Journal of Applied Veterinary Sciences, 9 (2): 01-17 (April, 2024).
ISSN: Online: 2090-3308, Print: 1687-4072
Journal homepage : https://javs.journals.ekb.eg

Ginseng Extract can alleviate The Induced-renal Toxicity of Titanium Dioxide Nanoparticles in a Rat Model

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ABSTRACT

Titanium dioxide nanoparticles (TiO_2-NPs) are widely utilized in cosmetics, food, and paintings. Although TiO_2-NPs may cause toxicity through a variety of routes, oxidative stress is by far the most common. Ginseng is employed in a variety of medical applications because of its potency as an antioxidant. Thus, the purpose of this study is to assess the protective and therapeutic benefits of Panax ginseng against TiO_2-NPs administration in the kidneys of male rats. Thirty-five mature male albino rats were divided into five groups of seven rats each at random. The experimental groups were as follows: Group I served as the control group; Group II received 200 mg/kg of ginseng orally; Group III received 200 mg/kg of TiO_2-NPs orally; Group IV served as the protective group; rats were pretreated with ginseng 1 hour before TiO_2-NPs at a dose similar to GII and GIII, respectively; and Group V served as the treatment group; rats received TiO_2-NPs for 14 days, then ginseng for another 14 days at a dose identical to GIII and GII, respectively. After 4 weeks, serum samples were collected, and kidney tissues were dissected for biochemical and histopathological examinations. Treatment with TiO_2-NPs elevated malonaldehyde (MDA), kidney biomarkers, and reduced glutathione (GSH) and glutathione peroxidase (GPx) levels. Furthermore, TiO_2-NPs induce upregulation of cysteine-aspartic acid protease (caspase3) and cyclooxygenase (COX-2). Histopathologically, TiO_2-NPs caused degenerative changes in renal tissue, including renal corpuscles, and showed hypertrophy with capillary congestion. Most renal tubules showed marked luminal dilation with epithelial cell flattening. Additionally, there was reduced immunoreactivity of Ki-67 in the kidney sections. Ginseng, on the other hand, substantially mitigated the detrimental impacts that TiO_2-NPs had on the rat renal tissues by down-regulating the genes for COX-2 and caspase3, restoring these biochemical and molecular parameters, and ameliorating the histological changes. In conclusion, ginseng could potentially be used to alleviate the renal toxicity brought on by TiO_2-NPs.

Keywords: COX-2, Ki-67Caspase-3, Ginseng, Histopathology, MDA, Titanium dioxide nanoparticles.

Original Article:
DOI:https://dx.doi.org/10.21608/javs.2024.253254.1296

Received : 05 December, 2023.
Accepted :28 January, 2024.
Published in April, 2024.

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J. Appl. Vet. Sci., 9(2): 01-17.

INTRODUCTION

Nanotechnology is a field of science concerned with the synthesis, characterization, and application of nanomaterials. These nanomaterials have at least one dimension in the nanorange (1–100 nm) **(Oberdörster *et al.*, 2005).** As nanoparticles (NPs) production and demand rose, the Organization for Economic Cooperation and Development (OECD) highlighted concerns about the safety of produced nanomaterials for human health and the environment. These concerns were created by a committee for NP operations and maintenance to test the toxicity of various manufactured NPs, such as titanium dioxide nanoparticles (TiO_2-NPs) **(No, 2016)**. Exposure to TiO_2-NPs mostly occurs by ingestion, inhalation, and dermal exposure **(Shah *et al.*, 2017)**. Additionally, it can cross biological barriers like the blood-brain barrier **(Zeman *et al.*, 2018)**. Administration of TiO_2-NPs through different routes results in their accumulation in various tissues, including the liver, kidney, brain, and spleen, with potential toxicological impacts **(Geraets *et al.*, 2014)**.

Numerous studies have assessed the toxicity of TiO_2-NPs with regard to oxidative stress,

membrane damage, inflammatory response, DNA damage, and apoptosis **(Latif *et al.*, 2019; Shirani *et al.*, 2020; Waris *et al.*, 2023)**. The liver and kidney are considered the most vulnerable target organs for TiO_2-NPs exposure **(Meena and Paulraj, 2012;Helmy *et al.*, 2015)**. The toxicity of sub-chronic TiO_2-NPs resulted in chronic nephritis, characterized by many clinical abnormalities including proximal cell death, renal cell necrosis, and renal fibrosis **(Gui *et al.*, 2011; Hong *et al.*, 2016)**. Another experimental investigation demonstrated that titanium caused significant pathological alterations in renal tissue **(Abdou *et al.*, 2019)**. TiO_2-NPs induce oxidative stress by increasing the production of reactive oxygen species (ROS) and lipid peroxides, which is activated by a reduction of anti-oxidative defense mechanisms **(Wani *et al.*, 2021)**.

In recent years, the usage of medicinal plants by many families has increased since they are safer and less expensive than modern medicine. Also, most of them have the ability to restore optimal balance and strengthen endogenous antioxidants **(Orazizadeh *et al.*, 2014; Azim *et al.*, 2015)**. East Asian nations have used ginseng, known as the "king of all herbs," as a traditional medicine to treat illness for thousands of years. Over the last three decades, it has risen to become one of the most extensively utilized herbs on a global scale **(Yu *et al.*, 2017)**. Many researchers have confirmed that ginseng roots possess anti-diabetic **(Chen *et al.*, 2019)**, hepatoprotective **(Abdelfattah-Hassan *et al.*, 2019)**, neuroprotective **(Huang *et al.*, 2019)**, anti-oxidative **(Park *et al.*, 2021)**, and anti-tumor effects **(Tao *et al.*, 2022).**

Ginseng extracts contain a number of active substances, including essential oils, saponins, triterpenes, fatty acids, aminoglycosides, alkaloids, peptidoglycans, polysaccharides, phenolic compounds, minerals, and vitamins. Ginsenosides are the most prominent active compounds in ginseng **(Kim, 2018).** The purpose of this study is to assess the protective and therapeutic benefits of Panax ginseng against the histological alterations, oxidative stress, and various mechanisms associated with the effects of oral TiO_2-NPs administration on the kidneys of male rats.

MATERIALS AND METHODS

1. Chemicals

The TiO_2-NPs powders were acquired from Nano Gate, Inc., Egypt. The company used a method for preparing NPs by precipitating anatase particles of TiO_2 from a homogeneous solution using titanium (IV) isopropoxide as a precursor. This process involved acidifying an aqueous solution using nitric acid to achieve a pH of 2, with a water-to-titanium mole ratio of around 200 **(Powell *et al.*, 2010)**. Ultrasonic vibration was used to disseminate the nanopowder for 15 minutes while it was suspended in distilled water. Dosing was done using a volume of suspension of 10 ml/kg of rat weight.

The ginseng extract (code: T9GNSG) obtained from Makin Company (which supplies natural materials for industries in food, pharmacy and cosmetics) in Egypt is in the form of a powder. It is certified as 100% organic and contains 42% ginsenosides. The extract originates from Spain. TiO_2-NPs and ginseng were dissolved in distilled water, freshly prepared, and orally administered by gavage. All compounds utilized in the current investigation were of the highest purity.

2. Characterization of TiO_2-NPs

An X-ray diffraction (XRD) pattern has been performed using the XPERT-PRO powder diffractometer system with 2 theta (20^O - 80^O), with a minimum step size of 2 theta: 0.001, and a wavelength (Kα) = 1.54614^O. The average size and shape were determined by Transmission Electron Microscope (TEM). TEM was performed on a JEOL JEM-2100 high resolution transmission electron microscope at an accelerating voltage of 200 kV, respectively.

