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Moringa oleifera Leaves Extract Protects Titanium Dioxide Nanoparticles-Induced Nephrotoxicity via Nrf2/HO-1 Signaling and Amelioration of Oxidative Stress

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Abstract

The efficacy of Moringa oleifera leaf extract (MO) in alleviating nephrotoxicity induced by titanium dioxide nanoparticles (TiO_2 NPs) was studied. Rats were divided into four groups. Group I received distilled water. Group II received TiO_2NPs. Group III received both TiO_2NPs suspension beside MO. Group IV received MO only. Kidney KIM-1, NF-κB TNF-α, and HSP-70 expression were significantly upregulated while both Nrf2 and HO-1were significantly downregulated in TiO_2NPs-treated rats. MO decreases expression of KIM-1, NF-κB, TNF-α, and HSP-70. In addition, MO has markedly upregulated the expression of Nrf2 and HO-1. In conclusion, MO can inhibit nephrotoxicity by suppressing oxidative stress and inflammation. These effects are suggested to be mediated by activating Nrf2/HO-1.The biochemical analysis and histopathological finding reinforced these results. These data support the antioxidant properties' nutraceutical role of MO against TiO_2NPs-induced toxicity.

Keywords Titanium dioxide nanoparticles · Moringa oleifera · Nephrotoxicity · Nrf2/HO-1 · Oxidative stress

Introduction

With the widespread applications of Titanium dioxide nanoparticles TiO_2NPs, a health concern has been created. TiO_2NPs is used in food colorant, cosmetics, plastic, and disinfectants [1]. Some studies reported that TiO_2NPs were accumulated in the kidney tissue, resulting in cell dysfunction and necrosis [2]. Studies have revealed several mechanisms by which these nanoparticles cause toxicity. TiO_2NPs may cause genetic toxicity through changing the structure of the molecular complex and the permeability of cell membrane [3, 4]. TiO_2NPs may produce oxidative stress. During oxidative stress, reactive oxygen species (ROS), such as hydroxyl radicals, are generated and cause DNA oxidation, generating 8-OHG, leading to errors and mutations in DNA replication [5, 6]. Also, TiO_2NPs induce different adverse effects on kidney as initiating inflammatory mechanisms and apoptosis and promote the oxygen-free radicals resulting in DNA damage, genetic instability, and cytotoxicity [7]. In other hands, high doses of TiO_2NPs significantly damaged the functions of the liver and kidney. TiO_2NPs caused damages in mitochondria and apoptosis of hepatocytes, generation of reactive oxygen species, and expression disorders of protective genes in the liver of mice [8]. The wide use of engineered nanomaterials in many fields, urged the scientific community to understand the processes behind their potential toxicity, in order to develop new strategies for human safety [9].

Moringa oleifera (MO) is one of the Moringaceae family that known as drumstick tree, all parts of the plant having a remarkable range of functional and nutraceutical properties [10]. Moringa oleifera leaves provide powerful benefits as they considered to have a highly significant source of protein, β-carotene, vitamins A, B, C, and E, riboflavin, nicotinic acid, folic acid, pyridoxine, amino acids, minerals, and various phenolic compounds [11].Several histological and immunostaining techniques could be used for identifying various pathological lesions in tissue sections, accurate quantification of the histopathological results considered to be critical for evaluation of nanoparticles toxicity [12]. The antioxidant and hepatoprotective activities of MO are possibly related to the free radical scavenging activity which might be due to the presence

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of total phenolics and flavonoids in the extract [13] KIM-1(Kidney Injury Molecule-1), a tubular protein that induced in response to a number of nephrotoxins and considered as a sensitive marker for proximal tubule injury [14]. Nrf2 (Nuclear factor erythroid 2-related factor 2) is a basic leucine zipper (bZIP) protein that regulates the transcription of several cytoprotective and antioxidant genes, therefore protects against oxidative damage triggered by injury and inflammation [15]. Heme oxygenase-1 (HO-1) is a protective gene in the kidney, entailed in the production of antiapoptotic metabolites, anti-inflammatory, and antioxidant [16]. The transcription factor, Nrf2 regulates ARE (antioxidant response element)-driven HO-1 gene expression [17]. Hence, the present study was designed to investigate the nephrotoxic effect triggered by TiO_2NPs in an attempt to ameliorate the renal affection by administration of Moringa oleifera leaves extract to male rats.

Materials and Methods

Chemicals

Titanium dioxide (Tio_2) was purchased from Sigma-Aldrich Chemical Co., USA. The titanium dioxide nanoparticles TiO_2NPs were prepared at Nanotechnology Unit, Faculty of postgraduate studies in advanced sciences, Beni-Suef University, according to the methods described by Farghali et al. [18]. The size range of TiO2-NPs is < 100 nm. Powder X-ray diffraction (XRD) pattern for NPs was measured using X-ray diffractometer XRD obtained using a Philips APD-3720 diffract meter (Cu Kα radiation, operated at 20 mA and 40 kV) in the 2 θ range of 5–70 at a scanning speed of 5°/min. Solutions of dispersed TiO2-NPs were prepared by ultrasonication for 15 min just before oral administration.

The leaves of Moringa oleifera (MO) were obtained from the farm of Egyptian Scientific Society of Moringa. The plant was identified by National Research Center; Giza, Egypt. The leaves were prepared and kept for ethanolic extraction [19].

