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Phoenix dactylifera seeds extract ameliorates the lipid profile fluctuations and the renal toxicity induced by silver nanoparticles in mice

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ABSTRACT

Applications of silver nanoparticles (AgNPs) include biosensing, cosmetics, and medicine. AgNPs may become deposited as particles in many tissues and vital organs after exposure. AgNPs toxicity could be removed from the body using natural products. This study aimed to address the effective of *Phoenix dactylifera* seed extract (PDSE) in treating renal and lipid profile alterations brought on by the exposure to AgNPs. The following four groups (n=10) of male CD1 mice were created: 200 µl of sterile saline has been injected intraperitoneal (i.p) into group 1 (Gp1). Gp2 had received PDSE (100 mg/kg) i.p. Gp3 had received 0.25 mg/Kg of AgNPs, i.p. every day for a month. Gp4 had received both AgNPs and PDSE treatment, as in Gp2 and Gp3. The kidney's histological changes and lipid profile were investigated. AgNPs caused a noticeable histological change in the kidney tissues as well as significant changes in the lipid profile. PDSE treatment after AgNPs injection reduced the toxicities on the kidneys` tissues and lipid profile. Together, PDSE treatment could be employed to decline the negative impacts that induced by AgNPs exposure on kidneys` tissues and lipid profile.

Keywords: Silver nanoparticles, *Phoenix dactylifera*, Seeds, Extract, Kidney function, Lipid profile.

1. Introduction

The range of nanoparticles (NPs) size is between 1–100 nm (Ema et al., 2017). Silver nanoparticles (AgNPs) are generally used in industrial, agricultural, medical and cosmetic applications. AgNPs, in particular, are used in preclinical trial and clinical settings for treatment of different diseases (Chen et al., 2013). Despite of the important role of AgNPs in different biomedical applications, it shows sort of toxicity on different vital organs (Vance et al., 2015). The toxicity of AgNPs to human cells appears to be induced by oxidative stress and inflammation (Wise et al., 2010). Therefore, several studies were reported different settings to ameliorate the induced.

toxicity of AgNPs (Akbarzadeh et al., 2016).

Phoenix dactylifera L is a flowering tree, belongs to Arecaceae palm family that is grown for its tasty sweet fruits (Krueger and Robert, 2018). PDSE showed a good promise in the treatment of diabetes due to the presence of polyphenols that have strong antioxidant properties (Mia and Al-Tareq, 2020). Dates fruit show a potential health advantage against a variety of cancers (Al-Sayyed et al., 2017). Free radical scavenging, antioxidant, antimutagenic, antibacterial, anti-inflammatory, gastro protective, hepatoprotective, nephroprotective, anticancer, and immune stimulant effects have been demonstrated in preclinical studies of *P. dactylifera* (PD) extracts (Khalid et al., 2017; El-Naggar et al., 2023; Al-Bagoury et al., 2023). This study aimed to address the potential efficacy of the treatment with PDSE to ameliorate changes induced by the toxic effects of AgNPs on the lipid profile and renal glomerular functions as

well as the kidney's tissue in experimental mice.

2. Materials and Methods

Chemicals

Silver nanoparticles

Silver nanoparticles (AgNPs) with average size (15 ± 3 nm) were purchased from Nanotech Egypt for Photo-Electronics (El-Wahaat Road, Dream Land City, Entrance 3, City of 6 October, AL Giza, Egypt). Vials were diluted by phosphate buffer saline (PBS) and the concentration was adjusted to 0.25 mg/kg, in 200 µl. (Sardareha et al., 2012). Cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), creatinine and urea kits were purchased from Bio diagnostic company, Egypt.