3.Animals

A total of 35 mature male albino rats weighing between 180 and 200 grams were obtained from the VACSERA facility located in Dokki, Egypt. The rats were housed at the animal facility located in the Department of Pathology, situated within the Faculty of Veterinary Medicine at the University of Cairo. Prior to the commencement of the experiment, the animals were subjected to a two-week period of observation in order to adapt to their surroundings. Subsequently, they were individually housed in propylene cages maintained at room temperature (25 ± 2 °C), with a humidity level of 70%, and subjected to a 12-hour light-dark cycle. The animals were fed with laboratory rat feed containing 22% protein, which was purchased from the Ibex group of companies in Egypt. Additionally, they were provided with unrestricted access to distilled water. In accordance with the authorized protocol (Vet CU 08072023738) of the Institutional Animal Care and Use Committee (IACUC) of Cairo University's Faculty of Veterinary Medicine, all animals were treated humanely**.**

4. Experimental design

The rats received the following care over a period of four weeks after being divided into five equal groups of seven animals each and seven animals per cage:

Group I acted as the negative control and was fed a basic diet and distilled water.

Group II (ginseng group): Rats were given ginseng (200 mg/kg bw) once daily, according to **Abd Eldaim *et al.*, (2020)**.

Group III : (TiO_2-NPs group), rats received (200mg/kg) of TiO_2-NPs according to **Grissa *et al.*, (2020)** once daily.

Group IV: (protective group: ginseng + TiO_2-NPs) rats pretreated with ginseng 1 hour before TiO_2-NPs at doses similar to groups II and III respectively.

Group V: (treatment group: TiO_2-NPs + ginseng) rats received TiO_2-NPs for 14 days, then ginseng till the end of the experiment at doses identical to groups III and II respectively.

5. Sample collection and preparation

After the end of the experiment, blood samples were taken from the orbital sinuses of the rats without using an anticoagulant, and the serum was separated by centrifuging the samples for 10 minutes at 3000 rpm. In order to evaluate the function of the kidneys, serum was stored at -20 ^{o}C. Rats were euthanized using cervical decapitation, and kidney samples were dissected out from each rat. Some kidney specimens were stored at -80 ^{o}C for evaluation of oxidative stress markers and quantitative real-time polymerase chain reaction (qRT-PCR). Other samples were fixed in a 10% neutral-buffered formalin (NBF) solution for histopathological and immuno-histochemical examinations.

6. Biochemical investigation

6.1. Kidney function tests investigation

Serum blood urea nitrogen (BUN) and creatinine levels were assayed using commercial reagent kits following the provided instructions (Bio-diagnostic Co., Giza, Egypt).

6.2. Biochemical determination of oxidative stress indices and antioxidant enzyme activity

Renal tissues were homogenized in ice-cold 0.1 M phosphate buffered saline (pH 7.4). At 15,000 rpm, the crude tissue homogenate was centrifuged for 15 min at 4 ^{o}C and then used for the determination of malondialdehyde (MDA) according to ***Ohkawa et al.*, (1979),** reduced glutathione (GSH) according to **Ellman (1959)**.

6.3. qRT-PCR analysis for *GPX, COX-2, and CASP 3* genes

The relative renal, GPX, COX-2, and CASP3 mRNA abundance was determined by qRT-PCR analysis using GAPDH as a housekeeping gene ***(Hassan et al., 2023)*.** Approximately 100 mg of kidney tissues were used for total RNA extraction using the total RNA Extraction Kit (Applied Biotechnology, EX02). After confirming RNA concentration and purity, RT-PCR was performed using the cDNA synthesis kit (Applied Biotechnology, AMP 11). The SYBR green PCR Master Mix (Applied Biotechnology, AMP 03) was used for qRT-PCR analysis **(Bashir *et al.*, 2021)**. A quantitative assessment of cDNA amplification for each gene was performed **(Hashim *et al.*, 2022)**. The primer sequence used for qRT-PCR analysis is showen in Table (1). In each experiment, negative controls that are template-free were included **(Elmosalamy *et al.*, 2022)**. Using the comparative $2^{-\Delta\Delta CT}$ method, the relative transcription levels were calculated **(Livak and Schmittgen, 2001) .**

Table 1: Primer sequence used for qRT-PCR

Gene symbol	Gene description	Accession number	Primer Sequence
GAPDH **(Hassan *et al.*, 2023)**	Glyceraldehyde3-phosphate dehydrogenase	NC_005103.4	F: - 5′-ACCACAGTCCATGCCATCAC-3′ R: - 5′-TCCACCACCCTGTTGCTGTA-3′
GPX **(Yasin *et al.*, 2022)**	Glutathione peroxidase	M21210.1	F:-5′-CTCTCCGCGGTGGCACAGT-3′ R: - 5-CCACCACCGGGTCGGACATAC-3′
COX 2 **(Atta *et al.*, 2023)**	Cycloxygenase 2	NM_017232.3	F: - 5′- AAA GCC TCGTCCAGATGCTA -3′ R: - 5′- ATGGTGGCTGTCTTGGTAGG -3′
CASP 3 **(Noshy *et al.*, 2023)**	Caspase 3	NM_012922.2	F: -5′-GGAGCTTGGAACGCGAAGAA-3′ R: -5′-ACACAAGCCCATTTCAGGGT-3′

7. Histopathological examination

7.1. Light microscopy (L.M.)

All the rats' kidneys were meticulously dissected. Following a 48-hour fixation in 10% NBF, samples were washed. Tissue samples were dehydrated in a series of escalating ethanol grades, clarified in toluene, and embedded in paraffin wax. Using a microtome, fine slices (3-4 μm) were cut from paraffin blocks. After that, the sections were dewaxed and stained with hematoxylin and eosin (H&E). Sections were light-microscopically imaged **(Bancroft and Gamble, 2013).**

7.2. Immune staining of Ki-67 protein (Marker of Proliferation Ki-67)

According to the manufacturer's instructions, thick deparaffinized renal sections (3–5 μm) were prepared for the immunohistochemical expression of Ki-67. Slides were blocked in 1% bovine serum albumin after being quenched in 3% hydrogen peroxide and washed in phosphate buffered saline (PBS). The sections were incubated with anti-mouse Ki-67 monoclonal antibody (Elabscience, CAT#E-AB-22027, clone: 8K5, dilution: 1:100) for 1hour; the slides were washed out and incubated with diaminobenzidine for 15min. The slides were then washed with PBS, counter-stained with haematoxylin, rehydrated, cleaned in xylene, and eventually observed via light microscopy to assess the intensity of immune expressions, and finally quantified using Leica Quin 500 software (Leica Microsystems, Switzerland) by calculating the mean area% in various slides (n = 5 fields/group) **(Kuduvalli *et al.*, 2023)**.

8. Statistical analysis

All data were initially inspected for normality, after which they were subjected to a one-way analysis of variance (ANOVA) to establish the mean significance between groups. The results were expressed as mean ± SE, which was then verified by an LSD post hoc test. Statistics were considered significant for P-values under 0.05. The SPSS statistical version 27 software programs (SPSS® Inc., USA) was used to perform the statistical analyses.

RESULTS

1. Characterization of TiO_2-NPs

The result of the XRD showed that, TiO_2-NPs used in this study, were anatase-phase (Fig. 1a). The measurement indicated that the average size of NPs was less than 15 nm. The TEM revealed that the TiO_2-NPs droplets were almost spherical in shape with a homogeneous nanometric size distribution (Fig. 1b).

