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Original Article

# The protective role of some natural antioxidants against some nanoparticles-induced subchronic nephrotoxicity in Wistar rats

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## ABSTRACT

The aim is to study the possible protection of fig fruit extract with olive oil and date-palm fruit extract (FOD) as natural antioxidants in decreasing the subchronic toxicity hazards of silicon oxide nanoparticles ($SiO_2$NPs), aluminum oxide nanoparticles ($Al_2O_3$NPs), or zinc oxide nanoparticles (ZnONPs) in male rats treated for 75 days. We used 80 male Wistar rats distributed into 8 groups (n = 10) according to the experiment. We used various antioxidant treatments at their recommended antioxidant doses. All nanoparticles were given orally and daily for 75 days at doses of 100 mg/kg. The oral administration of different nanoparticles (NPs) alone led to dramatic histopathological features, a significant increase in the levels of the TBARS, tumor suppressor p53, and inflammatory markers (TNF-α and IL-6) in the renal tissue. In addition, serum kidney function parameters elevated significantly in NPs treated groups compared with the control group. On the other hand, the renal TAC, GSH, SOD, and TBARS lowered significantly in the renal tissue of rats administered with different NPs compared to the control group. The FOD-NPs-treated groups recorded significantly reduced nephrotoxicity effects as compared to the groups treated with NPs alone. In Conclusion, administration of FOD provides considerable protective effects against NPs-induced subchronic nephrotoxicity in male Wistar rats.

**Keywords:** *$SiO_2$NPs; $Al_2O_3$NPs; ZnONPs; olive oil; extracts; oxidative stress; P53; kidney*.

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## 1. INTRODUCTION

Nanotechnology creates products that exhibit novel properties (particle size range from 1 to 100 nm) **[1]**. The spread of nanotechnology in industry causes the possible accumulation of nanomaterials in the human body exposed to these NPs **[2]**. Nanoparticles affect various physiological systems and may enter the human body via various routes and may even cause pathological disorders **[3]**.

The applications of $SiO_2$NPs in industry and biomedicine products are promising because they possess stabilities and easy modifications **[4]**. Toxicology studies have suggested that $SiO_2$ NPs can induce adverse effects in the liver, kidney, and brain **[5-7]**. $Al_2O_3$NPs have been used in industry **[8]**. The acute exposure to $Al_2O_3$NPs causes toxicity in experimental animals **[9]**. ZnONPs are used in food additives and other

pharmaceuticals **[10, 11]**. Subsequently, the increased use of ZnONPs causes more concerns about their toxicity and inflammatory effects **[12]**.

The Mediterranean diet contains natural products that produce a promising effect on the health state **[13]**. *Ficus carica* (fig) fruits (belonging to the Moraceae family), typically Mediterranean species have antioxidant properties. The fig fruits contain high levels of polyphenols, flavonoids, and anthocyanins **[14]**. Moreover, fig-trees possess multiple therapeutic and anti-inflammatory activities against different toxins **[15]**. Olive oil is an important constituent of the Mediterranean diet. It contains many antioxidants and active compounds responsible for its biological activities as oleic acid, phenolics (including hydroxytyrosol, tyrosol, and oleuropein), and squalene **[16]**. *Phoenix dactylifera* (belonging to the family Arecaceae*)* is one of the species of date palm and is considered one of the oldest cultivated fruit trees in the Middle East. The flesh of the dates contains a higher percentage of carbohydrates, vitamin C, vitamin A, vitamin E, vitamin B2, and dietary fibers. In addition, it is rich in iron, calcium, magnesium, phosphorus, potassium, sodium, zinc, and selenium and protects against oxidative stress **[17-19]**.

This study aims to explore the possible protective effects of FOD against different nanoparticles-induced subchronic nephrotoxicity in Wistar rats including renal oxidative stress, some biochemical, inflammatory, and histopathological alterations in addition to P53 content in the kidney tissue with a view to its possible applications in the clinical field.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

We used chemicals and reagents of high analytical grade from standard suppliers.

### 2.2. Nanoparticles

The silicon oxide nanoparticles ($SiO_2$NPs), aluminum oxide nanoparticles ($Al_2O_3$NPs), or zinc oxide nanoparticles (ZnONPs) were prepared and characterized in a private laboratory (Nanogate Laboratory, Cairo, Egypt).

### 2.3. Preparation of nanoparticles (NPs) treatments

The nanoparticles ($SiO_2$NPs, $Al_2O_3$NPs, and ZnONPs) were suspended in water. This suspension was vibrated by vortex for 5 min before injection to aid in preparing a homogeneous suspension. All nanoparticles were given orally by oral gavages for 75 consecutive days. All nanoparticles ($SiO_2$NPs **[20]**, $Al_2O_3$NPs **[21]**, and ZnONPs **[22]**) were given at doses of 100 mg/kg according to pilot studies in our lab (data not shown) and the given doses were found following previously published studies.

### 2.4. Plant materials and authorities

We purchased the extra-virgin olive oil from the Grup Pons Company (Spain), the fig fruit from Kafoods Ltd. (Turkey), and the date-palm fruit from the Al-MADINA AL-MUBARAK market (Saudi Arabia).