Collection of *P. dactylifera* seeds and preparation of their extract

P. dactylifera fruits were purchased from local market of Tanta city, Egypt. The plant materials were identified and authenticated by a taxonomist at Botany Department, Faculty of Science, Tanta University. *P. dactylifera* seeds were dried in shade then crushed in a mortar and the powder kept in suitable place for further use. 50 g of seed powder was mixed vigorously with 500 mL 70% (V/V) ethanol. The hydro-alcoholic extracts were filtered and the solvent was dried under air condition, then the extracts were washed and suspended in 0.9 % sterile saline for further processing as PDSE.

Animals

Forty male Swiss albino mice (20 ± 2 g) were allowed to acclimatize for a week in the animal house conditions of the Faculty of Science, Tanta University, before being divided into groups. The institutional animal care committee and Local Ethics Committee and Animals Research (Faculty of Science, Tanta University- Egypt) approved the experimental design and protocol (IACUC-SCI-TU- 0187) .

Experimental design

Mice were given drinking tap water and normal experimental pelleted animal food *ad libitium*. After 1 week of acclimation period in the animal facility to reduce the standard errors in the experimental groups, mice were separated into groups based on body weight. Male mice were divided into four groups (n=10) as follows: Group 1 (Gp1) was injected intraperitoneal (i.p) with 200 µl sterile saline. Mice in Gp2 were injected with PDSE (100 mg/kg), (i.p) for a month. Mice of Gp3 were injected with AgNPs (0.25mg/kg), (i.p) for one month. Mice in Gp 4 were injected with AgNPs as in Gp3 then, treated with PDSE as in Gp2.

Determination of kidney relative weight

All mice of the experiment were sacrificed after 30 days. Gross examinations were performed macroscopically on all mice during sacrifice. Percentages of absolute and relative kidney weights (kidney wt/b.wt × 100) of all mice were taken after organs being necropsied.

Serum and tissue samples preparation

Blood sampling from retro-orbital venous plexus of each group under light anesthesia using heparinized microhematocrit tubes were taken then centrifuged at approximately 1000 × g (or

3000 rpm) for 15 minutes. Sera were immediately separated and aliquot then stored at -20°C for biochemical analysis.

Histopathological investigations

Parts from kidney were preserved in 10% phosphate-buffered formalin at 4-5 mm3 thickness, dehydrated in graded alcohol series, cleared in xylene and embedded in paraffin blocks. 4-5 µm sections of the collected sections were stained with heamatoxylin and eosin (H &E) for histopathological examination (Bancroft and Stevens, 1996) .

Biochemical analysis

The total cholesterol level was measured according to the method of Abell et al. (1952). The plasma triglyceride was measured according to Buccolo and David (1973). Both HDL and LDL were estimated according to Friedewald et al. (1972). Creatinine and urea were assayed according to the method of Newman and Price (1999).

Statistical analysis

The data were expressed as mean ± SD. Comparison between groups was carried out using one-way ANOVA. If there is a significant difference between means, Turkey post hoc comparisons among different groups was performed. For all statistical tests, P values ≤ 0.05 was considered to be significantly different.

Data and statistical analysis were performed using Excel 2013 (Microsoft Corporation, USA), and Minitab (version 18).

3. Results

Treatment with PDSE after AgNPs restored the kidney relative weight.

Treatment with PDSE after AgNPs injection restored R.K.wt close to normal, as compared to control group (Gp1) (Fig 1). The treatment of naïve mice with PDSE significantly increased the R.K.wt ($p \leq 0.05$). The results showed that the injection of AgNPs for a month led to a significant decrease in R.K.wt ($p \leq 0.05$). Treatment with PDSE for a month after AgNPs injection led to significantly improvement in R.K.wt ($p \leq 0.05$).

Treatment with PDSE improved kidney functions in mice injected with AgNPs

The treatment of naïve mice with PDSE did not show any significant changes in the urea and creatinine levels ($p \geq 0.05$). The results showed that the injection of AgNPs for a month led to a significant decrease in total proteins and albumin level ($p \leq 0.05$). Treatment with PDSE for a month after the injection with AgNPs led to significantly improvement in total proteins and albumin level ($p \leq 0.05$) (Table 2).

