

Original Article

## The Impact of Silver Nanoparticles and its Combination with Black Seed (*Nigella sativa*) and Honey on Kidney Stones in Male Mice

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### HIGHLIGHTS

- Investigating the synergistic effects between honey, black seed, and silver nanoparticles on kidney stones.
- A detailed comparative study between these two herbal substances and silver nanoparticles in the elimination of kidney stones.

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### ABSTRACT

#### Introduction

This study aimed to evaluate how silver nanoparticles, in conjunction with black seed and honey, affected alterations in serum, urine, and renal variables in male mice with renal stones.

#### Methods

Our study considered four target groups: negative and healthy control groups, 125ppm silver nanoparticles treatment, 125mg/kg black seed with 125mg/kg honey, and 125ppm silver nanoparticles treatment. We introduced essential ingredients to the groups from the first to the last day of the experiment, adding 1% ethylene glycol to the drinking water. The researchers investigated serum variables, such as potassium, sodium, calcium, phosphorus, and magnesium, urine factors like citrate, oxalate, and calcium, and tissue factors like kidney weight and crystal count.

#### Results

The results of this paper revealed that the accumulation of calcium oxalate crystals in the treatment group with 125ppm of silver nanoparticles significantly increased compared to other groups ( $P$ -value $<0.001$ ). Biochemical examination of urine showed that urine oxalate in the group treated with 125ppm of silver nanoparticles increased by  $(0.03\pm 0.78)$  and in the combined group of honey and black seeds (125mg/kg) and silver nanoparticles (125ppm) increased by  $(0.05\pm 0.54)$ . It shows significance ( $P$ -value $<0.001$ ). In addition, the results of changes in serum and tissue parameters in both groups showed that they aggravate the formation of kidney stones.

#### Conclusions

According to the findings of this research, silver nanoparticles cause toxicity in serum, urinary, and kidney tissue factors. Therefore, they are not a suitable option for preventing and treating kidney stone patients.

**Keywords:** Serum and Urinary Factors; Kidney Stones; Silver Nanoparticles; Honey; Black Seed

#### Introduction

Nanotechnology includes nanoparticles and atom clusters with an average size of fewer than 100 nanometers (1). Nanomaterials offer unique qualities, including a large surface area to volume ratio, tiny size, and high carrier capacity, allowing them to be used in various

applications. Nanotechnology has permeated several disciplines in the last two decades, including treatments, medicine, the environment, drug development, and biotechnology (2). However, silver nanoparticles have an adverse impact on various organs, including necrosis of hepatocytes and aggregation of monocytes and neutrophils

in the portal region, which leads to functional problems. Silver nanoparticles appear to modify mitochondrial membranes by interacting with the thiol group in the inner membrane, according to Almofti et al., (3). According to a new study, nanoparticles' impacts on live cells depend on the nanoparticle's diameter, size, and form. Experiments on the impact of silver nanoparticles on the creatine kinase enzyme revealed that silver nanoparticles at concentrations of 25 and 50mg/l inhibit brain creatine kinase activity by 35 to 40% and skeletal muscle creatine kinase activity by 35 to 55 percent. Still, the heart creatine kinase is unaffected (4).

Different mechanisms have been developed to explain the effect of silver on microorganisms by scientists. Microbes cannot adapt to or resist silver due to the complexity of these systems. Thanks to nanotechnology, manufacturing silver particles at the nanoscale are now possible. Silver nanoparticles have allowed researchers to see the antibacterial properties of silver metal at extremely low concentrations. Several bacteria, fungi, and viruses have been studied to see how silver nanoparticles influence them. More than 650 different germs have been found, including AIDS and influenza viruses (5). Nanotechnology's various uses in therapy have been examined since its introduction in health. Silver nanoparticles have antibacterial capabilities and are among the most commonly employed. The activity of nanoparticles increases when the surface-to-volume ratio has been increased, so this rise can significantly impact their use in the therapy field (6). Herbal medicines such as black seed and honey are attracting much attention due to the high costs of treatment and the destructive consequences of modern treatment processes (7). The effects of silver nanoparticles and their combination with black seed and honey were investigated in the previous study. Thus, our aim in this paper is to examine the influence of silver nanoparticles and their combination with black seed and honey. It is possible that by altering the composition of silver nanoparticles, mixing them with plant extracts, and developing plant nanocomposites, their negative impacts will be lessened while their positive benefits will be amplified. As a result, the effects of silver nanoparticles and the combination of silver nanoparticles with hydroalcoholic extract of black seed and honey on reducing the formation of kidney stones in male mice caused by ethylene glycol were examined in this study.