### 2.5. Preparation of crude extracts

*Ficus carica* fruit extract was prepared and lyophilized according to a previous method **[23]**. The hydroalcoholic extract of the date fruit was made and lyophilized according to a previous method **[24]**.

### 2.6. Preparation of the antioxidant treatments

The extra-virgin olive oil was supplemented to rats by oral gavage. Every rat received oil in a concentration of 7 g/kg body mass **[25]**.

The fig and date-palm fruit extracts were prepared for supplementation in rats at a concentration of 1 g/mL just before the experimental use. The selected fig and date-palm

fruit doses were calculated based on the human recommended antioxidant doses **[26]** after conversion to rat doses.

### 2.7. The experimental animals

The experimental animals used in the present study were the mature male Wistar albino rats obtained from the Egyptian Holding Company for Biological Products and Vaccines (VACSERA, Giza, Egypt) and were housed at the animal facility, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt and provided with diet pellets and tap water. Five rats were placed into each cage and provided with standard diet pellets and drinking tap water *ad libitum* during the experimental period. Animals in all experiments remained 2-week acclimatization period before treatments. In addition, the animal masses were recorded regularly one time per week and the animal behaviors were monitored daily.

### 2.8. Animal welfare

The *in vivo* studies were conducted under the National Research Centre guidelines for the use and care of laboratory animals **[27]** and were approved by an independent ethics committee of the Faculty of Pharmacy, Ain Shams University.

### 2.9. Experimental design

A patch of 80 male Wistar albino rats average weight of 150-170 g were allowed to acclimatize for 2 weeks, and then divided into 8 groups as represented in **Table 1**

**Table 1**. The study design

| Groups | Treatment | Period |
|---|---|---|
| **1. Control** | diet pellets and tap water | 2 W + 75 days |
| **2. FOD** | extra-virgin olive oil (7 g/kg) and freshly prepared fig extract (1 g/kg) and date-palm fruit extract (1 g/kg) daily and orally. | 2 W + 75 days |
| **3. $SiO_2$NPs** | $SiO_2$NPs in doses of 100 mg/kg, daily and orally. | 75 days |
| **4. FOD-$SiO_2$NPs** | FOD and $SiO_2$NPs on the same schedule mentioned above in groups II and III. | 2 W + 75 days |
| **5. $Al_2O_3$NPs** | $Al_2O_3$NPs in doses of 100 mg/kg, daily and orally. | 75 days |
| **6. FOD-$Al_2O_3$NPs** | FOD and $Al_2O_3$NPs on the same schedule mentioned above in groups II and V. | 2 W + 75 days |
| **7. ZnONPs** | ZnO in doses of 100 mg/kg, daily and orally. | 75 days |
| **8. FOD-ZnONPs** | FOD and ZnONPs on the same schedule mentioned above in groups II and VII. | 2 W + 75 days |

**Note:** FOD, fig with olive oil and date-palm fruit extracts; $SiO_2$NPs, silicon oxide nanoparticles; $Al_2O_3$NPs, aluminum oxide nanoparticles; ZnONPs, zinc oxide nanoparticles; W; week

### 2.10. Collection of samples

Samples were collected from each animal under anesthesia from the retro-orbital venous plexus puncture at the end of the experiment. Blood samples were collected in non-heparinized tubes and centrifuged at 4000 rpm for 10 min. The sera were frozen at -80 ºC for the following measurements. After blood sampling, the animals were dissected and the kidneys were quickly removed and washed with saline, and divided into two portions one was used for biochemical analysis and the other was used for histopathology. The portion used for biochemical analysis was homogenized in ice-cold Tris–HCl lysis buffer, pH 7.4 using rotor-stator Homogenizer, fitted with a Teflon pestle (Omni International, Kennesaw, GA, USA). The homogenates were centrifuged under cooling at 3000 rpm for 20 min. The supernatants were aliquoted and stored at -80 °C until used.

### 2.11. Biochemical study

#### 2.11.1. Renal oxidative stress parameters

The measurements of oxidative stress markers of the renal tissue (renal total antioxidant capacity (TAC), renal reduced glutathione (GSH), and renal superoxide dismutase (SOD), thiobarbituric acid reactive substances (TBARS)) were estimated in the kidney tissue homogenate using kits from bio-diagnostic Co. for research kits, Egypt.

#### 2.11.2. Inflammatory markers

The tumor necrosis factor-alpha (TNF-α) was estimated in the kidney tissue homogenate of rats by ELISA technique using a kit purchased from MyBioSource, Inc. San Diego, CA 92195-3308: USA according to the manufacturer's instructions provided with the TNF-α assay kit (Catalog No: MBS2507393). The interleukin-6 (IL-6) was estimated in the kidney tissue homogenate of rats by ELISA technique using a kit purchased from Elabscience Biotechnology, Inc., Texas, USA according to the manufacturer's instructions provided with the IL-6 assay kit (Catalog No: E-EL-R0015).

#### 2.11.3. Apoptotic Biomarkers Estimation

The P53/Tumor Protein (p53/TP53) was estimated in the kidney tissue homogenate of rats by ELISA technique using a kit purchased from Cusabio Technology LLC, Houston, USA using methods outlined in the diagnostic kit.