Animals and Treatment

Eighty mature male albino rats, "weights 100–120 g," were supplied from the breeding unit of laboratory animal, Faculty of Veterinary Medicine, Beni-Suef University. All animals were housed in polypropylene cages, placed in a ventilated animal house, suitable temperature, relative humidity, and 12-h light/dark cycle, and properly maintained. Commercial food pellets for rats and water were available ad libitum. The rats were acclimated to the environment for 7 days. The rules of the ethics committee of Faculty of Veterinary Medicine, Beni-Suef University were followed (Institutional Animal Care and Use Committee, Beni-Suef University, Approval number 5/12/017). Rats were randomly assigned into four groups. Group I receive distilled water during the whole period of experiment and served as (−ve) control. Group II received TiO_2NPs suspension dispersed by ultrasonic vibration for 15 min via oral gavage in a dose of 500 mg/kg b.w (equal to 1/25 of LD50) [20]. Group III received TiO_2NPs suspension as in group II beside Moringa olifera extract at a daily oral dose of (400 mg/kg b.w) [21]. Group IV received Moringa olifera extract as described in group III. All treatments were given orally every other day for 60 days. Twenty four hours after the last dose, rats were weighed and anesthetized with an alcohol chloroform ether mixture, ACE mixture, in a ratio of 1:2:3 respectively, serum, and kidney samples were collected.

Biochemical Analysis

Biochemical Analysis in Serum

Total urea [22] and creatinine [23] levels were determined using reagent kits purchased from Diamond Diagnostic Chemical Company (Egypt).Total uric acid [24] and albumen [25] levels were determined using reagent kits purchased from Bio-diagnostic Chemical Company (Egypt).

Biochemical Analysis in Kidney Homogenate

Kidney samples were rapidly removed and rinsed from blood using distilled water, blotted between two damp filter papers, then weighted. The kidney parts placed in a pre-chilled glass tube with a calculated volume of cold buffer and the tube surrounding by cooling mixture "ice + sodium chloride + acetone" then homogenized by homogenizer. The homogenate is centrifuged, and the supernatants were used to estimate the level of lipid peroxidase (LPO) (malondialdehyde) which determined as thiobarbituric acid reactive substances (TBARS) [26], activity of superoxide dismutase (SOD) [27], glutathione (GSH) content [28], glutathione-S-transferase (GST) activity [29], glutathione peroxidase (GPx) activity [30], and total thiols content [31].

Hormonal Assessment

Plasma levels of renin were determined using rat ELISA kits (Rat Renin (REN) ELISA Cat. No. KT-27339).

Western Blot

Kidney samples kept at − 80 °C were used to investigate the effect of MOE on the expression levels Nrf2 (Nuclear factor erythroid 2-related factor 2), HSP-70(Heat shock proteins), and NF-κB (Nuclear factor kappa B) using β-actin as a loading control using chemiluminescence kit (BIORAD, USA) [32].

Table 1 Primer pairs used for PCR

Gene	GenBank accession number	Gene sequence (5′-3′)
NRF2	NM_031789.2	F: TTGTAGATGACCATGAGTCGC R: TGTCCTGCTGTATGCTGCTT
HO-1	NM_012580.2	F: GTAAATGCAGTGTTGGCCCC R: ATGTGCCAGGCATCTCCTTC
Kim-1	NM_173149	F: TGGCACTGTGACATCCTCAGA R: GCAACGGACATGCCAACATA)
β–actin	NM _007393	F: 5'TCACTATCGGCAATGTGCGG-3′ R: 5' GCTCAGGAGGAGCAATGATG-3′

Gene Expression

Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was used to detect the effect of TiO2-NPs and/or Moringa oleifera leaves on mRNA abundance of KIM-1, HO-1, andNrf2 and PPARγ according to Mahmoud [33]. c DNAs (Complementary DNA) were synthesized from 2 μg RNA and were amplified using SYBR Green master mix (Thermo Fisher Scientific, USA) with the primer sets outlined in Table 1. qPCR was performed and the 2-Ct method [34] was used to analyze the amplification data. The results were normalized to β-actin and presented as % of control.

Histological Preparations

Histopathology

Samples from kidneys were fixed in 10% buffered formalin for 48 h. Routine histological processing was carried out. Sectioning of 4–6 μm were stained with hematoxylin and eosin, Masson's trichrome stains for fibrous connective tissues identification, Periodic acid-Schiff (PAS) stain for mucopolysaccharides identification and to highlight basement membranes of glomerular capillary loops and tubular epithelium [35].

Micropathomorphological Analysis

Morphometric analyses of kidneys were carried out using optical microscope. Images were captured by a digital camera (Leica, DM2500 M). Image analyses were performed with a freeware version of Image-J (1.45 s) downloaded from the NIH website (http://rsb.info.nih.gov/ij) for measurements of