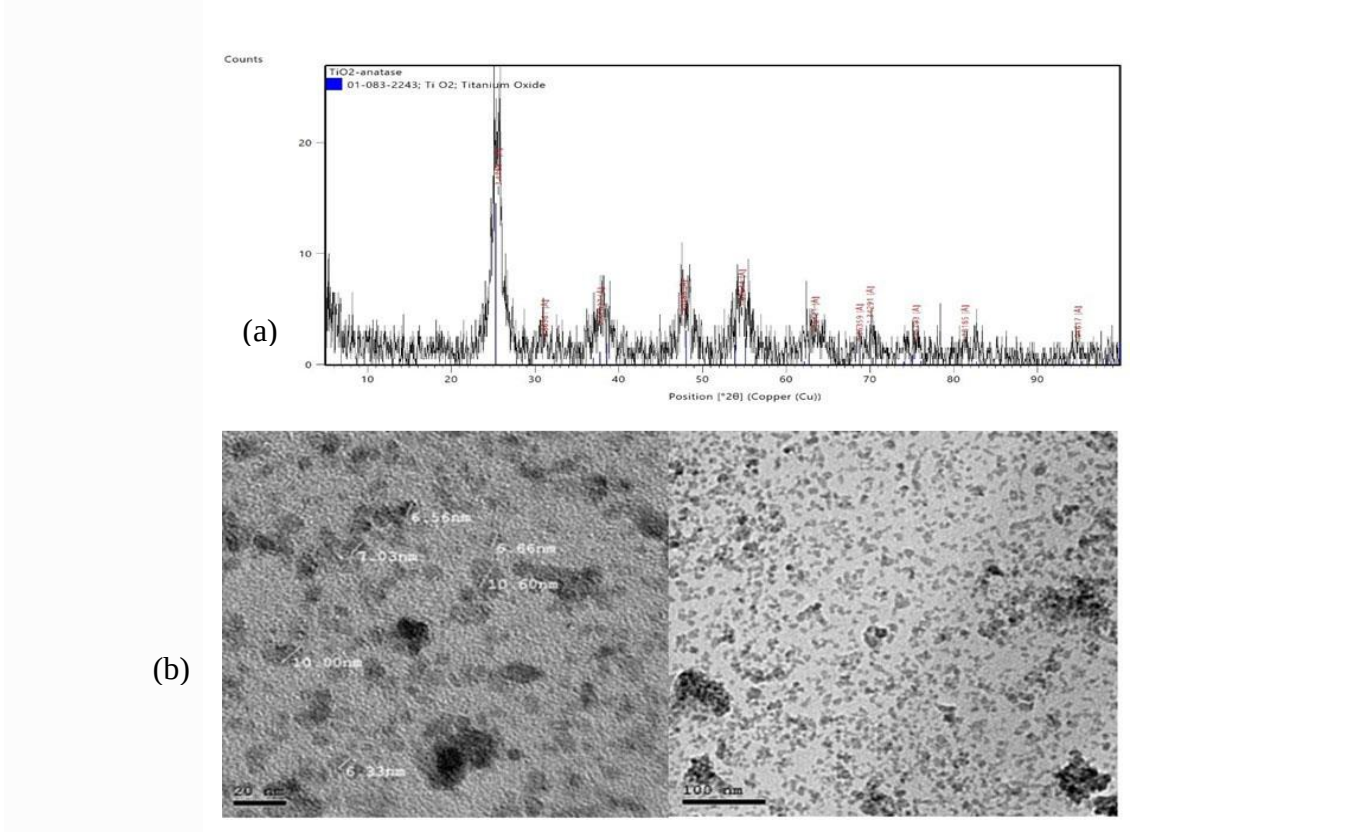


Fig. 1: XRD pattern of the prepared TiO_2-NP (a) and TEM images of the prepared TiO_2-NPs (b).

2. Biochemical examination

2.1. Kidney function investigation

Kidney function was estimated by determining serum BUN and creatinine levels. Figure (2) showed that TiO_2-NPs significantly increased serum BUN levels from 4.51 to 6.59 mg/dL and creatinine levels from 0.72 to 0.92 mg/dL when compared with the control group ($p \leq 0.05$). Protection with ginseng significantly reduced BUN to 4.56 and serum creatinine to 0.73 in comparison with the TiO_2-NPs group ($p \leq 0.05$). Also, ginseng treatment was able to significantly reduce both BUN and serum creatinine to 4.83 and 0.78, respectively.

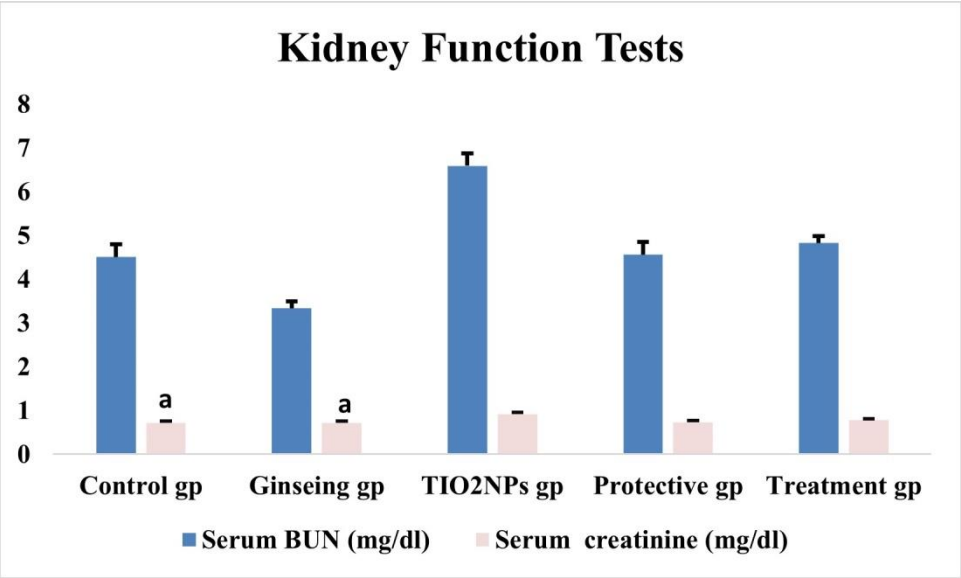
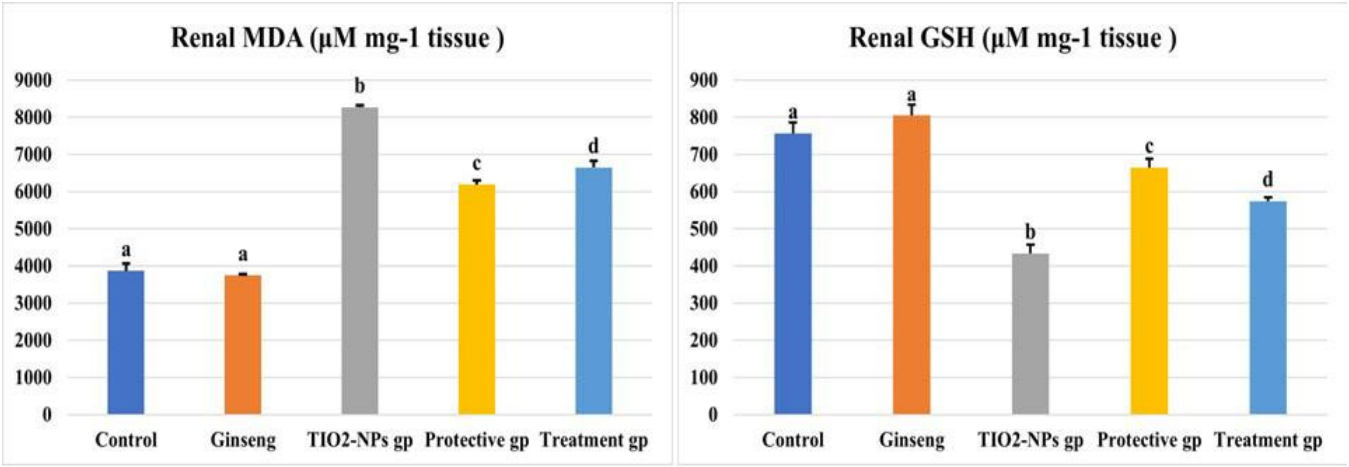


Fig. 2: Prophylactic and therapeutic effect of Ginseng against the induced renal damage by TiO_2-NPs in male rats. Data are represented as mean ± SEM. Groups having different letters are significantly different from each other at P<0.05. Groups having similar letters are non-significantly different from each other at P<0.05

2.2. Biochemical determination of oxidative stress indices and antioxidant enzyme activity

2.2.1. Renal MDA Content

According to the obtained data in **Fig. 3a**, TiO_2-NPs significantly elevated renal MDA content from 3866.67 to 8268.00 µM mg^{-1} tissue when compared with the control group ($p \leq 0.05$). Both ginseng-protective and treatment groups showed a significant reduction in renal MDA in comparison with the TiO_2-NPs group ($p \leq 0.05$).