#### 2.11.4. Kidney function tests

The measurements of serum kidney function parameters (creatinine, urea, blood urea nitrogen (BUN), and uric acid) were estimated in the blood serum using kits from bio-diagnostic Co. for research kits, Egypt.

#### 2.11.5. The histopathological study

The kidney tissue samples were fixed, processed, sectioned, and stained according to certain methods **[28]**.

### 2.12. Statistical analysis

The statistical analysis of the results was performed by using a statistical SPSS/PC program. All values were expressed as mean±SE. The results were analyzed using a one-way analysis of variance (ANOVA) test followed by the least significant difference (LSD) test for multiple comparisons. Differences were considered statistically significant at $p<0.05$.

## 3. RESULTS

### 3.1. The effects of fig with olive oil and date-palm fruit extracts (FOD) on the oxidative stress markers of the renal tissue of male rats treated with $SiO_2$NPs, $Al_2O_3$NPs, or ZnONPs for 75 days are represented in Table 2

The FOD and FOD-$SiO_2$NPs-treated groups show insignificant changes in the TAC, GSH, SOD, and TBARS in the renal tissue when compared with the control group.

The $SiO_2$NPs, $Al_2O_3$NPs, and ZnONPs-treated groups recorded a significant decrease ($P<0.05$) in the TAC (18.71%, 31.37%, and 36.17%, respectively), GSH (18.44%, 35.62%, and 41.65%, respectively), SOD (2.59%, 15.12%, and 13.51%, respectively) in contrast to a significant increase ($P<0.05$) in the TBARS (75.43%, 203.95%, and 208.94%, respectively) in the renal tissue when compared with their corresponding values in the control group.

Similarly, the FOD-$Al_2O_3$NPs and FOD-ZnONPs-treated groups recorded a significant decrease ($P<0.05$) in the TAC (19.10%, and 21.61%, respectively), GSH (25.04%, and 31.08%, respectively), SOD (10.00%, and 10.00%, respectively) in contrast to a significant increase ($P<0.05$) in the TBARS (144.55%, and 152.61%, respectively) in the renal tissue when compared with their corresponding values in the control group.

The FOD-$SiO_2$NPs, FOD-$Al_2O_3$NPs, and FOD-ZnONPs-treated groups recorded a significant increase ($P<0.05$) in the TAC, GSH, and SOD in contrast to a significant decrease ($P<0.05$) in the TBARS in the renal tissue when compared with their corresponding values in the $SiO_2$NPs, $Al_2O_3$NPs, and ZnONPs-treated groups respectively.

**Table 2.** The effects of fig with olive oil and date-palm fruit extract on the oxidative stress markers of the kidney tissue of male rats treated with silicon oxide nanoparticles, aluminum oxide nanoparticles, or zinc oxide nanoparticles for 75 days

| **Parameters** | **Experimental groups** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Control** | **FOD** | **$SiO_2$NPs** | **FOD-$SiO_2$NPs** | **$Al_2O_3$NPs** | **FOD-$Al_2O_3$NPs** | **ZnONPs** | **FOD-ZnONPs** |
| **Renal TAC (µmol/g tissue)** | 2.31±0.04 | 2.24±0.06 | 1.88±0.19[a] | 2.16±0.03[b] | 1.58±0.10[a] | 1.87±0.13[a,c] | 1.47±0.13[a] | 1.81±0.11[a,d] |
| | | -3.20% | -18.71% | -6.31% | -31.37% | -19.10% | -36.17% | -21.61% |
| **Renal GSH (mmol/g tissue)** | 5.42±0.06 | 5.40±0.10 | 4.42±0.15[a] | 5.24±0.14[b] | 3.49±0.22[a] | 4.06±0.24[a,c] | 3.16±0.22[a] | 3.73±0.26[a,d] |
| | | -0.39% | -18.44% | -3.32% | -35.62% | -25.04% | -41.65% | -31.08% |
| **Renal SOD (U/g tissue)** | 160±0.07 | 160±0.10 | 156±1.25[a] | 159±1.28[b] | 136±0.33[a] | 144±1.23[a,c] | 138±0.33[a] | 144±1.23[a,d] |
| | | -0.05% | -2.59% | -0.90% | -15.12% | -10.00% | -13.51% | -10.00% |
| **Renal TBARS (nmol/g tissue)** | 22.6±0.09 | 22.3±0.43 | 39.6±2.76[a] | 25.1±1.87[b] | 68.7±1.18[a] | 55.2±2.49[a,c] | 69.8±0.37[a] | 57.1±1.92[a,d] |
| | | -1.21% | 75.43% | 11.04% | 203.95% | 144.55% | 208.94% | 152.61% |

**Note:** Results are the mean ± standard error; %, percent of change from the control value; FOD, fig with olive oil and date-palm fruit extracts; $SiO_2$NPs, silicon oxide nanoparticles; $Al_2O_3$NPs, aluminum oxide nanoparticles; ZnONPs, zinc oxide nanoparticles; TAC, total antioxidant capacity; GSH, reduced glutathione; SOD, superoxide dismutase activity; TBARS, thiobarbituric acid reactive substances. **For each parameter:** [a]$p<0.05$, versus control group; [b]$p<0.05$, versus $SiO_2$NPs; [c]$p<0.05$, versus $Al_2O_3$NPs; [d]$p<0.05$, versus ZnONPs. **N.B:** The antioxidants (FOD) were used for 2 weeks before and during the administration of the nanoparticles (75 days).