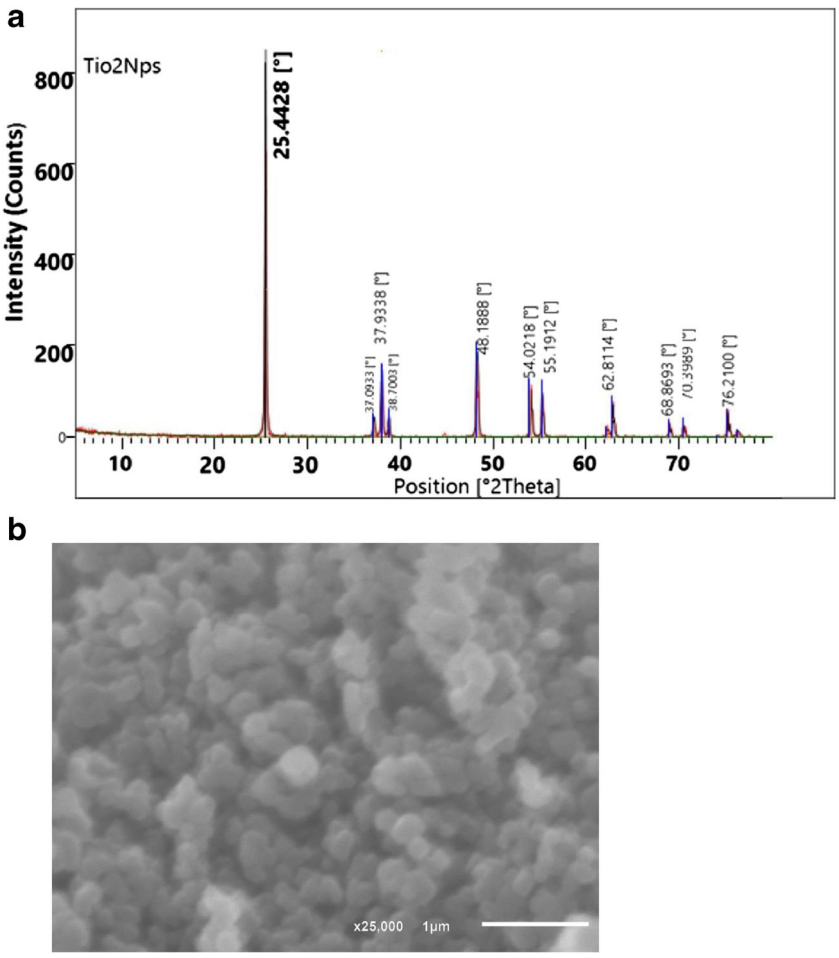

Fig. 1 **a** The X-ray diffraction peak of anataseTiO_2NPs. The average grain size was about 63.8 nm by calculation of Scherrer's equation. **b** Scanning electron microscope (SEM) image showing spherical shape of Tio2 NPs

Table 2 Effects of titanium dioxide nanoparticles (Tio_2NPs) and/or Moringa oleifera (MO) extract orally administered on serum creatinine, urea, uric acid, albumen, and renin levels of male rats (mean ± SD)

Groups	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)	Albumin (g/dL)	Renin (ng/ml/h)
Control	$0.32 \pm .10^{a}$	46.60 ± 2.60^{b}	$2.06 \pm .06^{b}$	$4.71 \pm .50^{b}$	13.10 ± 2.25^{a}
Tio2NPs	$0.69 \pm .16^{c}$	53.96 ± 4.50^{c}	$3.70 \pm .82^{c}$	3.81 ± .52a	55.50 ± 22.95^{b}
Tio2NPs + MO	$0.52 \pm .06^{b}$	41.28 ± 3.33^{a}	$2.62 \pm .10^{b}$	$4.84 \pm .51^{b}$	26.10 ± 4.85^{a}
MO	$0.24 \pm .15^{a}$	39.03 ± 3.63^{a}	$1.21 \pm .19^{a}$	4.54 ± .42 b	$12.46 \pm .56^{a}$

Values are the mean ± SD for ten rats in each group

The means within the same column and bearing different lowercase superscript letters are significantly different at $P < 0.05$

glomerular area (GA) and perimeters, measurements of Bowman's capsule, quantification of collagen fibers area percentages in renal cortex and medulla by using Masson's trichrome stain, quantification of area percentages and integrated intensities (pixels) of mucopolysaccharides using Periodic acid-Schiff (PAS) stain, and 25–30 captured microscopic images at × 400 magnification fields were evaluated using image J software.

Statistical Analysis

Data obtained from our study is expressed as mean ± SD of ten rats per group, and statistical significance was evaluated by SPSS version 21 software package (SPSS, Inc., USA) through one-way ANOVA followed by Duncan's test for making multiple comparisons among the groups and values where significance when $P \leq 0.05$.

Results

Characterization of TiO_2 NPs

The result of the X-ray diffraction (XRD) shows the TiO_2 NPs that used in this study, was anatase phase with the size of 63.8 nm (Fig. 1a, b). The shape of TiO_2 NPs was described by SEM images (Fig. 1b).

Serum Kidney Function Biomarkers

There is a significant increase in creatinine, urea, and uric acid in rats exposed to TiO2 NPs when compared to control group. In the meantime, TiO_2NPs intoxicated rats reflected an obvious significant decrease in albumen when compared with rats control (Table 3). It is worthy noted that administration of Moringa oleifera leaves extract (MO) with TiO_2NPs showed a significant suppression in all the above-examined parameters in comparison with rats administered TiO_2NPs only (Table 2).

Renin Hormone

A significant increase in serum renin level in rats exposed to TiO_2NPs, while administration of MO with TiO_2NPs showing a marked decrease in renin levels in comparison with rats administered TiO_2NPs only (Table 2).

Antioxidant Enzyme Activities

The concentrations of MDA, SOD, GST, GSH, GPx, and total thiols in kidney homogenate of experimental rats were recorded in Table 3. It was shown that oral administration of TiO_2NPs to male rats induced a significant decrease in the concentration of SOD, GST, GSH, GPx, and total thiols and a marked elevation in MDA content when compared with control rats. Nevertheless, the TiO_2NPs intoxicated group treated with MO returned these levels to near control value.