### Methods

The animal house at Shahrekord Azad University provided 45 male mice weighing 25-30g. The Islamic Azad University Ethical committee approved the study (IR.IAU.NAJAFABAD.REC.1397.075). All rats were housed in ideal temperature and humidity conditions at Shahrekord Azad University's animal nest. Animals have full access to water and food throughout this period.

We gave drinking water containing 1% ethylene glycol to 8 groups of mice throughout this time. Sawdust was put on the cage floors, and the cages were cleaned twice a week to prevent mice from becoming unwell and maintain proper hygiene.

The animals were regarded as 4 experimental groups with 5 mice in every group. These groups are: Healthy control group (1): 1% of distilled water was added to the drinking water of this group during the experiment. Negative control group (2): We added 1% ethylene glycol to the drinking water of mice group during 30 days of treatment.

Treatment group (3) Mice from the first day to the end of the experiment with the administration of 1% ethylene glycol in drinking water for 15 days (every other day) daily 0.5ml of silver nanoparticles with The concentration of 125ppm was given by intraperitoneal injection.

Treatment group (4): In this group, mice from the first day to the end have the administration of 1% ethylene glycol in drinking water, 125mg/kg of black seed hydroalcoholic extract with gavaging 125mg/kg of honey, and also during 15 Day (every other day) 0.5ml of silver nanoparticles with a concentration of 125ppm were injected intraperitoneally daily.

### Preparation of silver nanoparticles solution

Sigma provided spherical nanoparticles with a normal diameter of 10nm and a storage solution concentration of 1000ppm. Citrate reduction was used to make silver nanoparticles chemically. The required dilutions were then generated using the dilution series approach from the primary storage solution. The mice were dissected, collected for pathological examination, weighed, and stored in 10% formalin Hematoxylin and. eosin staining were performed on the collected samples at a pathology facility.

### Statistical Analysis

In the biochemical analysis phase, SPSS software was used to assess the result statistically (8), one-way variance analysis (ANOVA), and Tukey post hoc tests. The data is provided as Mean Standard Error Mean in all cases. The results were significantly considered as (P-value<0.001), (P-value<0.01), and (P-value<0.05).

### Results

Mice treated with 125 ppm silver nanoparticles had the highest serum potassium (5.7±0.16mg/dl). Compared to the healthy group, Mean serum potassium of the negative control group (5.4±0.29mg/dl) increased (4.7±0.22). The data obtained from the serum sodium level of different groups of treated mice showed that the sodium level in all groups decreased negatively compared to the control group. The Mean serum calcium of healthy control group (8.30±0.37mg/dl) decreased compared

to a negative control group ( $10.34 \pm 0.33$  mg/dl). Serum phosphorus levels of all treated groups were very close to each other. Serum phosphorus levels of healthy control ( $5.48 \pm 0.50$  mg/dl) and negative ( $5.48 \pm 0.60$  mg/dl) were equal, respectively. Compared to the negative control (P-value > 0.05), Serum magnesium levels of the control group decreased significantly (Table 1).

#### Results of determination of urinary parameters in mice

Mean urine citrate decreased in the negative control group ( $1.98 \pm 0.15$  mg/dl) compared with a healthy control group ( $2.58 \pm 0.07$  mg/dl). The treatment group had 125 ppm silver nanoparticles reducing the amount of urinary oxalate ( $0.78 \pm 0.03$  mg/dl). In the treatment group with three studied factors, namely black seed, honey, and silver nanoparticles, the amount of oxalate equal to  $0.54 \pm 0.05$  mg/dl was obtained. Urine oxalate levels in healthy control group ( $0.4 \pm 0.01$  mg/dl) decreased compared to negative control group ( $0.86 \pm 0.03$  mg/dl). The analysis of the results showed that the group treated with 125 ppm silver nanoparticles had the highest urinary calcium content ( $12.56 \pm 0.54$ ) compared to the other group (Table 2).

#### Results of determining the weight of all mice

In this study, the weight of all mice was determined after treatment with different compounds alone or in combination. Compared with the negative control group that had the highest kidney weight ( $0.67 \pm 0.01$  g), the group treated with 125 ppm silver nanoparticles had the highest kidney weight ( $0.65 \pm 0.005$  g) among the treatment groups (Table 3).

