this induce VEGF expression which is a potent regulator of angiogenesis, vascular survival, and vascular permeability[42]. Also, previous published data stated that high amounts of NO are released from the inducible NO synthase (iNOS) isoform in response to inflammatory stimuli from a variety of cell types[43]. Renal proximal tubule and inner medullary collecting duct cells can produce NO via expression of an inducible isoform of nitric oxide synthase[44]. Mesangial cells and invading immune cells are capable of expressing iNOS upon stimulation with TNF-α and IL-1b. The release of large amounts of NO in the glomerulus may lead to the progression of renal failure during several forms of glomerulonephritis[45]. The over production together with expression of various tissue factors, cytokines, and chemokines in response to tissue injury, stimulate VEGF synthesizing cells such as platelets, immune cells, and inflammatory cells[43, 46]. It was found that stimulation of angiogenesis may contribute to the transition from acute to chronic inflammation. Recent studies revealed that expression of this factor may increase the permeability of newly generated vessels. High permeability may decrease the functionality of these neovessels and may in turn facilitate renal injury in chronic renovascular disease by allowing the leakage of injurious cytokines to the extra-vascular space. Beside, VEGF has been shown to promote tumor growth and it has been suggested to play a role in promoting atherosclerosis[47, 48]. Additionally, some investigations demonstrated that TNF-α and VEGF expressions were significantly linked. Both TNF- α and VEGF may promote a procoagulant state, by increasing expres-

sion of tissue factor on endothelial cells and/or monocytes[49, 50]. Increased tissue factor expression is thought to play a significant role in the development of multi-organ system failure in acute injury[50]. This suggests the possibility that TNF- α and VEGF might act synergistically to potentiate renal injury and/or systemic organ dysfunction[51]. Co-ingestion of the studied agents each alone markedly reduced the dramatic increase in serum NO and the angiogenic biomarker in sera of TiO_2-NPs intoxicated rats which may related to their anti-inflammatory beneficial action.

A number of studies have suggested that oxidative stress induced by the imbalance between the antioxidants and production reactive oxygen species (ROS) is the major cause of TiO_2-NPs-induced cytotoxicity[6, 30, 52]. In our study, the low and high doses of TiO_2-NPs caused GSH depletion in renal tissue. Similar effect was obtained by some authors who reported the depletion of this non-enzymatic antioxidant in cultured BEAS-2B cells and human epidermal cells after exposure to TiO_2-NPs. The authors attributed this finding to the over production of ROS in response to TiO_2-NPs toxicity suggesting free radical species generated by this NPs seemed to reduce the levels of cellular antioxidants significantly and associated with an increase in lipid hydroperoxide, the hallmark of membrane damage[6, 52]. Amelioration of renal GSH level of TiO_2-NPs intoxicated rats by treatment with either quercetin or idebenone may related to antioxidant potential effect of both agents. Quercetin has protective effect against renotoxicity, induced by chemical toxins, via increased the levels of reduced glutathione, catalase, glutathione peroxidase and superoixde dismutase but decreased formation of lipid peroxidation and preventing the histological damage in kidney[12]. Idebenone has been shown to act as membrane-associated antioxidant that can prevent the formation of reactive oxygen species and reactive radicals. Idebenone could protect membranes and mitochondria from oxidative damage through inhibiting lipid peroxidation[13, 14, 53].

In conclusion, the present study, demonstrated the severity of cytotoxic impacts of TiO_2-NPs on renal tissue of rats was dose-dependent. This was pronounced from the elevation in serum renal function biomarkers, elevation in inflammatory mediators (TNF, IL6, and CRP), IgG, VEGF, and NO. These toxic effects were more evident in rats received high repeated doses of TiO_2-NPs and were supported by the histopathological examination. Treatment with either quercetin or idebenone was more effective in alleviating the damaging impacts of the used meta oxide-NPs in rats intoxicated with low repeated doses than counterparts intoxicated with high repeated doses. This may be related to their ability to neutralize the liberated ROS and inhibit the inflammatory cytokines induced by such nanoparticles.

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