Table 3 Effects of titanium dioxide nanoparticles (Tio_2NPs) and/or Moringa oleifera (MO) extract orally administered on antioxidant concentration in kidney homogenate of male rats (mean ± SD)

Groups	Lipid peroxidation (nmol MDA/100 mg tissue/h)	SOD (mU/100 mg tissue)	GST (U/100 mg tissue)	GSH (nmol/100 mg tissue)	GPx (mU/100 mg tissue)	Total thiols (nmol/100 mg tissue)
Control	$8.04 \pm .78^{a}$	67.52 ± 1.94^{b}	54.18 ± 2.12^{b}	15.15 ± 2.99^{b}	176.32 ± 1.78^{b}	23.58 ± 1.98^{b}
Tio2NPs	10.04 ± 1.06^{b}	62.53 ± 2.19^{a}	43.69 ± 2.65^{a}	11.50 ± 2.82^{a}	128.24 ± 10.2^{a}	16.30 ± 3.54^{a}
Tio2NPs + MO	7.92 ± 1.36^{a}	67.75 ± 2.07^{b}	51.02 ± 5.19^{b}	20.82 ± 2.23^{c}	133.70 ± 2.23^{a}	20.64 ± 2.71^{b}
MO	6.61 ± 1.01^{a}	72.78 ± 3.06^{c}	66.70 ± 4.13^{c}	24.40 ± 2.62^{c}	169.20 ± 13.02^{b}	$23.51 \pm .99^{b}$

Values are the mean ± SD for ten rats in each group

The means within the same column and bearing different lowercase superscript letters are significantly different at $P < 0.05$

Western Blot

Figure 2a, b and Table 4 provide the expression of TNF-α, NF-κB, and HSP70 proteins respectively in the kidney of control, TiO_2NPs, and MO-treated rats as shown by western blotting. TiO_2NPs-treated rats showed significant ($P < 0.05$) upregulation of protein expression as compared with the corresponding control rats while MO significantly ($P < 0.05$)