#### Determining the number of crystals in mice

Negative control group). In this group, the cortical region is not regular, and the capsular space in the renal bodies

is much wider than in the control group. In the medullary regions, we observed clear pathological changes so that the central spaces of the tubules are filled. This finding results from forming oxalate crystals that fill most of the central space of all tubules. In this group, The equivalent of  $40.7 \pm 1.29$  crystal units in 10 fields was randomly counted in this group. The changes in the pathology of this group are quite obvious in the form of filling the central spaces of the tubules (Figure 1).

In this group, the cortex area, which contains renal bodies (glomerular network and Bowman's capsule) and Pct and Dct tubules, is normal. The medulla area is also usual in this group, and no detrimental influence is observed in the tissue. No traces of calcium oxalate crystals were seen in every slice. In the group (125 ppm silver nanoparticles), extensive tissue destruction was observed in the cortex and medulla. Calcium oxalate crystals are abundantly deposited in the urinary tract of these two areas, which are large but slightly smaller than the control group. The renal bodies have been destroyed and lost their order after treatment with silver nanoparticles. Pct and Dct tubules showed fewer changes than renal bodies. We randomly counted the equivalent of  $35.5 \pm 1.56$  crystal units in 10 fields in this group. The combined group of silver nanoparticles, honey, and black seed) due to the association of honey and black seed with silver nanoparticles, the changes in pathology observed in this group were less than in the group of silver nanoparticles alone. The tubules have an almost normal appearance, and no significant pathological changes were observed in the medulla oblongata. Calcium oxalate crystals were mainly present in the bodies. The equivalent of  $12.6 \pm 1.41$  crystal units in 10 fields was randomly counted.

The results showed that in the 125 ppm group, silver nanoparticles of  $35.5 \pm 1.96$  crystals were observed, which had the highest number of crystals in this group compared

**Table 1.** Compare serum parameters in mice of the negative control group with other groups.

Serum parameters	Negative Control	Control	N.silver 125ppm	N. Sativa, Honey (125 mg/ml) & N.silver 125 ppm
Serum potassium	$5.4 \pm 0.29$	$4.7 \pm 0.22$	$0.16 \pm 5.7$	$4.6 \pm 0.35$
Serum sodium	$181.2 \pm 1.56$	$139.8 \pm 3$	$152.80 \pm 6.68$	$14.2 \pm 3.30$
Serum calcium	$10.34 \pm 0.33$	$8.3 \pm 0.37$	$9.86 \pm 0.31$	$8.94 \pm 0.37$
Serum phosphorus	$5.48 \pm 0.60$	$5.48 \pm 0.50$	$6.08 \pm 0.53$	$5.9 \pm 0.33$
Serum magnesium	$1.52 \pm 0.13^*$	$1.99 \pm 0.11$	$1.73 \pm 0.22$	$1.73 \pm 0.11$

\*P-value < 0.01

**Table 2.** Compare of urinary parameters in the negative control group with other groups

Urinary parameters	Negative Control	Control	N.silver 125ppm	N. Sativa, Honey (125 mg/ml) & N.silver 125ppm)
Urinary citrate	$1.98 \pm 0.15$	$2.58 \pm 0.7$	$2.04 \pm 0.19$	$2.68 \pm 0.2$
Urinary oxalate	$0.86 \pm 0.03$	$0.4 \pm 0.01$	$0.78 \pm 0.03$	$0.54 \pm 0.05$
Urinary calcium	$11.26 \pm 0.65$	$9.80 \pm 0.32$	$12.56 \pm 0.54$	$9.50 \pm 0.54$

**Table 3.** Compare the Kidney weight of the negative control group with other groups

Variable	Negative Control	Control	N.silver 125ppm	N. Sativa, Honey (125 mg/ml) & N.silver 125ppm)
Kidney weight	0.67±0.01	0.25±0.01	0.65±0.005	0.31±0.04

**Table 4.** Compare the mean number of negative control group crystals with other groups

Variable	Negative Control	Control	N.silver 125ppm	N. Sativa, Honey (125mg/ml) & N.silver 125ppm
Number of crystals	40.7±1.29	0±0	35.5±1.96	12.6±1.41

to the other groups (Table 4).