(a) (b)

Fig.3: Prophylactic and therapeutic effect of Ginseng against renal oxidative stress biomarkers MDA (µM mg-1 tissue) (a) and GSH (µM mg-1 tissue) (b), induced by TiO_2-NPs in male rat. Data are represented as mean ± SEM. Groups having different letters are significantly different from each other at P<0.05. Groups having similar letters are non-significantly different from each other at P<0.05.

2.2.2. Renal GSH content

Our current results in Fig.3b indicated that, TiO_2-NPs significantly depressed renal GSH content from 756.59 to 433.41 µM mg^{-1} tissue when compared with the control group ($p \leq 0.05$). Ginseng protection and treatment succeeded in significantly elevating renal GSH content in comparison with the TiO_2-NPs group ($p \leq 0.05$).

2.3. qRT-PCR analysis for *GPX, COX-2 and CASP 3* genes

2.3.1. Renal mRNA relative expression of GPx gene

TiO_2-NPs exerted a significant down-regulation of renal mRNA expression of the GPX gene to 0.12 when compared to the control group ($P<0.05$). Both prophylactic and therapeutic Ginseng administration significantly elevated the mRNA GPX gene expression to 0.68 and 0.65, respectively, when compared with TiO_2-NPs ($p \leq 0.05$), as shown in Fig. (4a).

2.3.2. Renal mRNA relative expression of COX2 gene

TiO_2-NPs induced a significant up-regulation of renal mRNA expression of the COX2 gene to 5.9 when compared to the control group ($P<0.05$). Both prophylactic and therapeutic Ginseng administration significantly decreased the mRNA COX2 gene expression to 2.76 and 2.90, respectively, when compared with TiO_2-NPs ($p \leq 0.05$), as shown in Fig. (4b).

2.3.3. Renal mRNA relative expression of CASP 3 gene

TiO_2-NPs triggered a significant up-regulation of renal mRNA expression of the CASP3 gene to 5.20 when compared to the control group ($P<0.05$). Both prophylactic and therapeutic Ginseng administration significantly modulate the mRNA CASP3 gene expression to 1.90 and 2.10, respectively, when compared with TiO_2-NPs ($p \leq 0.05$) (Fig.4c).

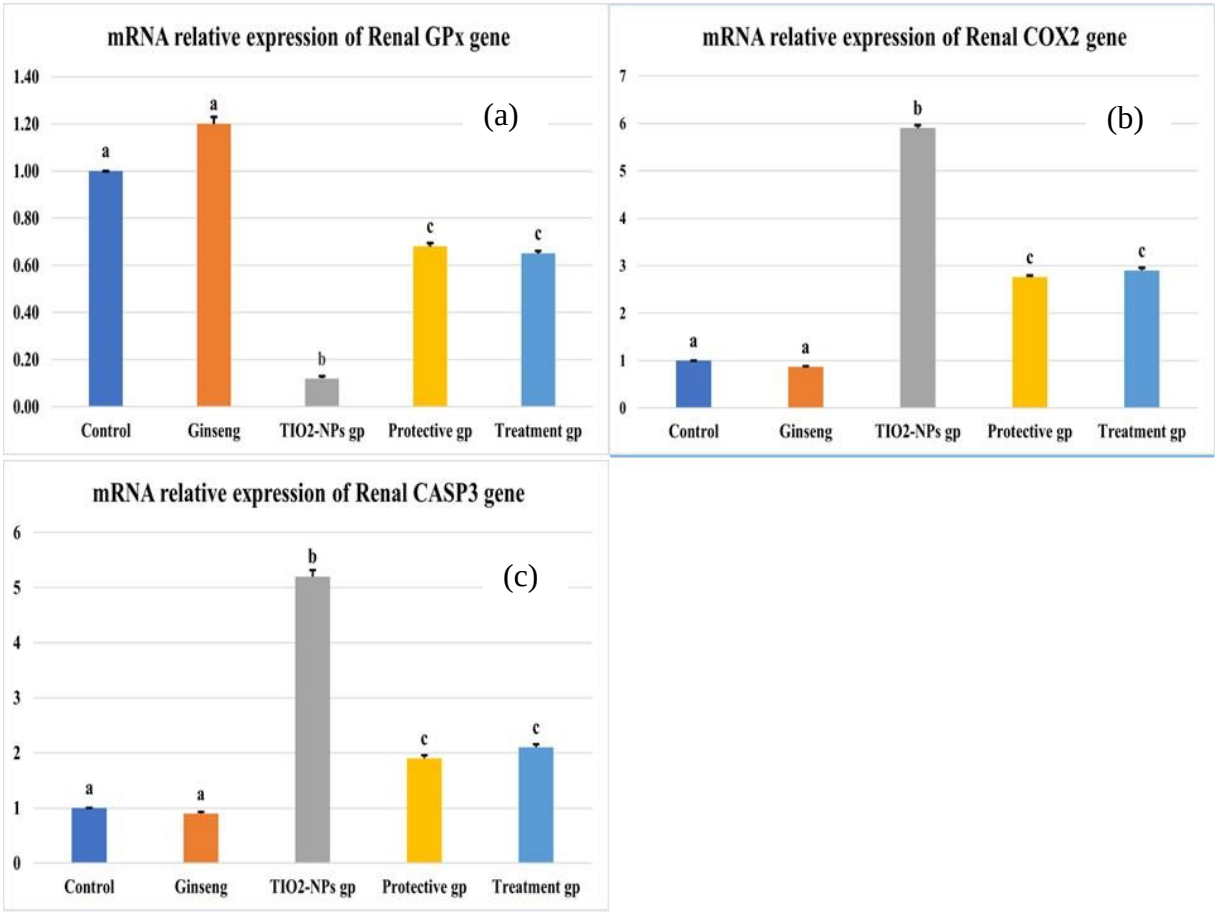


Fig. 4: Prophylactic and therapeutic effect of Ginseng against renal mRNA relative expression of GPx gene (a), COX2 gene (b), and CASP 3 gene (c) induced by TiO_2-NPs in male rat. Data are represented as mean ± SEM. Groups having different letters are significantly different from each other at $P<0.05$. Groups having similar letters are non-significantly different from each other at $P<0.05$.