### 3.2. The effects of fig with olive oil and date-palm fruit extracts (FOD) on the tumor suppressor p53 and inflammatory markers in the renal tissue of male rats treated with $SiO_2$NPs, $Al_2O_3$NPs, or ZnONPs for 75 days are represented in Figs. 1-3

The FOD and FOD-$SiO_2$NPs-treated groups show insignificant changes in the p53, TNF-α, and IL-6 in the renal tissue when compared with control values.

The $SiO_2$NPs, $Al_2O_3$NPs, and ZnONPs-treated groups recorded a significant increase ($P<0.05$) in the p53 (7.87%, 32.96%, and 39.04%, respectively), TNF-α (8.17%, 72.76%, and 132.26%, respectively), and IL-6 (11.77%, 36.65%, and 29.70%, respectively) in the renal tissue when compared with their corresponding values in the control group.

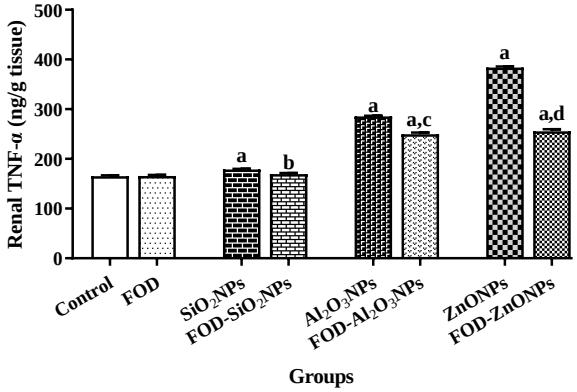


**Fig. 1.** The effects of fig with olive oil and date-palm fruit extracts (FOD) on the renal tumor necrosis factor-α (TNF-α) in rats treated with different nanoparticles (NPs) for 75 days. [a]$p<0.05$, versus control group; [b]$p<0.05$, versus $SiO_2$NPs; [c]$p<0.05$, versus $Al_2O_3$NPs; [d]$p<0.05$, versus ZnONPs. **N.B:** The antioxidants (FOD) treatments were used for 2 weeks before and during the administration of the nanoparticles.

Similarly, the FOD-$Al_2O_3$NPs and FOD-ZnONPs-treated groups recorded a significant increase ($P<0.05$) in the p53 (23.88%, and 25.20%, respectively), TNF-α (50.97%, and 54.66%, respectively), and IL-6 (21.02%, and 17.00%, respectively) in the renal tissue when compared with their corresponding values in the control group.

The FOD-$SiO_2$NPs, FOD-$Al_2O_3$NPs, and FOD-ZnONPs-treated groups recorded a significant decrease ($P<0.05$) in the p53, TNF-α, and IL-6 in the renal tissue when compared with their corresponding values in the $SiO_2$NPs, $Al_2O_3$NPs, and ZnONPs-treated groups, respectively.

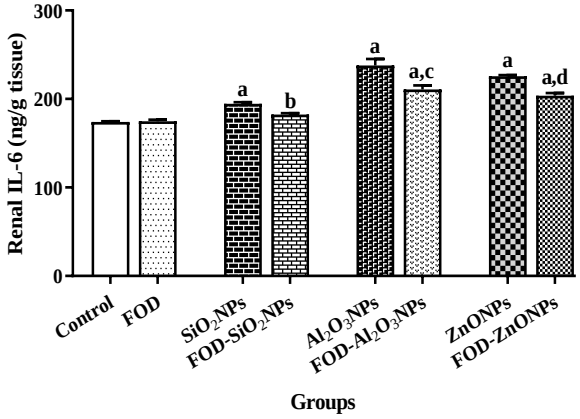


**Fig. 2.** The effects of fig with olive oil and date-palm fruit extracts (FOD) on the renal interleukin-6 (IL-6) in rats treated with different nanoparticles (NPs) for 75 days. [a]$p<0.05$, versus control group; [b]$p<0.05$, versus $SiO_2$NPs; [c]$p<0.05$, versus $Al_2O_3$NPs; [d]$p<0.05$, versus ZnONPs. **N.B:** The antioxidants (FOD) treatments were used for 2 weeks before and during the administration of the nanoparticles.