### Discussion

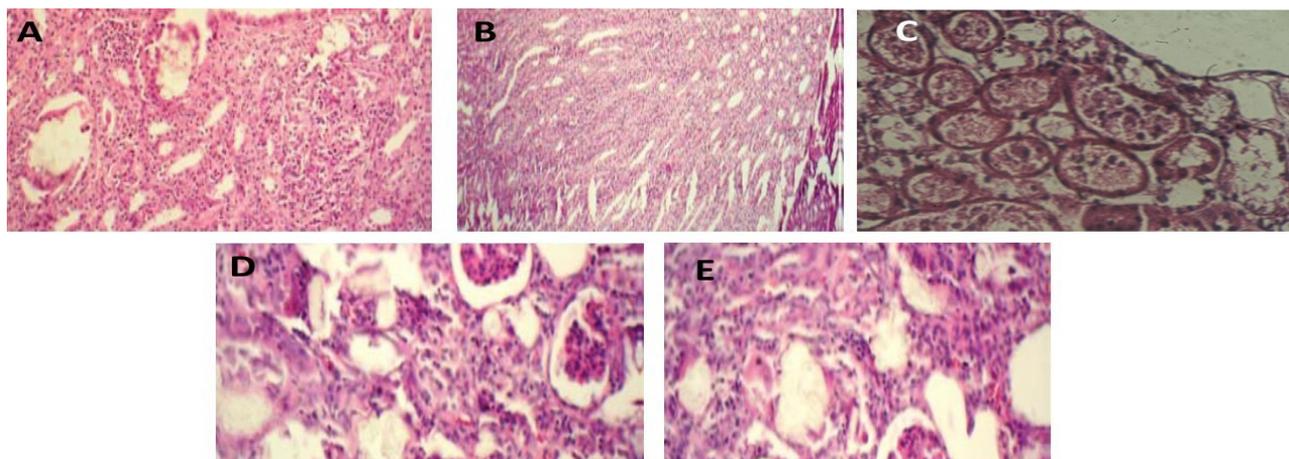
Heavy metals have long been used as an antiseptic to prevent bacteria from growing. These materials are now being proposed for application at the nanoscale. Antimicrobial silver nanoparticles are becoming increasingly popular. The group given 125ppm silver nanoparticles had the highest serum potassium level, greater than the control group. These findings demonstrated that silver nanoparticles did not affect kidney stone reduction. The blood sodium level in the group treated with silver nanoparticles (152.80 6.68mg/dl) was more significant than in the other groups treated with honey and black seed. It can be concluded that nanoparticles had little effect on lowering kidney stones, while the combination of honey and black seed can be decreased serum sodium and examined in the treatment of renal stones, according to our latest previous. The treatment group with silver nanoparticles alone (9.86±0.31mg/dl) showed the highest serum calcium. Silver nanoparticles alone could not significantly increase the amount of citrate (2.04±0.19mg/dl), which indicates the lack of a significant effect of this factor on the amount of citrate and the fight against kidney stones. In the treatment group with silver nanoparticles, a high amount of oxalate (0.78±0.03mg/dl) was obtained, indicating that this factor is ineffective against kidney stones. Several treatment policies are considered for kidney stones with several challenges and complications (9).

In addition, silver nanoparticles had no effect in lowering urinary calcium or preventing the production of kidney stones. In the study, the kidney weight in the negative control group (0.67±0.01g) was very close to that in the group treated with silver nanoparticles (0.65±0.005g). The number of crystals in the group treated with silver nanoparticles (35.5±1.96) was higher than in the other composite groups. Combining silver nanoparticles with pumpkin hydroethanolic extract on wound healing in mice indicated the cumulative effect. The combined impact of these two compounds is more than each, which shows that the designed nanocomposite has synergistic restorative effects. In this study, silver nanoparticles