PDSE treatment after AgNPs injection restored the lipid profile near to normal levels

Treatment of naïve mice with PDSE showed no significant changes in total cholesterol, triglyceride, and LDL levels ($p \geq 0.05$). The injection of AgNPs for a month led to a significant increase in cholesterol, triglycerides and LDL levels ($p \leq 0.05$). Treatment with PDSE for a month post AgNPs injection led to significant improvements in cholesterol, triglycerides and LDL levels ($p \leq 0.05$).

PDSE ameliorated AgNPs-induced renal tissue alterations in mice

Histological structure of the kidney of control mice (Gp1) showed normal structure indexed by intact renal Bowman's capsule and proximal and distal convoluted tubule.

Bowman's capsule appears well developed with an outer mantle layer and an inner mantle that surrounds a mass of blood capillaries known as glomerulus (Fig 2A). No histopathological changes were noticed in kidney sections of mice that treated with PDSE alone (Gp2) as compared to control group. However, few degenerated cells with darkly stained nuclei and cytoplasmic vacuolations were seen in walls of the renal tubules (Fig 2B). The injection of AgNPs (Gp3) resulted in histopathological alterations in the renal tissue that manifested by atrophy of the renal corpuscles, disorganized bowman's space, and pyknotic epithelial cells with darkly stained nuclei. Moreover, several convoluted tubules are disintegrated and also had irregular dilated with flattened epithelial lining.

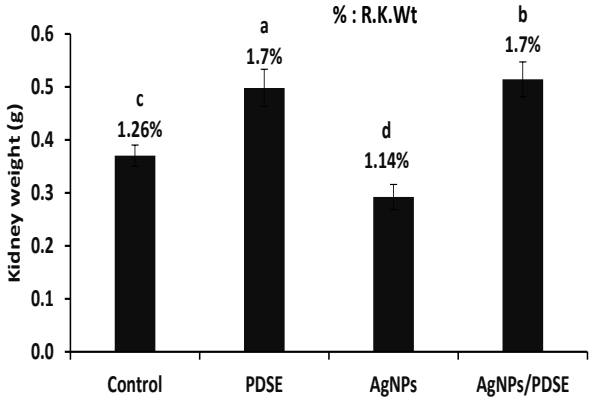

Fig. 1. Effect of PDSE and AgNPs on the relative weight of mice kidney in different groups

Congested blood vessels, cytoplasmic vacuolations, focal per tubular inflammatory cellular infiltration were determined in the affected areas (Fig 2C). Kidney sections of the AgNPs / PDSE treated group (Gp4) exhibits nearly normal histological appearance and structure, which displayed normal glomeruli and renal tubules compared to (Gp3).

Moreover, few disorganized renal tubules with destructed lining epithelia and cytoplasmic vacuolar degeneration were observed (Fig 2D). That is to say, PDSE treatment stopped further damage in the glomerular and tubular tissues, but however, few lesions that are still found may be tissue residues induced by former AgNPs administration.

Table 1. Effect of *P. dactylifera* seeds extract on the Kidney weight and kidney relative weight of different treatment groups

Groups	Kidney wt. (g)	R.K.wt. (%)
Control	0.37 ± 0.02^{c}	1.26%
PDSE	0.50 ± 0.03^{a}	1.70%
AgNPs	0.29 ± 0.02^{d}	1.14%
AgNPs/PDSE	0.51 ± 0.03^{b}	1.70%

The values represented as mean ± SD; PDSE: *P. dactylifera* seed extract; AgNPs: Silver nanoparticles; R.K.wt: Relative kidney weight. Means that share letters are significantly different at *P* value < 0.05; while those that do not share letters are not significantly different

Table 2. Effect of *P. dactylifera* seeds extract on the creatinine and urea levels of different treatment groups.