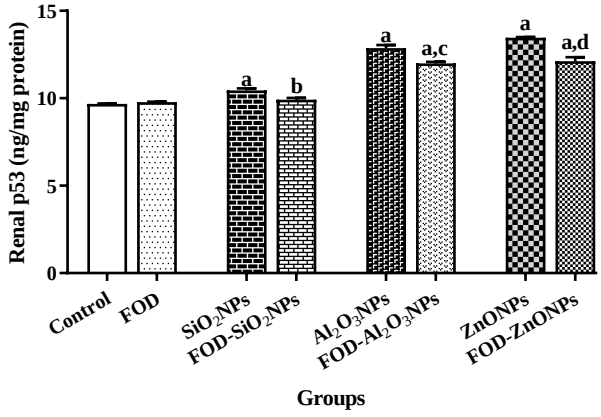


**Fig. 3.** The effects of fig with olive oil and date-palm fruit extracts (FOD) on the renal tumor suppressor p53 (p53) in rats treated with different nanoparticles (NPs) for 75 days. [a]$p<0.05$, versus control group; [b]$p<0.05$, versus $SiO_2$NPs; [c]$p<0.05$, versus $Al_2O_3$NPs; [d]$p<0.05$, versus ZnONPs. **N.B:** The antioxidants (FOD) treatments were used for 2 weeks before and during the administration of the nanoparticles.

### 3.3. The effects of fig with olive oil and date-palm fruit extracts (FOD) on the serum kidney function parameters of male rats treated with $SiO_2$NPs, $Al_2O_3$NPs, or ZnONPs for 75 days are represented in Table 3

The FOD and FOD-$SiO_2$NPs-treated groups show insignificant changes in the creatinine, urea, BUN, and uric acid in the serum when compared with control values.

The $SiO_2$NPs, $Al_2O_3$NPs, and ZnONPs-treated groups recorded a significant increase ($P<0.05$) in the creatinine (19.78%, 44.51%, and 62.64%, respectively), urea (23.71%, 43.14%, and 34.57%, respectively), BUN (19.33%, 31.91%, and 43.13%, respectively), and uric acid (10.63%, 19.41%, and 21.80%, respectively) in the renal tissue when compared with their corresponding values in the control group.

Similarly, the FOD-$Al_2O_3$NPs and FOD-ZnONPs-treated groups recorded a significant increase ($P<0.05$) in the creatinine (26.92%, and 44.51%, respectively), urea (24.29%, and 16.00%, respectively), BUN (15.84%, and 25.65%, respectively), and uric acid (8.88%, and 9.95%, respectively) in the renal tissue when compared with their corresponding values in the control group.

The FOD-$SiO_2$NPs, FOD-$Al_2O_3$NPs, and FOD-ZnONPs-treated groups recorded a significant decrease ($P<0.05$) in the creatinine, urea, BUN, and uric acid in the renal tissue when compared with their corresponding values in the $SiO_2$NPs, $Al_2O_3$NPs, and ZnONPs-treated groups, respectively.

**Table 3.** The effects of fig with olive oil and date-palm fruit extract on the serum kidney function parameters of male rats treated with silicon oxide nanoparticles, aluminum oxide nanoparticles, or zinc oxide nanoparticles for 75 days

| Parameters | Experimental groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | FOD | $SiO_2$NPs | FOD-$SiO_2$NPs | $Al_2O_3$NPs | FOD-$Al_2O_3$NPs | ZnONPs | FOD-ZnONPs |
| **Serum Creatinine (mg/dL)** | 0.18±0.00 | 0.17±0.01 | 0.21±0.00[a] | 0.19±0.01[b] | 0.26±0.00[a] | 0.23±0.01[a,c] | 0.29±0.02[a] | 0.26±0.01[a,d] |
| | | -5.49% | 19.78% | 8.24% | 44.51% | 26.92% | 62.64% | 44.51% |
| **Serum Urea (mg/dL)** | 35±2.49 | 34.7±2.27 | 43.3±1.85[a] | 38.4±2.42[b] | 50.1±2.40[a] | 43.5±1.45[a,c] | 47.1±2.23[a] | 40.6±2.30[a,d] |
| | | -0.86% | 23.71% | 9.71% | 43.14% | 24.29% | 34.57% | 16.00% |
| **Serum BUN (mg/dL)** | 19.1±1.01 | 19.0±0.94 | 22.8±1.30[a] | 21.1±1.06[b] | 25.2±1.19[a] | 22.2±0.66[a,c] | 27.4±1.16[a] | 24.0±1.37[a,d] |
| | | -0.52% | 19.33% | 10.20% | 31.91% | 15.84% | 43.13% | 25.65% |
| **Serum Uric acid (mg/dL)** | 2.05±0.08 | 1.99±0.10 | 2.26±0.09[a] | 2.14±0.06[b] | 2.44±0.05[a] | 2.23±0.06[a,c] | 2.49±0.06[a] | 2.25±0.06[a,d] |
| | | -2.88% | 10.63% | 4.59% | 19.41% | 8.88% | 21.80% | 9.95% |

**Note:** Results are the mean ± standard error; %, percent of change from the control value; FOD, fig with olive oil and date-palm fruit extracts; $SiO_2$NPs, silicon oxide nanoparticles; $Al_2O_3$NPs, aluminum oxide nanoparticles; ZnONPs, zinc oxide nanoparticles; BUN, blood urea nitrogen. **For each parameter:** [a]$p<0.05$, versus control group; [b]$p<0.05$, versus $SiO_2$NPs; [c]$p<0.05$, versus $Al_2O_3$NPs; [d]$p<0.05$, versus ZnONPs. **N.B:** The antioxidants (FOD) were used for 2 weeks before and during the administration of the nanoparticles (75 days).