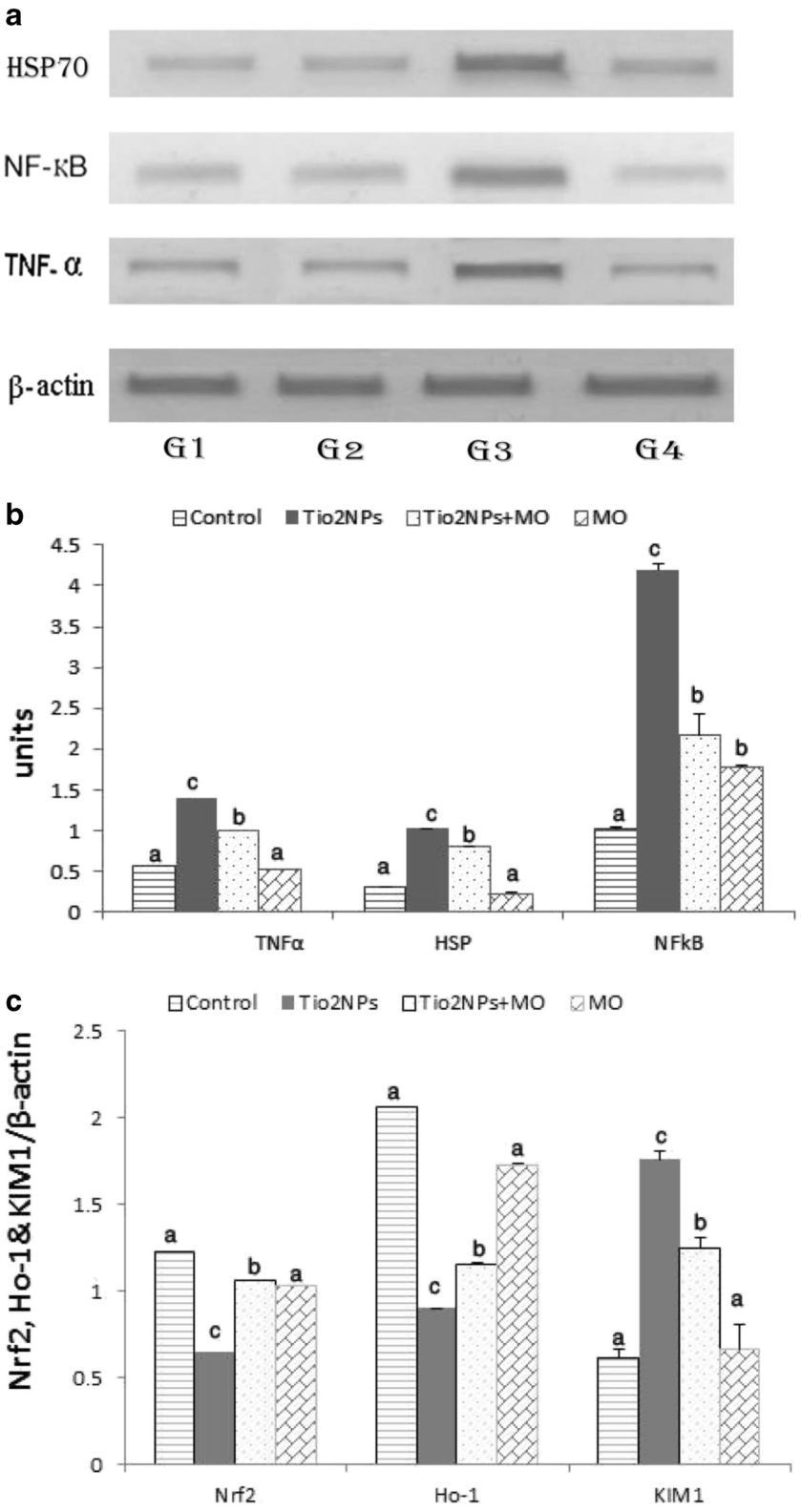

Fig. 2 **a** Effect of titanium dioxide nanoparticles (Tio_2NPs) and /or Moringa oleifera (MO) extract on TNF-α, NF-κB, and HSP70 protein expression in the kidney. **b** Western blot of the expression pattern of HSP70, NF-κB, and TNF-α respectively. Lanes G1, G2, G3, and G4 represent control, Moringa oleifera (MO) extract, Tio_2NPs, and Tio_2NPs + MO-treated groups correspondingly. The expression of β-actin acts as a loading control. Quantitative data were expressed in relative intensity arbitrary units. The bar represents the standard deviation of the mean. Values not sharing the same letter was differed significantly ($P < 0.05$). **c** Effects of titanium dioxide nanoparticles (Tio_2NPs) and/or Moringa oleifera (MO) extract orally administered on Nrf2, KIM1, and HO-1 gene expression in the kidney

Table 4 Effect of titanium dioxide nanoparticles (Tio_2NPs) and/or Moringa oleifera (MO) extract on TNF-α, NF-κB, and HSP70 protein expression in the kidney

Groups	TNFα	HSP	NFκB
Control	$0.57 \pm .02^{a}$	$0.31 \pm .11^{a}$	$1.03 \pm .03^{a}$
Tio_2 NPs	$1.39 \pm .06^{c}$	$1.03 \pm .03^{c}$	$4.20 \pm .36^{c}$
Tio2NPs + MO	$1.01 \pm .26^{b}$	$0.81 \pm .09^{b}$	$2.16 \pm .47^{b}$
MO	$0.53 \pm .02^{a}$	$0.23 \pm .05^{a}$	$1.77 \pm .24^{b}$

Values are the mean ± SD for ten rats in each group

The means within the same column and bearing different lowercase superscript letters are significantly different at $P < 0.05$

decreases the level of TNF-α and NF-κB protein expression as compared with TiO_2NPs-treated rats. In addition, HSP70 protein expression in the kidney was decreased significantly at ($P < 0.05$) in a group treated with MO. Group treated with MO only were not shown a significant effect at all studied protein.

Gene Expression

Nrf2 and HO-1 mRNA abundance in the kidneys of Tio2NPs-treated rats showed significant ($P < 0.05$) downregulation when compared with the corresponding control. Oral treatment of the rats with MO produced significant upregulation of Nrf2 ($P < 0.05$) and HO-1 ($P < 0.05$) mRNA expression as represented in Table 5. KIM1 mRNA expression exhibited the opposite expression pattern; they were significantly ($P < 0.05$) upregulated in the kidneys of TiO_2NPs-treated rats, Oral treatment with MO produced significant ($P < 0.05$) downregulation of KIM1mRNA expressions depicted in Fig. 2c. Group treated with MO only was not shown a significant effect on all studied genes.

Histopathological Studies

Glomerular Area (GA) and Perimeter

The glomerular areas were ranged from 3300 to 3800 μm^2 in different groups, while the glomerular perimeter was ranged from 210 to 230 μm. A high significant difference could be detected between them in both glomerular areas and perimeters ($P < 0.0001$, 0.001 respectively). There was a significant decrease in both glomerular areas and perimeters of Tio2NPs + MO groups in comparison with control negative, TiO_2NPs, and MO groups (Figs. 3 and 4 (7A) $P = 0.0001$, 0.0001, and 0.004) in the former and (Fig. 4 (7B), $P = 0.0001$, 0.0001, and 0.005) in the latter. Statistically, no significant difference of both glomerular areas, and perimeters could be detected between control negative, TiO_2NPs, and MO groups (Fig. 4 (7A), $P = 0.846$ and 0.107) in the former and (Figs. 3 and 4 (7B), $P = 0.725$ and 0.364) in the latter.

Table 5 Effects of titanium dioxide nanoparticles (Tio_2NPs) and/or Moringa oleifera (MO) extract orally administered onNrf2, KIM1, and HO-1 gene expression in the kidney

Groups	Nrf2	Ho-1	KIM1
Control	1.23 ± 0.047^{a}	2.067 ± 0.257^{a}	0.616 ± 0.04^{a}
Tio2NPs	0.65 ± 0.049^{c}	0.9 ± 0.059^{c}	1.763 ± 0.066^{c}
Tio2NPs + MO	1.06 ± 0.06^{b}	1.16 ± 0.0556^{b}	1.25 ± 0.043^{b}
MO	1.033 ± 0.15^{a}	1.733 ± 0.0556^{a}	0.66 ± 0.07^{a}

Values are the mean ± SD for ten rats in each group

The means within the same column and bearing different lowercase superscript letters are significantly different at $P < 0.05$

Measurements of Bowman's Capsule Space

The average thickness of Bowman's spaces in different groups were 3.7, 2.8, 3.1, and 3.3 μm in control negative, TiO_2NPs, TiO_2NPs + MO, and MO groups respectively; a highly significant difference could be detected between them (Fig. 4(7C).The maximum thickness of Bowman's capsule could be detected in control negative group with a significant difference in comparison with other groups. No significant difference between TiO_2NPs and TiO_2NPs + MO groups ($P = 0.06$).