with an average of 10 nm with concentrations of 250, 125, and 500 ppm and hydroethanolic pumpkin extract with concentrations of 70%, 50%, and 25% / kg and a combined concentration of silver nanoparticles of 125 ppm and extract / kg/mg was 25% (10). The impact of silver nanoparticles on serum phosphofructokinase enzyme and skeletal muscle tissue structure in male Wistar rats was examined in this study. The results demonstrated that silver nanoparticle concentrations of 50, 100, 200, and 400 ppm had no effect on the activity of this enzyme. The 400 ppm silver nanoparticles reduced skeletal muscle tissue order and tore some myofibrils with comparison to the control group (11). Susan et al., demonstrated that the distribution of nanoparticles in bodily tissues and their effects alter as their diameter and concentration change. Another study found that histopathological alterations linked to kidney dysfunction caused by nanoparticles' direct influence on cells, such as the creation of reactive oxygen species (ROS), are essential in the emergence of structural and functional abnormalities in the kidneys of male albino rats (12). The use of black seed aids in the reduction of this urine component. In the negative control group, the level of calcium oxalate in the black seed treatment group dropped. Numerous studies have examined the impact of several medicinal herbs on preventing kidney stones. Honey can also help in the treatment of kidney and urinary tract problems. Because it has no salt and just a little amount of protein, it is a good therapy for renal pain (13).

In a study conducted by Kim et al., When rats were treated with silver nanoparticles (size 42 nm, doses of 0.25, 0.5 and 1mg/kg) for 28 days, there was no significant increase in BUN and creatine levels, but Tissue damage was observed in the liver and kidney at very low doses of silver nanoparticles, but inflammatory responses were observed in the kidney. An excessive inflammatory response in the kidney appeared to indicate damage to the kidney's filtration capacity (14). In the present design, damage was observed in the kidney tissue by intraperitoneal injection of 125ppm to mice.

This study observed significant changes in serum sodium and potassium levels. This result is similar to the study conducted by Oberdo and Lam. Some studies report that silver and silver salts can be distributed throughout



**Figure 1.** A: Residues of stones in renal calyces in the negative control group (10×). B: Kidney-free medulla in the control group (10×). C: Normal renal cortex in the control group (40×). D: Total accumulation of calcium oxalate in urinary tubules in the group of 125ppm silver nanoparticles (40×). E: Damage to tubules and accumulation of calcium oxalate crystals in the combination group of 125mg/kg honey, black seed, and silver nanoparticles (40×).

the body and accumulate in the liver and kidney. Studies show that silver makes the cell membrane more permeable to sodium and potassium and disrupts the activity of the sodium-potassium ATPase pump and mitochondria, and changes sodium and potassium levels in blood serum (15). Compared to the negative control group, the average serum sodium level in the combined group of honey and black seed (125mg/kg) and silver nanoparticles decreased by 125ppm. In the present study, the average serum level of potassium in the group treated with the concentration of 125 ppm of silver nanoparticles was the highest ( $5.7 \pm 0.16$ mg/dl) compared to the negative control group. The results of this research are consistent with the studies mentioned earlier, and nanoparticles cause disturbances in pumped activity and increased potassium retention inside the cell, and changes in sodium and potassium levels were observed.

Kim et al., reported that when silver nanoparticles enter the body through ingestion, inhalation, or skin, they are transported into the bloodstream and accumulate in some organs (16). Silver nanoparticles cause DNA damage in mammalian cells through oxidative stress-related mechanisms and significantly increase cell death (17).

Stepien et al., investigated the toxicity of silver nanoparticles during oral administration in guinea pigs. The results showed that silver nanoparticles could enter the bloodstream and accumulate in some organs, including the liver and kidney, which induces the toxicity of hepatocytes and kidneys, and if contact with silver nanoparticles is prolonged, it may lead to death (18).

Silver nanoparticles are absorbed and distributed in different tissues after oral ingestion, inhalation, and skin contact in different tissues of living organisms, so the toxicity of silver nanoparticles for different body tissues

after absorption needs further study. Studies have shown that intravenous injection of nanoparticles (50nm) in rats damages the liver, kidney, and lung tissues. The results of this plan are consistent with the current plan (19).

### Conclusions

The present study observed the toxic effect of silver nanoparticle solution with a concentration of 125 ppm and black seed and honey (125mg/kg) on serum and urinary. Kidney tissue parameters, and in short, it can be concluded that silver nanoparticles directly affect the normal activation process of body cells and cause disruption in the functioning of body organs. Therefore, silver nanoparticles can not be recommended for treating kidney stones because the results achieved in the single state and combined with honey and black seed were to change the factors to intensify the state of stone formation.

### Authors' contributions

All authors contributed equally.

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### Conflict of interest

All authors declare that there is no potential competing or conflict of interest.

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### Ethics statement

The present study was run under the Ethics Committee

of Islamic Azad University (IR.IAU.NAJAFABAD. REC.1397.075).

#### Data availability

Data will be provided on request.

#### Abbreviations

ROS Reactive oxygen species

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