### 3.4. The effects of fig with olive oil and date-palm fruit extracts (FOD) on the kidney histopathological characters of male rats treated with $SiO_2$NPs, $Al_2O_3$NPs, or ZnONPs for 75 days

The control kidney showed a normal morphological appearance. However, the antioxidant-treated NPs-administered groups (FOD-$SiO_2$NPs, FOD-$Al_2O_3$NPs, and FOD-ZnONPs) recorded significantly ameliorated histopathological characters in the kidney tissue when compared with their corresponding values in the non-antioxidant-treated NPs-administered groups ($SiO_2$NPs, $Al_2O_3$NPs, and ZnONPs, respectively) as indicated in **(Table 4 and Fig. 4)**.

**Table 4.** The effects of fig with olive oil and date-palm fruit extract on the kidney histopathological results of male rats treated with silicon oxide nanoparticles, aluminum oxide nanoparticles, or zinc oxide nanoparticles for 75 days

| | G | BS | Tubules | | | Interstitium | Medulla |
|---|---|---|---|---|---|---|---|
| | | | Lining | Brush border | Lumen | | |
| **Control** | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **$SiO_2$NPs** | + | + | + | 0 | + | + | + |
| **FOD-$SiO_2$NPs** | 0 | 0 | 0 | 0 | 0 | + | 0 |
| **$Al_2O_3$NPs** | + | + | + | 0 | 0 | + | + |
| **FOD-$Al_2O_3$NPs** | 0 | 0 | + | 0 | 0 | + | + |
| **ZnONPs** | ++ | + | ++ | ++ | ++ | ++ | + |
| **FOD-ZnONPs** | 0 | 0 | + | 0 | + | + | + |

FOD, fig with olive oil and date-palm fruit extracts; $SiO_2$NPs, silicon oxide nanoparticles; $Al_2O_3$NPs, aluminum oxide nanoparticles; ZnONPs, zinc oxide nanoparticles.

# Glomeruli (G): (0, average; +, edematous/congested; ++, small-sized/atrophied).
# Bowman's spaces (BS): (0, average; +, widened/dilated; ++, obliterated).
# Tubules
- Lining: (0, average; +, edematous/apoptotic; ++, atrophied/necrotic).
- Brush border: (0, preserved; +, partial loss; ++, complete loss).
- Lumen: (0, free; +, few/scattered casts; ++, marked intra-tubular casts).

# Interstitium: (0, average; +, dilated/congested BV; ++, markedly dilated BV/interstitial hemorrhage).
# Medulla: (0, average; +, congested capillaries/scattered hyaline casts; ++, marked hyaline casts).