Micropathomorphological Analysis

Area Percentages of Collagen Fibers in Both Renal Cortex and Medulla Using Masson's Trichrom Stain

The lowest area percentages of collagen fibrous connective tissue proliferation could be detected in control negative group, while the highest percentages could be detected in TiO_2NPs group. High significant difference in both renal cortex and medulla between four groups (Fig. 3, Fig. 4(7D, 7E)).Our data showed a significant difference of collagen fiber proliferation between TiO_2NPs and control negative, TiO_2NPs + MO, and MO groups (Fig. 4(7D), $P = 0.0001$, 0.002, and 0.001 respectively) in the renal cortex and (Fig. 4(7E), $P = 0.0001$, 0.004, and 0.0001 respectively) in the renal medulla. No significant of fibrous connective tissue proliferation could be found between control negative, TiO_2NPs + MO, and MO groups (Fig. 4(7D), $P = 0.063$ and 0.127) in the renal cortex and (Fig. 4(7E), $P = 0.101$ and 0.507) in the renal medulla.

Area Percentages and Integrated Intensities of Positive Reactions of Mucopolysaccharides Using Periodic Acid Schiff's (PAS) Stain

Normally, positive reaction for PAS stain could be detected due to the presence of mucopolysaccharides which found in different portions including basement membranes of

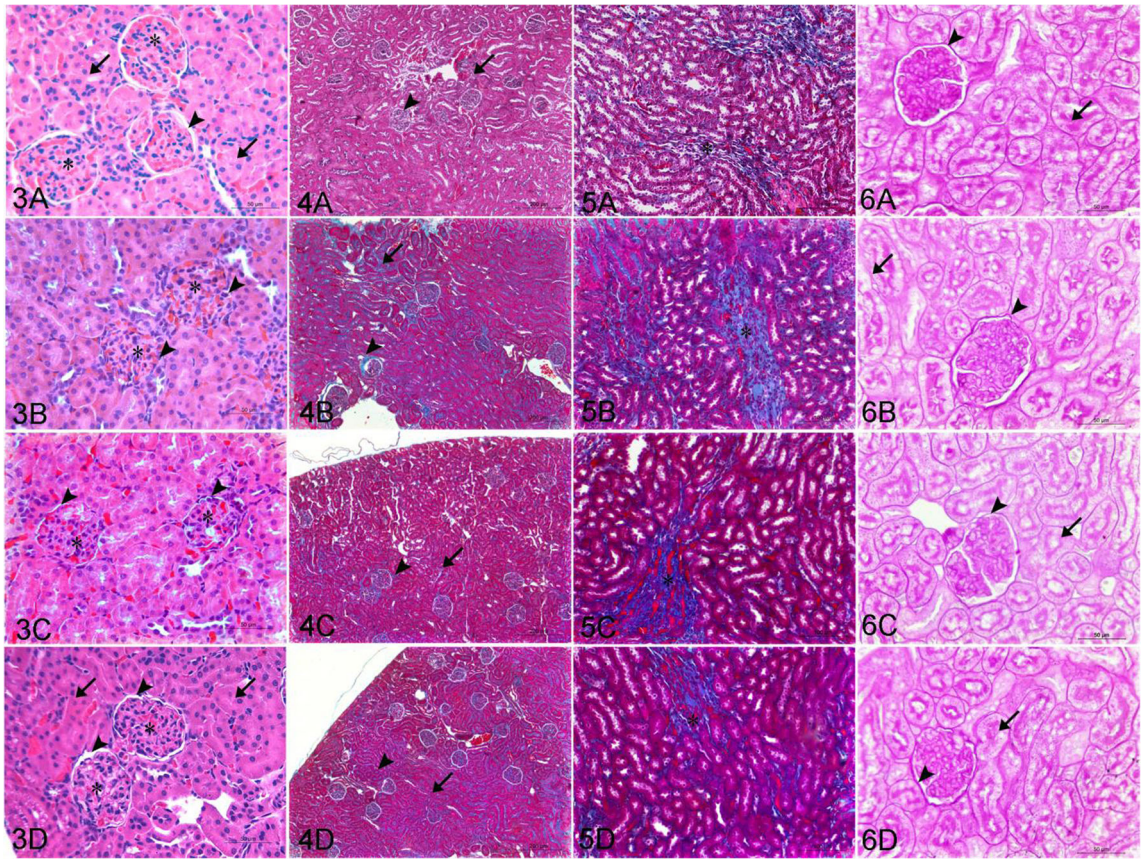

Fig. 3 (part 1) Sections of rat's renal cortex routinely stained with hematoxylin and eosin stain from (3A) control negative group showing a normal histological structure of Glomeruli (*) with normal Bowman's capsule (arrowheads) and renal tubules (arrows). (3B) Tio_2NPs showing hypercellularity of the glomerular tuft (*) associated with severe narrowing of Bowman's capsule (arrow heads). (3C) Tio_2NPs + MO group showing a decrease in the glomerular area (*) associated with moderate narrowing of Bowman's space (arrowheads). (3D) MO group was shown more or less normal histological structure of glomeruli (*), Bowman's space (arrowheads), and renal tubules (arrows). (part 2) Sections of rat's renal cortex stained with Masson's trichrome stain from (4A) control group showing minimal collagen fibers amount present around the renal tubules (arrow) and glomeruli tuft (arrow head). (4B) Tio_2NPs was shown a marked increase of the collagen fibers around renal tubules (arrow) and glomerular tuft (arrowhead). (4C) and (4D) Tio_2NPs + MO group and MO group respectively, showing moderate proliferation of collagen fibers around renal tubules (arrow) and glomeruli (arrowhead). Masson's trichrome. (part 3) Sections of rat's renal medulla stained with Masson's trichrome stain from (5A) control negative group showing minimal collagen fibers (*). (5B) Tio_2NPs showing marked increase of the collagen fibers. (*) (5C) Tio_2NPs + MO group was shown a moderate proliferation of collagen fibers (*). (5D) MO group was shown a mild proliferation of fibers (*). Masson's trichrome ×200. (part 4) Sections of rat's renal cortex stained with PAS stain from (6A) control negative group was shown a positive reaction in basement membranes of glomerular tuft (arrow head) and brush border of proximal convoluted tubules (arrow). (6B), (6D) Tio_2NPs, MO groups showing moderate positive reaction in glomerular membrane (arrow head) and renal tubules (arrow). (6C) Tio_2NPs + MO group showing mild positive reaction of basement glomerular basement membrane (arrowhead) and renal tubules (arrow) PAS