3. Histopathological examination

3.1. Light microscopy observations

The architecture in the kidneys of all rats in the control and ginseng groups (GI and GII) was well preserved and kept intact, showing normal histological components of the glomeruli, renal tubules, and interstitial tissues of both the cortex and medulla. The glomerular capillaries and Bowman's capsules that make up the renal (Malpighian) corpuscles in the rats' renal cortex had a normal histological appearance. The Bowman's capsule consisted of two layers: the external parietal layer, which was composed of simple squamous epithelium, and the internal visceral layer. The urinary space stood between the two layers. The proximal convoluted tubule (PCT) lumina was narrow. They were lined with high pyramidal acidophilic cells and low cuboidal cells lining the distal convoluted tubules (DCT) **(Fig.5a,b).**

On the contrary, the kidneys of rats orally administered TiO_2-NPs at a dose of 200 mg/kg showed renal histological alterations, including the glomeruli, renal tubules, and interstitial tissue of both the cortex and medulla. There was congestion and dilation in the glomerular capillaries, with a narrowing of the urinary space. Some renal corpuscles showed hypertrophy **(Fig.5c)** and others showed atrophy with wide urinary space. Most of PCT and DCT showed marked luminal dilation with epithelial cell flattening as well as necrosis of tubules, as demonstrated by pyknotic nuclei. There was a desquamation of epithelial cells into the lumen and an accumulation of NPs (brown colour) inside the tissue (Fig.**5d).** Mononuclear cell infiltrations **(Fig.5d)** and congestion of interstitial capillaries were present among degenerated tubules and renal corpuscles (Fig.5c). The renal cortex of animals from the protective group (GIV) showed partial enhancement; the renal corpuscles were nearly normal in size; there was mild congestion in glomerular capillaries; and the majority of PCT were nearly normal, with the exception of a few showing dilatation. The majority of renal tubules were ameliorated, but some tubular cells had pyknotic nuclei. Furthermore, the administration of ginseng + TiO_2-NPs ameliorated interstitial infiltration of inflammatory cells and congestion **(Fig.5e).** However, the renal cortex of animals from the treatment group (GV) showed the same enhancement as GIV except for moderate interstitial congestion, but the majority of renal tubules and glomeruli were nearly normal **(Fig.5f).**

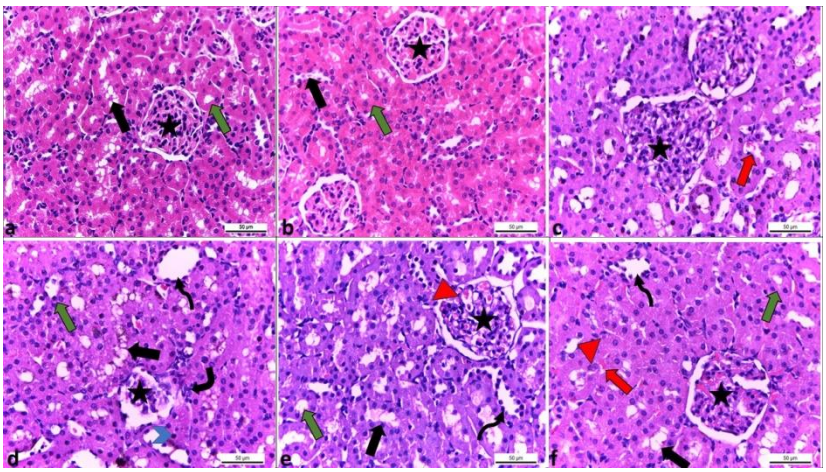


Fig. 5: Histological sections of renal cortex from albino rats stained with H&E (magnification power X400, scale bar = 50 µm). (a): Negative control group (GI) (b) Ginseng group (GII) demonstrating typical renal cortex which contain typical renal corpuscle with normal glomeruli (star), distal convoluted tubule (DCT) (black arrow) as well as proximal convoluted tubule (PCT) (green arrow). (c & d): TiO_2-NPs treated group (GIII) highlighting degenerative changes (c) Renal glomerular hypertrophy and congestion with narrowing of urinary space (star) and congestion of interstitial capillaries (red arrow) (d) Glomerular atrophy with wide urinary space (star), mononuclear cell infiltrations among degenerated tubules (bent arrow), intra-luminal exfoliation of epithelial cells with accumulation of NP with brown colour inside DCT (black arrow) and marked luminal dilation in PCT (green arrow) and DCT with epithelial cell flattening (curved arrow) and nuclear pyknosis (arrow head). (e): Protective group (GIV) observing ameliorative renal cortex with nearly normal size of glomeruli and urinary space with few congestions (star), some PCT appear normal (green arrow) but other still degenerated with pyknotic nuclei (triangle head) as well as DCT appear nearly normal (black arrow) but other still degenerated also with pyknotic nuclei (curved arrow). (f): Treatment group (GV) showing glomeruli with nearly normal size but also with few congestion (star), moderate interstitial congestion (red arrow), some PCT appear normal (green arrow) but other still degenerated also (triangle head) as well as DCT appear nearly normal (black arrow) but other still degenerated also with pyknotic nuclei (curved arrow).

Sections from the control group (GI) and ginseng group (GII) showing normal renal medulla, in which the renal tubules were lined by cuboidal cells and loops of Henle lined with flat squamous cells, and the interstitial connective tissue had blood capillaries **(Figs.6a,b).**

The renal medulla was also affected in the TiO_2-NPs treated group (GIII) in the form of renal tubule degeneration and epithelial cell deterioration with dark pyknotic nuclei. Luminal dilatation with epithelial cell flattening were observed in most renal tubules **(Fig.6c).** Interstitial haemorrhage with extensive inflammatory cell infiltration **(Fig.6d).** The supplementation of ginseng enhanced the architecture of the renal medulla in GIV. Moreover, the tubules had clear signs of regeneration of their lining epithelial cells, with their lumens appearing clear but some tubules still dilated with epithelial cell flattening also. Inflammatory cell infiltration apparently disappeared as compared to (GIII) and the interstitial haemorrhage was still seen **(Fig.6e).** On the other hand, the treatment effect of ginseng (GV) showed more enhancement in the renal medulla as signs of tubular regeneration were observed in most tubules and with the exception of a few tubules still degenerated ($p \leq 0.05$). Absence of interstitial haemorrhage and inflammatory cells infiltration were also noted **(Fig.6f).**

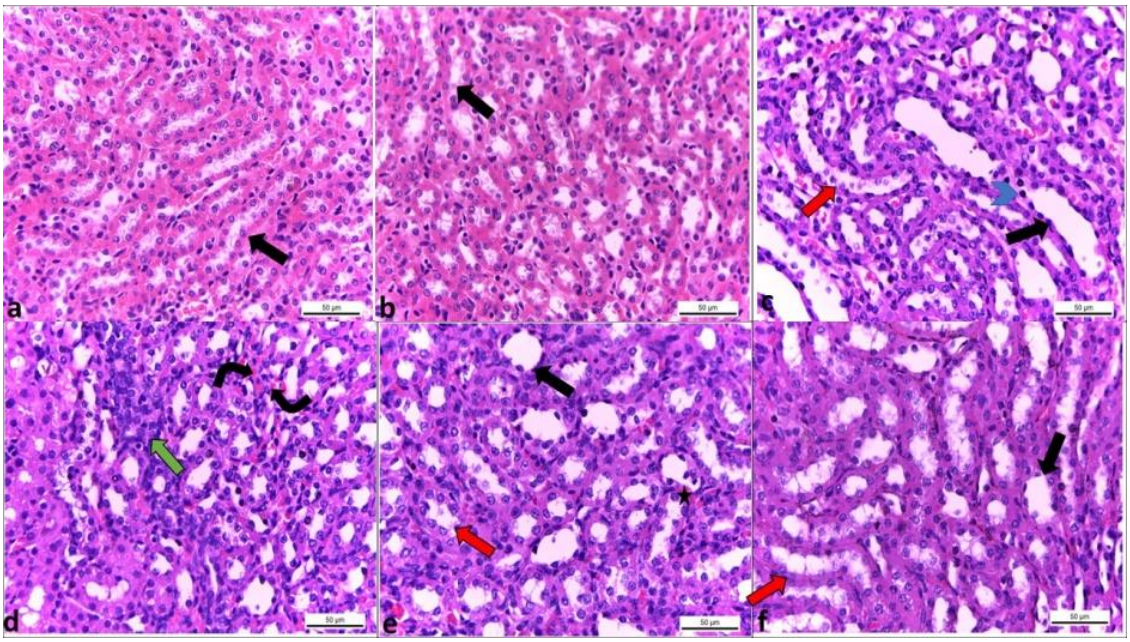


Fig. 6: Histological sections of renal medulla from albino rats stained with H&E (magnification power X400, scale bar = 50 µm). (a) Negative control group (GI) (b) Ginseng group (GII) showing typical renal medulla contain normal renal tubules (black arrows). (c & d): TiO_2-NPs treated group (GIII) demonstrating degenerative changes (c) Dilatation in renal tubules with epithelial cell flattening (black arrow), epithelial desquamation (red arrow) and pyknotic nuclei (arrowhead) (d) Extensive inflammatory cells infiltration (green arrow) and interstitial haemorrhage (bent arrow). (e): Protective group (GIV) marking improvement in renal medulla some renal tubules appear normal (red arrow) but other still dilated (black arrow). Also, interstitial haemorrhage is still seen (star). (f): Treatment group (GV) highlighting best improvement most of renal tubules appeared intact (red arrow) except few still observe luminal dilatation with epithelial cell flattening (black arrow). Notice absence of interstitial haemorrhage or inflammatory cells infiltrations