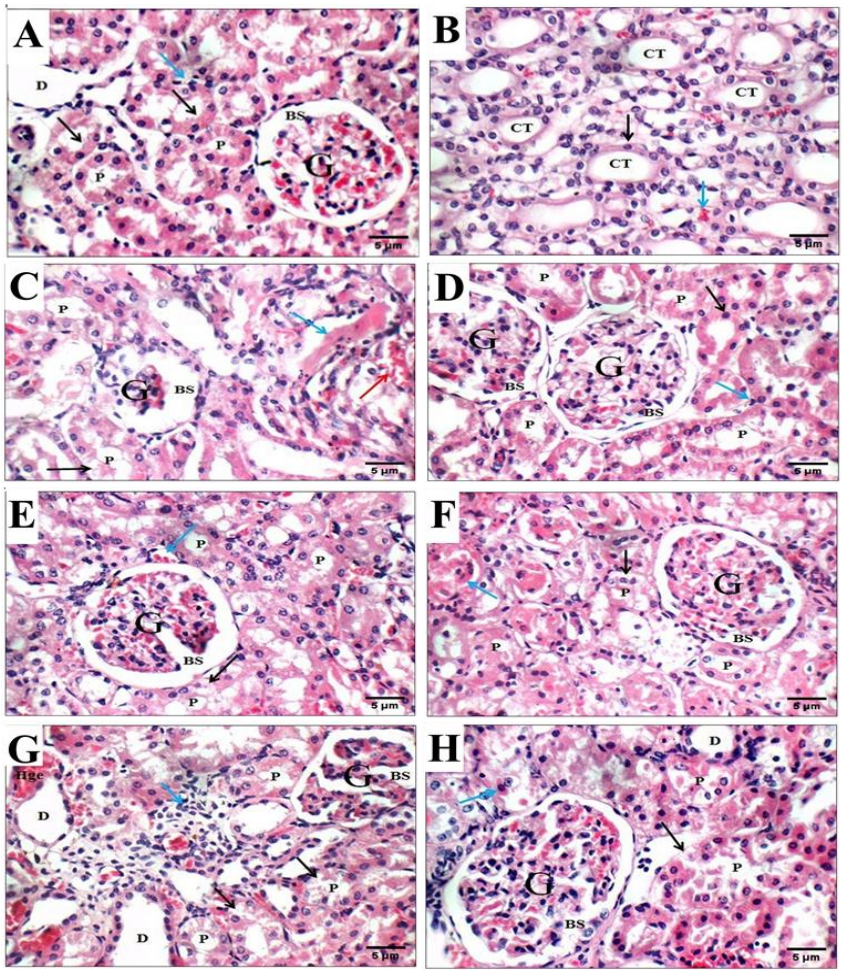


**Fig. 4.** The effects of fig with olive oil and date-palm fruit extracts (FOD) on the microscopic histological appearance of livers treated with different nanoparticles (NPs) for 75 days. **A**, higher power view of the control kidney showing average glomeruli (G) with average Bowman's spaces (BS), average proximal tubules (P) with preserved brush borders (black arrows), average distal tubules (D), and average interstitium (blue arrow); **B**, FOD-treated group renal medulla showing average collecting tubules (CT) with average epithelial lining (black arrow) and average peri-tubular capillaries (blue arrow); **C**, $SiO_2$NPs-treated group showing atrophied glomerulus (G) with widened Bowman's space (BS), proximal tubules (P) with mildly edematous epithelial lining (black arrow), intra-tubular hyaline casts (blue arrow), and mildly congested blood vessels (red arrow); **D**, FOD-$SiO_2$NPs-treated group showing average glomeruli (G) with average Bowman's spaces (BS), proximal tubules (P) with average epithelial lining (black arrow), and average interstitium (blue arrow); **E**, $Al_2O_3$ NPs-treated group showing average glomerulus (G) with average Bowman's space (BS), proximal tubules (P) with mildly edematous epithelial lining (black arrow) and mildly congested interstitial blood vessels (blue arrow); **F**, FOD-$Al_2O_3$NPs-treated group showing average glomerulus (G) with average Bowman's space (BS), proximal tubules (P) with mildly edematous (black arrow) and apoptotic epithelial lining (blue arrow); **G**, ZnO NPs-treated group showing atrophied glomerulus (G) with widened Bowman's space (BS), proximal tubules (P) with edematous epithelial lining (black arrow), and areas of interstitial hemorrhage (Hge) with mild inflammatory infiltrate (blue arrow); **H**, FOD-ZnO NPs-treated group showing average glomerulus (G) with average Bowman's space (BS), proximal tubules (P) with mildly edematous (black arrow) and apoptotic epithelial lining (blue arrow).

## 4. DISCUSSION

Recently, more nanomedicines have been already approved by the U.S. Food and Drug Administration (FDA) for human use **[29]**. With the progress in the nanotechnology field, there may be an increase in the exposure of humans to nanoparticles, so further urgent studies are required to study the possibility of any detrimental health impacts, target organs damage as well as their mechanisms **[30]**.

The present study confirmed the renal toxicity of rats treated with $SiO_2$NPs, $Al_2O_3$NPs, or ZnONPs **[31-34]** which indicated that these toxicities may be mediated through induction of oxidative stress, and lipid peroxidation, systemic inflammation, and renal toxicity. Due to their small size, the NPs can travel to different organs of the body when ingested. The mechanism(s) of the kidney cytotoxicity induced by nanoparticles might be due to enhanced production of reactive oxygen species ROS leading to oxidative stress and lipid peroxidation and induction of inflammatory pathways **[3, 35]**.

Regarding the oxidative stress markers, the $SiO_2$NPs, $Al_2O_3$NPs, and ZnONPs-treated groups recorded a significant depletion of TAC, GSH, SOD, and CAT and a significant increase of TBARS as compared to the control group. These results are in agreement with previous studies **[22, 32, 34, 36]** which reported a significant depletion in the antioxidant system accompanied by a dramatic fall in the antioxidants after $SiO_2$NPs, $Al_2O_3$NPs, or ZnONPs administration.

The lipid peroxidation products (TBARS) are correlated with increased production of ROS so, considered a useful marker of oxidative stress. In addition, TBARS played an important role in determining the NPs ($SiO_2$NPs, $Al_2O_3$NPs, or ZnONPs) toxicity **[32, 36]**. The cells are equipped with several natural enzymatic (SOD and CAT) and non-enzymatic (GSH) endogenous antioxidant defenses to combat oxidative stress **[37]**. The treatments with $SiO_2$NPs, $Al_2O_3$NPs, or ZnONPs lead to the depletion of these endogenous antioxidants **[22, 32]**. In agreement, Guan *et al*. **[38]** and Zhang *et al*. **[3]** recorded that $SiO_2$NPs, $Al_2O_3$NPs, or ZnONPs administration cause oxidative stress leading to DNA damage and cytotoxicity in the kidney.

The results obtained in the present study showed that the extra-virgin olive oil with fig and date-palm fruit extracts are quite safe products and they have not resulted in any adverse effects when administered to rats **[39-43]**.

The present study demonstrated that the antioxidant-treated NPs-administered groups (FOD-$SiO_2$NPs, FOD-$Al_2O_3$NPs, and FOD-ZnONPs) recorded significantly ameliorated oxidative stress markers in the kidney tissue when compared with their corresponding values in the non-antioxidant-treated NPs-administered groups ($SiO_2$NPs, $Al_2O_3$NPs, and ZnONPs, respectively). The treatment with FOD revealed the presence of numerous and varied antioxidants and those diverse natural antioxidants acted synergistically **[40, 44]**. The free radical toxicity in the present study was counteracted by the FOD treatment which might be attributed to the protective activity of the plant antioxidants since they are complex mixtures of many chemicals **[45]**.