Groups	Creat (mg/dl)	Urea (mg/dl)
Control	0.48 ± 0.02^{b}	35.33 ± 0.58^{b}
PDSE alone	0.47 ± 0.04^{b}	36.33 ± 2.08^{b}
AgNPs alone	0.87 ± 0.02^{a}	51.67 ± 1.53^{a}
AgNPs/PDSE	0.47 ± 0.01^{b}	37.67 ± 2.5^{b}

The values represented as mean ± SD; PDSE: *P. dactylifera* seed extract; AgNPs: Silver nanoparticles*;* Creat: Creatinine. Means that share letters are significantly different at *P* value < 0.05; while those that do not share letters are not significantly different

Table 3. Effect of *P. dactylifera* seeds extract on the cholesterol, triglycerides, HDL and LDL levels in different treatment groups

Groups	Chol. (mg/dl)	Tri. (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	143 ± 1.0^{a}	92.3 ± 2.5^{c}	56.33 ± 1.53^{a}	$52.2 \pm 1.53^{a, b}$
PDSE alone	133 ± 1.5^{b}	108 ± 2.0^{b}	58.00 ± 1.00^{a}	53.67 ± 1.53^{a}
AgNPs alone	156 ± 10.0^{c}	177 ± 2.0^{a}	43.67 ± 1.53^{b}	72.38 ± 5.62^{c}
AgNPs/PDSE	136 ± 1.53^{b}	95.6 ± 1.5^{c}	58.33 ± 1.53^{a}	48.93 ± 1.47^{b}

The values represented as mean ± SD; PDSE: *P. dactylifera* seed extract; AgNPs: Silver nanoparticles*;* Tri: Triglycerides; Chol: Cholesterol; HDL: High density lipoprotein; LDL: Low density lipoprotein. Means that share letters are significantly different at *p* value < 0.05; while those do not share letters are not significantly different.

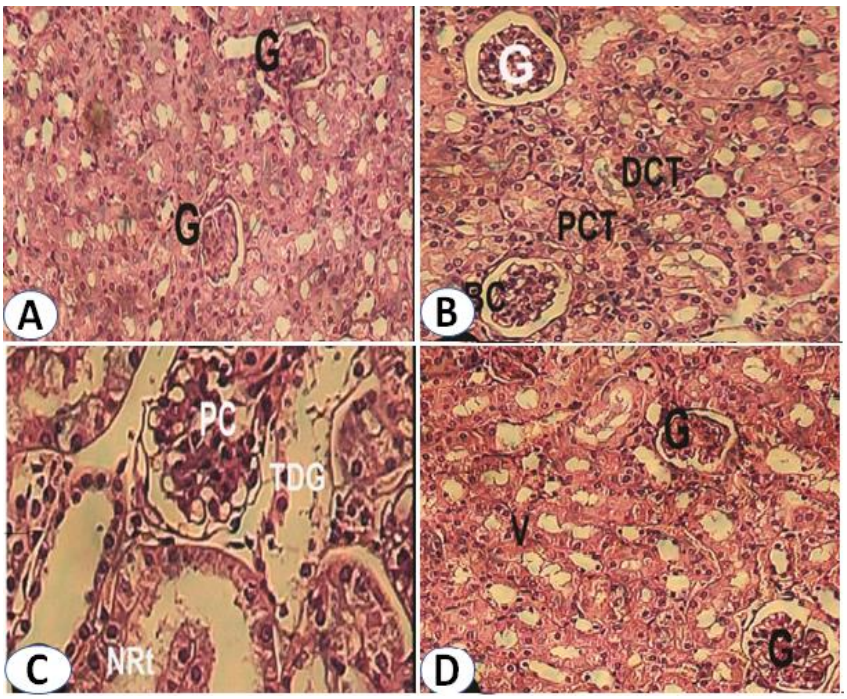

Fig. 2. Photomicrographs of H and E staining kidney section of all groups. A) Kidney sections of the control group showing normal cortex region, glomeruli (G). B) PDSE kidney sections showing normal glomeruli (G), bowman's space (B) renal tubules (PCT-DCT). C) AgNPs kidney sections showing atrophy of glomeruli (DG), degeneration of corpuscles (CDG), renal edema (RED) and tubular degeneration (TDG). D) kidney sections treated with PDSE after AgNPs injection exhibiting a normal like structure of the kidney tissue with normal glomeruli (G) and few disorganizations renal tubules (PCT-DCT) (H &E stain) (X100 - X400).