glomerular capillary loops and certain tubular epithelium (Figs. 3 and 4(7F, 7G)). The highest area percentages and intensities of PAS-positive reaction could be detected in control negative group; the high significant difference could be detected between different four groups (Fig. 4(7F, 7G).There was a highly significant difference of area percentages and integrated intensities of PAS-positive reactions between control negative group and other groups. No significant difference could be detected between TiO_2NPs and TiO_2NPs + MO, and MO groups ($P = 0.423$ and 0.734 respectively) of area percentage positive reactions and ($P = 0.055$ and 0.731) of integrated intensities of PAS-positive reactions.

Discussion

TiO_2NPs has been widely used in industry and medicine. However, the safety of TiO_2NPs exposure remains unclear. In the present study, we investigated the potential toxicity of TiO_2NPs and attempt to decrease the toxic effect by MO. In the present study, the biochemical finding reinforced the kidney damage through a significant increase in serum urea, creatinine, and uric acid levels [20]. The concentration of urea nitrogen in the blood (BUN) reflects glomerular filtration and urine-concentrating capacity [36, 37]. The massive nonselective proteinuria is ascribed to a various disorders of the glomerular

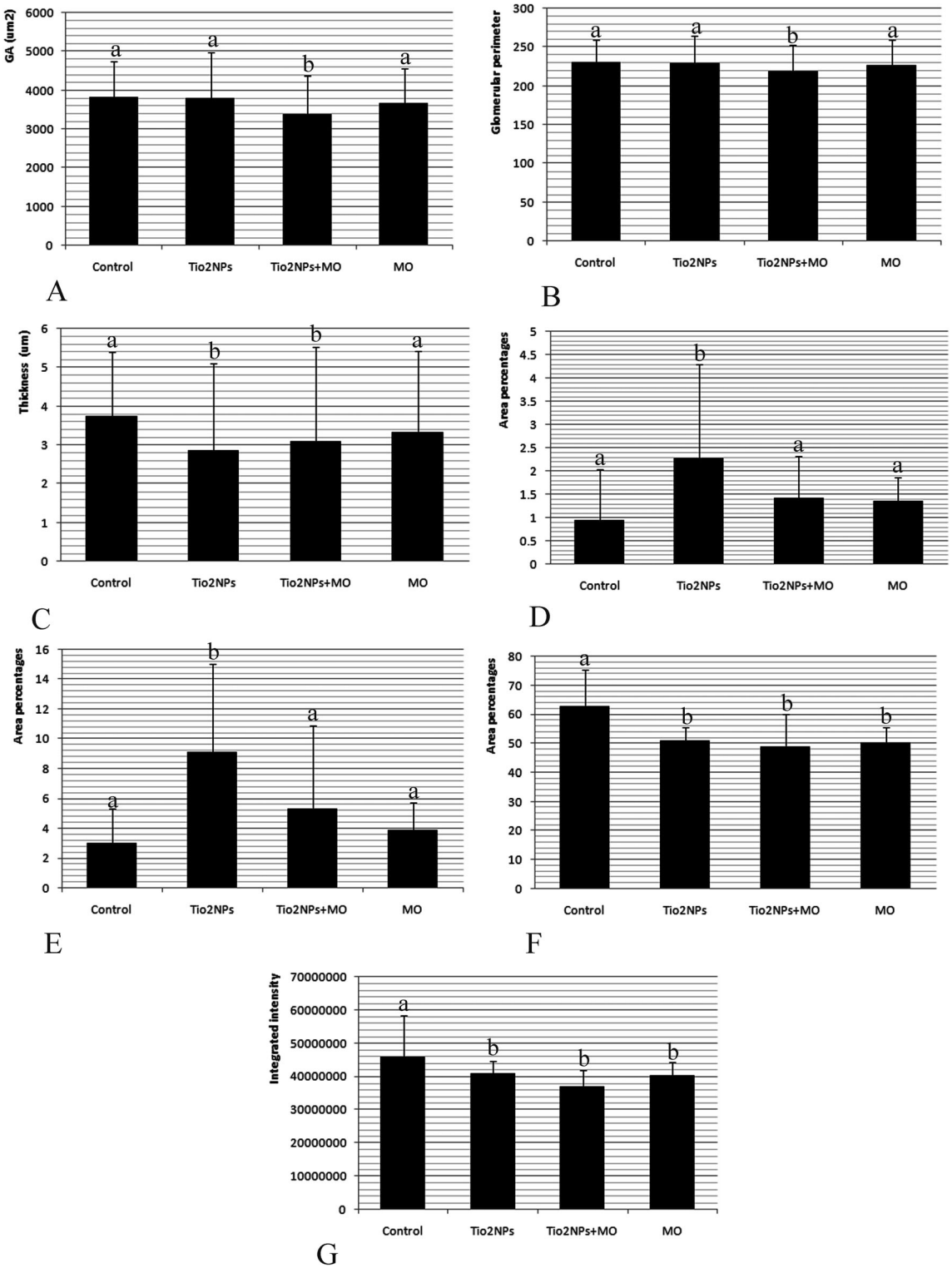

Fig. 4 (7A) glomerular area (GA), (7B) glomerular perimeter, (7C) thickness of Bowman's spaces, (7D) area percentages of collagen fibers in renal cortex using Masson's trichrome stain, (7E) area percentages of collagen fibers in renal medulla using Masson's trichrome stain different groups, (7F) area percentages of PAS positive reactions and (7G) integrated intensities of PAS-positive reactions in different groups. Values not sharing the same letter are significantly different ($P < 0.05$)