The $SiO_2$NPs, $Al_2O_3$NPs, and ZnONPs-treated groups recorded a significant increase in the renal TNF-α and renal IL-6 when compared with their corresponding control values which revealed the inflammatory reaction appeared after rats exposed to these NPs as agreeing with **[46]** who concluded that the pro-inflammatory cytokines (TNF-α, IL-6, and IL-Iβ) could play an important role in regulating immunity. In agreement, Faddah *et al*. **[47]** found that the elevation in serum inflammatory markers including TNF-α, IL-6, and CRP due to NPs

ingestion induced nephrotoxicity.

The $SiO_2$NPs, $Al_2O_3$NPs, and ZnONPs-treated groups recorded a significant increase in the p53 when compared with their corresponding control values. It is agreeing with the fact that oxidative stress can stimulate apoptotic cell death **[48]**. Tumor suppressor genes regulate diverse cellular activities including programmed cell death. In addition, p53 known as a potent prooxidant protein, down-regulates intracellular ROS levels, thus reducing the probability of genetic alterations and apoptosis **[49, 50]**. In agreement, different NPs such as $SiO_2$NPs or ZnONPs caused DNA damage and apoptosis in the kidney tissues **[32, 51]**.

Our results showed that FOD effectively reduced inflammatory markers and p53 induced by the NPs-administration. These results revealed that FOD contains a strong antioxidant effect, and since they contained high amounts of phenolic compounds, they have been reported as free radical scavengers and have therapeutic effects against tissue inflammatory, cancer (tumor suppressor), and cardiovascular diseases **[40, 52, 53]**.

The kidney function parameters of urea, creatinine and uric acid levels were markedly increased by $SiO_2$NPs, $Al_2O_3$NPs, or ZnONPs administration in the present study. In kidney damage, these biomarkers were released into the bloodstream from the proximal cells. In agreement, the elevated concentration of these biomarkers indicates proximal cell destruction **[54]**, and the inflammatory cells were infiltrated into the renal tissue after oral ingestion of $SiO_2$NPs, $Al_2O_3$NPs, or ZnONPs in rats **[55]**. Also, in agreement, Yan *et al*. **[51]** and Yousef *et al*. **[22]** demonstrated that ZnONPs and $Al_2O_3$NPs exhibited toxicological symptoms implying the potential renal damage. In addition, the *in vitro* research indicated the NP's cytotoxicity to kidney cells **[56, 57]**.

The most sensitive biochemical markers in the diagnosis of renal damage are the retention of creatinine and urea in the blood **[58]**; therefore, the significant increase in these biomarkers indicates kidney functional damage **[59]**. The higher improvement in the antioxidant markers by concomitant administration of FOD in the present study could be explained by Allouche *et al*. **[60]** who stated that olive oil by its richness in carotenoids, tocopherols, vitamin E, and polyphenols as potential antioxidants properties acting as ROS scavenger as well as increasing the activity of antioxidant enzymes. In agreement, the hydroxytyrosol of FOD was reported to be an effective scavenger. Administration of hydroxytyrosol to the albino rats prevented kidney toxicity **[61]**. The decrease in uric acid level may be due to FOD treatment that enhances GSH concentration and inhibits XDH conversion to XO that catalyzes purine degradation, hypoxanthine, and xanthine metabolism to uric acid **[40]**.

The kidney damage caused by $SiO_2$NPs, $Al_2O_3$NPs, or ZnONPs in the present study is further confirmed by histopathological examination, which showed many pathological features such as degeneration of the epithelial cells of various renal tubules, distorted corpuscles, capillaries shrinkage, and inflammatory infiltration in various tubules. These data are in line with the previous studies **[22, 62, 63]**.

The significantly ameliorated histopathological characters recorded at the FOD-$SiO_2$NPs, FOD-$Al_2O_3$NPs, and FOD-ZnONPs when compared with their corresponding values in the non-antioxidant-treated NPs groups could be attributed to the antioxidant effect of FOD due to their varied phenolic compounds, which were able to donate a hydrogen atom to the free radicals, thus stopping the propagation chain reaction during the lipid peroxidation process

**[40, 64]**.

## Conclusion

According to the results obtained in the present study, the administration of extra virgin olive oil with fig and date palm extracts (FOD) provides considerable protective effects against different NPs-induced subchronic nephrotoxicity in male Wistar albino rats. In addition, the administration of FOD revealed a synergistic effect of the combination between them to produce a broad spectrum of antioxidative activities that creates an effective defense system against the free radical attack.

## Declarations

### Ethics approval and consent to participate

The study protocol was approved by the Research Ethical Committee, Faculty of Pharmacy, Ain Shams University, and conducted according to the regulations and recommendations of the ethical guidelines and complied with the guide for the care and use of laboratory animals.

### Consent to publish

All authors have read and agreed to the published version of the manuscript.

### Availability of data and materials

Data analyzed during this study are all included in the main manuscript.

### Competing interests

No competing interests were declared by the authors.

### Funding statement

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