Discussion

Silver nanoparticles (AgNPs) are introduced to the human body by different routes, causing damage to various body systems, including digestive, respiratory, and reproductive organs (Volker et al., 2013). The assessment of bio-distribution, elimination, and accumulation of AgNPs in potential organ toxicity were studied before (Xiu et al., 2014). Natural products are possible antioxidants that can protect chronic diseases from oxidative damage. *P. dactylifera L* contains anthocyanins, phenolic, sterols, carotenoids, procyanidins, and flavonoids, which possess free radicals scavenging, anti-oxidant, anti-microbial, anti-inflammatory, anti-hyperlipidemic, gastroprotective, hepatoprotective, nephroprotective, anticancer, and immunostimulant activities (El-Far et al., 2016). The present study recorded significant increases in creatinine and urea levels of AgNPs group. Other studies showed that AgNPs induced renal functions impairment through the significant increase in serum creatinine and urea levels (Yarmohammadi et al., 2014; Kim et al., 2018).

Accordingly, treatment with PDSE ameliorated AgNPs induced renal dysfunction, as indicated by decrease in serum creatinine and urea levels. An important report, in a gentamicin-treated nephrotoxicity rat model, extracts of PD fruit significantly reduced plasma creatinine and urea levels and ameliorated the proximal tubular damage (Al-Qarawi et al., 2018). In addition, diabetic rats' with increased serum glucose, cholesterol and triglyceride levels were dramatically reduced by the PDSE treatment (Khan et al., 2016).

The histopathological examination revealed several histological alterations in the kidney tissues of AgNPs group. These alterations were in renal tissue that manifested by renal corpuscles atrophy, disorganized bowman's space, and tubular pyknotic cells with darkly stained nuclei. Previous studies demonstrated that AgNPs caused swelling of podocytes that affect glomerular filtration (Guo et al., 2016; Tiwari et al., 2017; Moradi-Sardareh et al., 2018). It has been shown that AgNPs were able to induce geno-toxicity and tissue damage in the

kidney (Alarifi et al., 2016). The obtained results were consistent with results of other work in which silver accumulation did not modify renal function, because AgNPs appeared to be located in the basement membranes of the medulla and cortex, not the lumens of nephrons (Genter et al., 2012). This result is in agreement with a previous report that described the distribution of AgNPs in multiple tissues including kidneys (Park et al., 2011).

Treatment with PDSE following AgNPs injection revealed well improved histological appearance and structure, which displayed normal glomeruli and renal tubules. The results showed that the PDSE may play a crucial role in the treatment and management of various nephrons dysfunction and tissue toxicity lesions induced by AgNPs. In a study using a CCl4-induced toxicity model in rats, a hydro acetone, PDSE was demonstrated to confer a noticeable protection on the kidney in a dose dependent manner. The nephron-protective ability of this extract may be explained by its ability to scavenge effectively the free radical generated during CCl4 metabolism, probably because the extract contains a high quantity of pro anthocyanin's, which exhibited high antioxidant activity (Ahmed et al., 2015**).** Other study revealed that prolonged administration of PDSE aqueous extract ameliorated the progressive decline in renal dysfunction among the treated rats **(**Hasan and Mohieldein, 2016). The present results showed that PDSE has the ability to protect renal tissues from the damage and ameliorated the lipid profile against AgNPs toxicity.

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Conflict of interest

All authors declared that they have no conflicts of interest.

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