3.2. Immunohistochemical observations "Ki-67"

The renal cortex samples from the control rats (GI) and ginseng group (GII) stained for ki-67 showed a strong positive reaction upon immunohistochemical analysis **(Figs.7a,b).** On the contrary, the TiO_2-NPs-treated group (GIII) induced a significant decrease in ki-67 immunoreaction to 1.27 compared with the negative control group ($P<0.05$) **(Fig.7c).** In both the protective (GIV) and treatment (GV) groups of rats, there was a moderate immunoexpression of ki-67 to 7.12 and 8.18 respectively, in comparison with the TiO_2-NPs group ($p \leq 0.05$) **(Figs.7d,e).**

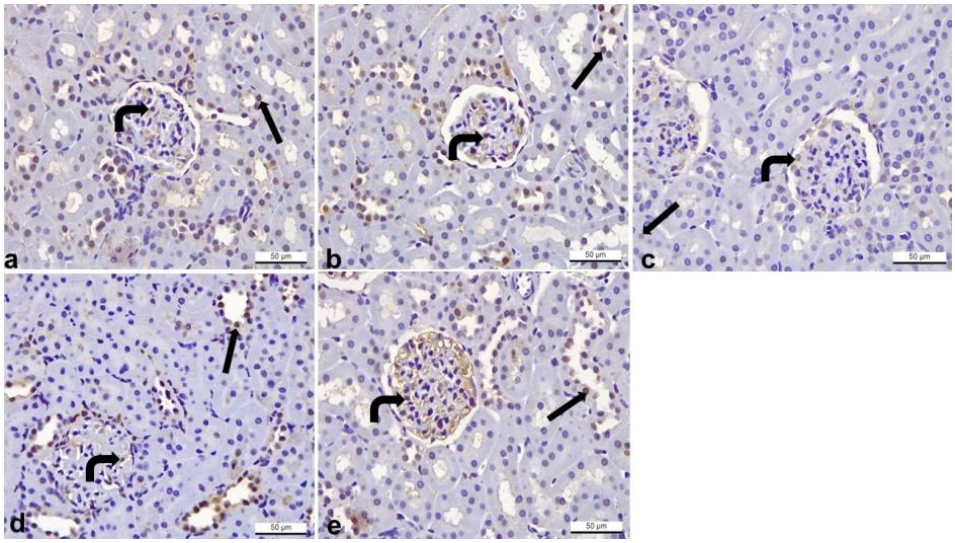


Fig. 7: Immunohistochemically Ki-67-stained renal cortex sections (X400) (scale bar= 50µm). (a): Negative control group (GI) and (b): Ginseng group (GII) showing high positive nuclear expression of Ki_67 within renal tubules (arrow) and renal glomeruli (bent arrow). (c): TiO_2-NPs treated group (GIII) demonstrating significant decrease in nuclear expression of Ki-67 within renal tubules (arrow) and renal glomeruli (bent arrow). (d): Protective group (GIV) and (e): Treatment group (GV) highlighting temperate Ki-67 expression within renal tubules (arrow) and renal glomeruli (bent arrow).

The same results were observed in specimens of renal medulla for all groups, as showen in renal cortex (Figs.8a,b,c,d and e).

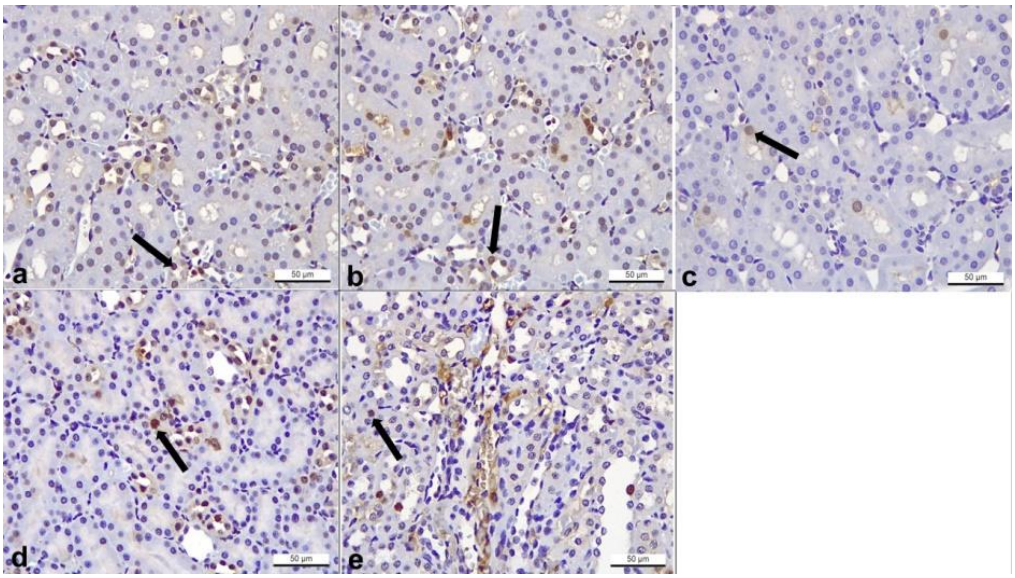


Fig. 8 Immunohistochemically Ki-67-stained renal medulla sections (X400) (scale bar= 50µm). (a): Negative control group (GI) (b): Ginseng group (GII) showing strong positive nuclear expression of Ki-67 (arrow). (c): TiO_2-NPs treated group (GIII) revealing low nuclear expression of Ki-67 (arrow). (d): Protective group (GIV) and (e): Treatment group (GV) highlighting moderate Ki-67 expression within the renal tubules (arrow).

The immune histochemical analysis and scoring of Ki-67 immunoreactivity in the kidney cortex and medulla confirmed that control rats (GI) and the ginseng group (GII) revealed the highest nuclear reactivity without a significant difference between them ($P>0.05$). Meanwhile, the TiO_2-NPs-treated group (GIII) highlighted the lowest nuclear reactivity with a significant difference from other groups ($P<0.05$). On the other hand, protective (GIV) and treatment (GV) groups exhibited moderate nuclear reactivity of Ki-67 without significant differences between them ($P>0.05$), and with high significant differences between GI, GII, and GIII ($P<0.05$) (**Fig.9).**