filtration barrier, including podocytes detachment, glomerular basement membrane rupture, glomerulonephritis, and elevation of intrarenal Ang II which induces proteinuria accompanied by progressive injury of the glomerular filtration barrier and podocytes that reflected on rennin level [38]. MO presented a noteworthy decrease in the levels of serum urea and creatinine

indicating anti nephrotoxic potential as the leaves of this plant are a good source of phenolic compounds, β carotene carotenoids, vitamins, minerals, glycosides, alkaloids, flavonoids, and polyphenols [39]. The proximate analysis showed that Moringa leaves are rich in fiber, protein, carbohydrate, and energy contents (11.23 ± 0.16, 9.38 ± 0.23, 56.33 ± 0.27 g 100 g^{-1}, and 332.68 ± 0.06 KCal respectively). Moringa is a good source for essential amino acids especially lysine (69.13 ± 0.13 mg 100 g^{-1}); essential minerals such as Na (289.34 ± 0.35), K (33.63 ± 0.24), Mg (25.64 ± 0.25), Ca (486.23 ± 0.11), P (105.23 ± 0.32), and Fe (9.45 ± 0.16) mg 100 g^{-1} respectively; and vitamins (A = 13.48 ± 0.51, B1 = 0.05 ± 0.28, B2 = 0.8 ± 0.25, B3 = 220 ± 0.42, C = 245.13 ± 0.46, and E = 16.80 ± 0.24 mg 100 g respectively, and a high level of phenolic content and flavonoids (28.56 ± 0.03 mg GAE g^{-1} and16.33 ± 0.12 mg g^{-1})) [40].

TiO2NPs induced a marked elevation in LPO values in the kidney homogenate of the exposed rats, a significant decrease in activity of SOD, and significant depletion in the concentration of GSH as compared with control. ROS was generated from the catalytic properties of TiO2NPs that producing the hydroxyl radical which resulted in cellular injury, protein damage, DNA fragmentation, lipid peroxidation, and alteration of the antioxidant defense system [41]. GSH, GPX, and GST decline resulted in decreasing cellular defense against free radicals that induced cellular injury which leads to cell death [42]. Although Oyagbemi et al. [43] reported that chronic administration of MO leaves might predispose to kidney damage, in our study, we detect that MO leaves have not induced a toxic effect in all parameter measured. Our results were in agreement with Falowo et al. [10] who reported that the application of MO is regarded as safe and can provide consumers with healthy and functional food products. In the present study, Tio_2NPs administration with MO were significantly improved the SOD, total thiols, GSH, GPX, and GST levels and decreased LPO, as MO contains a potent antioxidant [44]. TiO_2 NPs induce upregulation of KIM-1 and downregulation of Nrf2 and HO-1 and upregulation of NF-κB TNF-α and HSP-70. Nrf2 could play a role in regulating HO-1 expression; also a higher level expression of HSP-70 is often associated with a cellular response to a harmful stress [45, 46]. MO could effectively suppress the HSP-70 probably through its antioxidant and cytoprotective actions and distinctly suppressed KIM-1 expression in the kidney.MO could also enhance the antioxidant defense through the induction of HO-1, as observed in our study, probably via ERK/Nrf2 signaling and thereby acts as a potential therapeutic agent in preventing renal injury [47].

The increased expression of NF-κB in TiO2 NPs-treated rats could be attributed to increased oxidative stress, and elevated NF-κB could in turn, induce the synthesis of other inflammatory-related molecules that enhance kidney damage [48]. Exposure to TiO2 NPs showed an increase in the level of TNF-α. Our RT-PCR results of Nrf2 revealed its upregulation at the transcriptional level TiO_2NPs-treated animals suggesting the involvement of inflammation. Treatment with MO attenuated the inflammation probably via TNF-α. The anti-inflammatory effect of MO might be associated with diminished NF-κB expression in TiO_2NPs-induced nephrotoxicity [2]. The present results revealed the possibility that Nrf2-mediated nephroprotective effect may be achieved by the suppression of NF-κB. The ameliorative effect of MO may be due to the presence of various phytochemicals [49]. Tubulointerstitial fibrosis could be evaluated by Masson's trichrome staining for collagen [50]. In the current study, the significant increase of collagen fiber proliferations in TiO_2NPs group was due to the increase in the caspase-3 reaction which indicated the occurrence of apoptosis [51]. Positive reaction for PAS stain could be detected due to the presence of mucopolysaccharides present in the renal basement membranes of glomeruli and tubular epithelium; TiO_2NPs-treated groups showed lower PAS-positive reactions which attributed to degenerative changes of the glomeruli tuft and renal tubules [52].

Conclusion

In conclusion, the data presented by this study suggests that MO provides new insights via attenuating nephrotoxicity induced by TiO_2NPs through alleviating some gene expression and improving kidney function which is corroborated by the well-marked kidney histopathological observation.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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