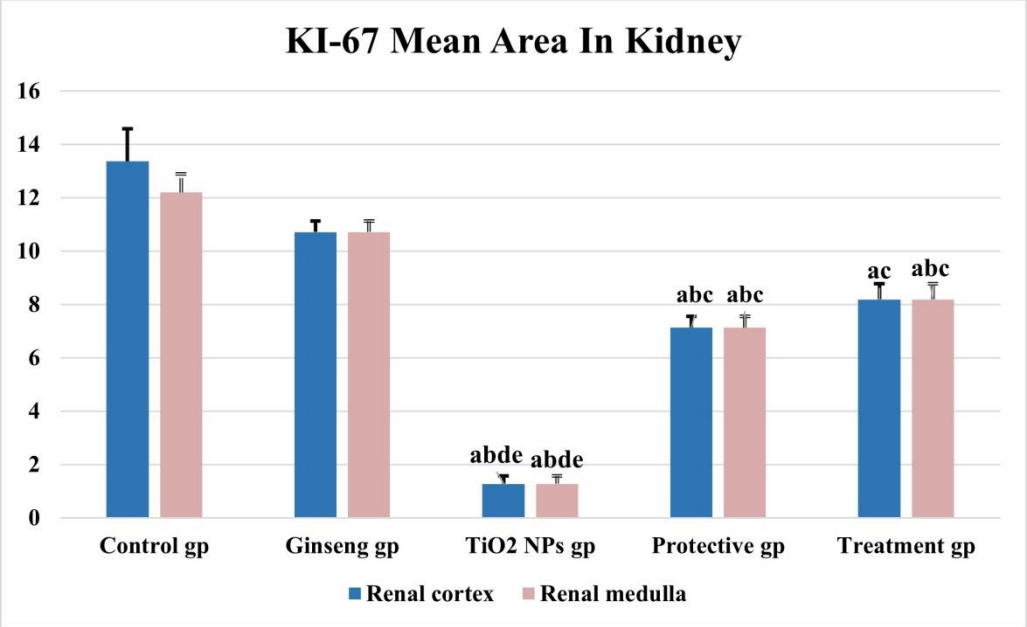


Fig. 9: Data analysis of Ki-67 immunoreactivity in the renal cortex and renal medulla. Results were expressed as mean ± SEM; superscript values refer to (a) significance from GI, (b) significance from GII, (c) significance from GIII, (d) significance from GIV, and (e) significance from GV at P<0.05

DISCUSSION

Oral exposure to NPs increases as a result of a dramatic increase in products containing them. Consequently, the gastrointestinal tract (GIT) becomes a potential route of exposure that could lead to systemic exposure if the intestinal barrier is breached **(Vieira *et al.*, 2022).** The reason for choosing kidney tissues in this study is that the kidney is one of the most highly susceptible organs in the body to hazardous substances because of its high blood flow and tendency to concentrate waste **(L'azou *et al.*, 2002)**. The current study aimed to investigate the possible protective and therapeutic role of ginseng supplementation against TiO_2-NPs-induced renal damage. In the present study, TiO_2-NPs-induced renal damage was evaluated by biochemical determination of renal biomarkers (BUN and creatinine) and histopathological analysis. BUN is considered the main renal biomarker that increases in response to any renal injury. Serum creatinine is another kidney biomarker that is elevated due to a hindered glomerular filtration rate **(Ahmed *et al.*, 2022).** Our current results indicated a significant increase in both renal biomarkers in TiO_2-NPs group ($p \leq 0.05$). These findings were in harmony with **Al-Doaiss *et al.*, (2019) and Bakour *et al.*, (2021)**. TiO_2-NPs-induced renal damage might be attributed to the initiation of oxidative stress.

This literature revealed a significant renal reduction in GSH content, relative mRNA expression of GPx, and elevation in MDA. GSH is an important endogenous nonenzymatic cell antioxidant molecule that is involved in the neutralization and scavenging of ROS. GPx is one of the antioxidant enzymes that acts by scavenging ROS and reducing lipid peroxides. Depression of the antioxidants results in the accumulation of ROS, with subsequent induction of lipid peroxidation and the formation of MDA **(Su *et al.*, 2019)**. TiO_2-NPs is a potent initiator of oxidative stress via depression of the antioxidant mechanism, as reported by **Liu *et al.*, (2010); Wang *et al.*, (2011) and Hamida *et al.*, (2020)** The current results indicated that TiO_2-NPs induced a significant upregulation of renal mRNA expression of the *COX2* gene.

The present records support the previous findings conducted by **Kumar *et al.*, (2016); Dinesh *et al.*, (2017) and Kim *et al.*, (2019).** The assessment of renal proapoptotic effect of TiO_2-NPs was evaluated by the relative mRNA expression of the *CASP3* gene. *CASP-3* is the main effector of apoptosis due to its role in coordinating the degradation of cytoskeletal proteins or the destruction of cellular structures **(Sibarani *et al.*, 2020)**. In addition to its stimulation by the intrinsic (mitochondrial) pathway, it is also triggered by the extrinsic (death ligand) pathway **(Wang, 2014)**. In the current study, oral ingestion of TiO_2-NPs significantly upregulates the relative mRNA expression of *CASP3* in renal tissues. The current results are in harmony with earlier studies conducted by **Shukla *et al.*, (2014) and Abbasi-Oshaghi *et al.*, (2019)** who revealed that TiO_2-NPs can induce cell apoptosis via the caspase-dependent signaling pathway. The expression of the genes for Bax,

caspase-3, p53, and Bcl-2 is altered by the injection of TiO_2-NPs **(Sallam *et al.*, 2023)**. These findings demonstrated that the creation of ROS following TiO_2-NPs exposure stimulates a number of receptors, which then activate signaling pathways to reduce the antioxidant via ROS synthesis **(Baranowska-Wójcik *et al.*, 2020)**. Also, **Salem *et al.*, (2017)** found that the cytoplasm of the tubular cells in rats treated with TiO_2-NPs at a dose of 150 mg/kg showed highly expressed caspase-3 immunoreactivity. Moreover, severe histopathological alterations in the renal tissues of rats exposed to TiO_2-NPs were identified in the present work, which reinforced these biochemical and molecular results.

Results of the existing research showed that TiO_2-NPs have deleterious effects on the kidney, as evidenced by marked congestion and dilation in glomerular capillaries with a narrowing of urinary space. **Morgan *et al.*, (2017) and Abdel-Wahhab *et al.*, (2021)** confirmed these findings. According to **Al-Doaiss *et al.*, (2019)** who explained that exposure to TiO_2-NPs can result in glomerulonephritis, which eventually results in renal failure due to glomerular injury characterized by protein leakage into urine. A study by **Yang *et al.*, (2017)** revealed that the renal glomerular basement membrane is delicate and vulnerable to the harmful effects of NPs. Additionally, the findings of our research revealed atrophy and hypertrophy of the renal corpuscle. Most of the PCT and DCT displayed prominent luminal dilatation, epithelial cell flattening, and necrosis of tubular cells with pyknotic nuclei. **Helmy *et al.*, (2015); Niu *et al.*, (2017) and Salem *et al.*, (2017)** supported these findings. According to research by **VelmaandTchounwou (2010) and Aslam *et al.*, (2013)** lipid peroxidation is the main cause of tubular dilatation.

As evidenced by **Guicciardi and Gores (2013),** proteolytic enzymes are released into the cell as a result of lysosomal membrane breakdown. These enzymes cause the breakdown of the cellular constituents, which results in cell death. **Helmy *et al.*, (2015)** revealed that most likely fibroblasts are the cells with flattened nuclei found lining some tubules. The majority of fibroblasts develop in the interstitial tissue via a process known as epithelial-mesenchymal transition. A change in the equilibrium of the local cytokine concentrations triggers the transformation of tubular epithelial cells into mesenchymal phenotypes. Fibroblasts multiply and release too much extracellular matrix when the renal parenchyma is damaged repeatedly and is chronically inflamed. As a result, the kidney's typical interstitial architecture is destroyed **(Ross and Pawlina, 2007)**. Furthermore, in the present study, epithelial cells were desquamated into the tubular lumina, and NP was accumulating within the tissue. These observations come in contact with **Abdel-Wahhab *et al.*, (2021) and Bakour *et al.*, (2021)**.

Desquamation of cells, according to **Racusen (1995),** is caused by changes to cell microfilaments and/or surface attachment proteins that affect cell adherence. Additionally, among the deteriorated tubules and renal corpuscles, mononuclear cell infiltrations and interstitial congestion were observed in our study. This alteration indicated that TiO_2-NPs could compete with the antioxidant defense mechanism and promote oxidative stress in the renal tissue, resulting in the initiation of an inflammatory response **(Al-Doaiss *et al.*, 2019)**. Cell infiltration has reportedly been linked to chronic renal disorders as a symptom of tubular cell atrophy **(Sadek *et al.*, 2016)**. Additionally, the renal medulla in the TiO_2-NPs-treated group (GIII) was harmed, as evidenced by degenerated tubules and deteriorated epithelial cells. Marked dilatation of the tubular lumen and flattening of epithelial cells were also detected. **Abdelhalim and Jarrar, (2011)** explained that TiO_2-NPs may interact with proteins (enzymes) in renal tissue, disrupting the antioxidant defence mechanisms and generating ROS, as evidenced by the development of cytoplasmic degeneration and nuclei in tubular cells. The aforementioned turn might cause cells to experience oxidative stress, which would encourage necrosis or apoptosis, two types of cell death.

Many medicinal herbs receive special attention for their antioxidant properties. Ginseng, for example, has been used extensively in traditional Chinese medicine for thousands of years as a cure for weakness and exhaustion **(Mahady *et al.*, 2000)**. Ginseng supplementation possesses potent antioxidant and anti-inflammatory properties due to its components, "Ginsenoside, polysaccharides, proteins, phenols, polyacetylenes, sesquiterpenes, and alkaloids which exhibit their antioxidant activity by metal ion chelation as well as by scavenging of free radicals **(Yang *et al.*, 2022).** Our current results indicated that both treatment and protective regimens of ginseng supplementation succeeded in restoring serum biochemical parameters, improving cell regeneration, and ameliorating renal oxidative stress, inflammation, and apoptosis induced by TiO_2-NPs. These results were supported by a significant reduction in serum urea and creatinine levels. The antioxidant properties of ginseng were also demonstrated by a notable decrease in renal MDA level as well as a concurrently significant increase in GSH content and GPx activity. The renal tissue architectural changes brought on by TiO_2-NPs were lessened by the antioxidant actions of ginseng, which decreased caspase-3 and COX-2 gene expression in renal tissue. Moreover, renal protective effects of

ginseng were confirmed against other hazardous agents such as carbon tetra chloride (CCL4) **(Ghamry *et al.,* 2022)**, silicon dioxide nanoparticles **(El-Demerdash *et al.,* 2021)**, fipronil (FPN) **(Abd Eldaim *et al.,* 2020)**, and cisplatin **(Yousef and Hussien, 2015; Baek *et al.,* 2017).** Renal protective effects may be attributable to oxidative stress inhibition since ginseng can increase the activity of many antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), GPx, glutathione S-transferase (GST), and hemeoxygenase-1, mostly through inhibition of the mitogen-activated protein kinase signaling network **(Bak *et al.,* 2012)**. A crucial transcription factor known as nuclear factor kappa B (NF-κB) regulates the generation of cytokines and DNA transcription **(Salminen *et al.,* 2008)**. Tissue injury is caused by an overexpression of these factors.

Ginseng strongly inhibits NF-κB, indicating that its protective effects may work through NF-κB's signalling pathway, as documented by **Song *et al.,* (2012)**, which may be the major cause of COX-2's gene expression being significantly reduced in the current study.

Also, **Abd Eldaim *et al.,* (2020)** revealed that ginseng's antioxidant actions diminished caspase-3 and COX-2 expression in hepatic and renal tissues, which alleviated FPN-induced changes in hepatic and renal tissue architectures. These findings were supported by **Abdel Dayem *et al.,* (2020)** who demonstrated that ginseng has an anti-inflammatory impact because it reduces inflammatory cell infiltration and significantly reduces COX-2 expression in the omeprazole group. Ginseng also lessens oxidative stress and apoptosis in rat renal tubular cells, which serves to reduce gentamicin and lambda-cyhalothrin-induced nephrotoxicity **(Lee *et al.,* 2013; El-Bialy *et al.,* 2020)**. Administration of ginseng in conjunction with TiO_2-NPs **(**GIV&GV) demonstrated the efficacy of ginseng in shielding the kidneys from the detrimental effects of TiO_2-NPs simply because ginseng combination with TiO_2-NPs in **GIV&GV** revealed significant improvements at the level of both the renal cortex and medulla in the form of the renal corpuscles, which had regained their usual size, whereas most of the PCT, DCT, and tubules of the renal medulla seemed nearly normal as a control group. The observations of this study were supported by a number of studies that revealed the nephroprotective effects of ginseng **(El-Bialy *et al.,* 2020; Maher *et al.,* 2023; Yousef and Hussien, 2015; Zidan *et al.,* 2015)**. This can be attributed to the components of Panax ginseng, which are free radical scavengers, reduce lipid peroxidation, and shield cells and tissues from oxidative stress brought on by free radicals **(Huu Tung *et al.,* 2012)**.

The current study confirmed the potent protective and therapeutic effects of ginseng extract against TiO_2-NPs intoxication at the level of renal tissue, as in addition to their antioxidant properties, ginseng components have been shown to have anti-inflammatory and anti-apoptotic effects that help protect the kidney. In particular, ginseng components may stimulate mesangial cell proliferation and prevent cell death, which may be related to the stimulation of Bcl-2 and the suppression of caspase-3 expression **(Jin *et al.,* 2021)**. Also, the administration of 200 mg/kg ginseng two hours prior to the administration of fipronil (FPN) modulated the histopathological effects in renal tissues of rats in the form of moderate congestion in renal tissue, a notable decrease in the number of inflammatory cell infiltrates, and necrosis restricted to sparse cells **(Abd Eldaim *et al.,* 2020).** Furthermore, the coadministration of ginseng with malathion displayed almost normal renal histological structure with minor hydropic epithelial cell degenerations and mild inter-tubular capillary congestion **(Ghamry *et al.,* 2022)**.

Among a range of organisms, immunohistochemistry has emerged as a useful diagnostic and investigative tool. The cell proliferation rate can be measured using the Ki-67 marker **(Jurková *et al.,* 2016)**. In the present study, exposure to TiO_2-NPs greatly reduced the immunoexpression of Ki-67 in the renal tissues in comparison to the control group, indicating that TiO_2-NPs dramatically diminished the mitotic index in TiO_2-NPs-exposed rats. These findings are in agreement with **Márquez-Ramírez *et al.,* (2012)** who showed that TiO_2-NPs inhibited the proliferation of C6 and U373 cell lines. On the other hand, administration of ginseng with TiO_2-NPs in GIV and GV significantly increased Ki-67 immunoexpression, indicating greater replicative activity; this may be ascribed to cellular regeneration (regenerative proliferation). This came into contact with **Faghani *et al.,* (2022)** who found that co-treatment with ginseng decreased the expression of apoptotic markers while increasing the proliferative index (Ki-67) in granulosa and theca cells in pre-antral and antral follicles, as well as in stroma cells. Therefore, ginseng, as a protective and therapeutic agent, reduced cellular damage and improved cell regeneration when supplied along with TiO_2-NPs.

CONCLUSION

Current research suggests that TiO_2-NPs may impair kidney function by producing oxidative damage, altering antioxidant defense levels, changing biochemical and molecular markers, and causing histological alterations. Ginseng inhibits oxidative

stress, apoptosis, and inflammation in rat renal tissues, demonstrating its antioxidant and anti-inflammatory capabilities. We recommend administering ginseng as a potential prophylactic and therapeutic agent against renal toxicity induced by TiO_2-NPs. More study is required to practically validate whether this treatment is appropriate for humans. Another limitation of our work was using a rat model. Unlike the rat model, human exposure levels are unknown. Future use of ginseng requires testing various dosages. Thus, we recommend further study to fill this gap.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This work did not receive funding from any organization.

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How to cite this article:
Heba Abuzaied, Dina W. Bashir, Eman Rashad, Maha M. Rashad and Hany El-Habback, 2024. Ginseng Extract can alleviate The Induced-renal Toxicity of Titanium Dioxide Nanoparticles in a Rat Model. Journal of Applied Veterinary Sciences, 9 (2): 1-17.
https://dx.doi.org/10.21608/javs.2024.253254.1296