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The Effect of Silver Nanoparticles Produced from Extract of Medicinal Plant (*Kelussia*) on Kidney Function Tests

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Abstract

Background & Objectives: *Kelussia odoratissima* is involved in the treatment of kidney stones, irritation of the urinary tract and kidney and bladder cleansing. It is useful for gout patients and is used to treat kidney and ureteral stones. The aim of this study was to investigate the effect of *Kelussia odoratissima* on renal function tests in male rats.

Materials & Methods: In this study, 20 rats were randomly divided into one control group and three treatment groups (n=5 per group). The treatment groups received a 40, 80, and 120 mg/kg silver nanoparticles produced by the extracts of *Kelussia*, orally once a day for 6 days. After taking a blood sample, a kidney function test was performed. SPSS software version 21 was used for data collection, and the results were presented as mean and standard deviation (mean±SEM).

Results: Oral administration of silver nanoparticles produced by *Kelussia* extracts at 80 mg/kg dose significantly reduced bilirubin and urea compared to the control group ($P<0.05$). The 120 mg/kg dose also significantly reduced urea compared to the control group ($P<0.05$). There was no significant effect on total protein, albumin and urea.

Conclusion: Silver nanoparticles produced by the *Kelussia* extracts did not show any negative effect on kidney function, but had some positive effect on waste removal.

Keywords: *Kelussia*, Kidney, Rat

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Introduction

Herbal medicines contain a large number of naturally occurring chemicals (constituents) that have some type of biological activity. Constituting less than 0.1% of the dry weight of the plant, these plants are potent repositories of secondary metabolites and the primary active ingredients of many drugs. And environmental factors cause changes in the growth of medicinal plants as well as in the quantity and quality of active compounds such as alkaloids, glycosides, and essential oils (1). With increasing awareness of the side effects of chemical drugs, people have become more inclined to use herbal remedies and natural herbal products (2).

Kelussia odoratissima Mozaff. (Keluss) is an endemic medicinal plant of Central Zagros

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region of Iran (3) and It has medicinal and nutritional aspects (4). The herbaceous perennial belongs to the Umbelliferae family at a height of 120 to 200 cm. The local name of this plant is Keluss, which is native to the western region of Iran. This medicinal plant grows naturally in rangelands in so many snow areas in Zagros Mountains and Isfahan, Chaharmahal Bakhtiari, Kohgiluyeh and Boyer Ahmad, Fars, Lorestan (5, 6). Calming property (7), it improves memory (8) and blood coagulant (9). *Kelussia* root is used in root treasure syrup along with asparagus, ruscus, fennel and parsley to treat chronic albuminuria and kidney stone excretion, and diuretics. The presence of flavonoids such as rutin, apigenin and luteolin, flanol, caffeic acid and a variety of phthalidic volatile essential oils have been demonstrated in *Kelussia* (10). extract of *Kelussia* has nutritional indices as well as anticoagulant activity and antioxidant properties (11). Terpin is a fragrant substance in *Kelussia* that prevents from pain, microbial contamination and formation in your kidney stones or urinary tract, in addition to potassium in the gland and its trunk, stimulates the flow of urine and cleanses the kidney and bladder. It is useful for patients with gout because the etheric oil present in *Kelussia* dilates the kidneys so that uric acid is better excreted (10). Results of Rabbani et al. (2011) study on the chemical composition of the essential oil of *Kelussia odoratissima* Mozaff. Mice showed that the most important known major constituent of the essential oil of this drug 3-butene was 4, 5-dihydrofataldehyde (85.9%)(7).

Nanotechnology is one of the most dynamic and advanced sciences with high capacity, efficiency and abundance for use in various sciences, including agriculture and environment. Important applications and uses of this technology in Iran, especially in agricultural and environmental issues, make this technology not a choice but a necessity. Silver nanoparticles are a new antimicrobial compound and have the potential to eliminate many contains water-borne bacteria (12).

Urea, the end product of protein catabolism in the body, uric acid, the product of purine metabolism, and creatinine, an excreted chemical derived from creatine metabolism, are indicators of kidney function. Increased serum levels of these substances indicate decreased clearance and inability of the kidneys to excrete these substances through the bloodstream. Therefore, serum levels of these substances can be used as an indicator to measure renal efficiency and function (13). According to the findings of Mostaanzadeh et al. (2019) research entitled "investigating the interaction of *Apium graveolens* L. and *Kelussia odoratissima* Mozaff extracts on calcium oxalate with the purpose of kidney stone treatment", these herbal teas could be a good choice for treating diseases and preventing calcium oxalate formation in people predisposed to kidney stones (5). Since no study has been conducted on the effect of native *Kelussia* of *Kohgiluyeh* region on renal function indices, this study aimed to investigate the effect of silver nanoparticles produced by *Kelussia* extract on renal function tests.

Materials and Methods

Animals

Adult male rats (6 weeks old), weighing approximately 185 to 213 g, were obtained from a research center affiliated with the University of Medical Sciences and were housed under appropriate hygienic conditions, with an equal ratio of 12 hours of light and 12 hours of darkness, humidity, and The temperature was maintained at 22˚C to 26˚C for 6 days to meet the conditions of the animal. Rats had access to purified municipal water, free food prepared by the companies Pars Animal Feed Co.

Preparation of Herbal Extract

The extractions were carried out with methanol at constant temperature. 200 grams *Kelussia* aerial branches shredded, it was prepared from the cold region of Kohgiluyeh city, The methanol was added so that the solvent was completely covered

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in the percolator, after 72 hours the methanol extract was removed from the percolator and repeated with the addition of methanol to the extract and filtered with funnel and filter paper.

Preparation of Nanoparticles from Plant Extract

Silver nanoparticles from salt silver nitrate ($AgNO_3$) One millimolar is used. Dissolve 0.849 g of silver nitrate in 50 mL of distilled water to make a one-tenth molar solution. The extract was added to 200 mL and then heated at 50°C for 10 minutes. The resulting solution was placed in a dark place. The color change of the extract to dark brown indicates the formation of silver nanoparticles. The sediment was then centrifuged, washed with water and ethanol, and dried in an oven at 0°C for 3 hours. Complete resuscitation of Ag^+ ions to silver nanoparticles by color change of solutions and spectroscopy For 4 consecutive days, every 24 hours, an optical inspection and spectrophotometer were analyzed, and the best time for the formation of silver nanoparticles was determined. UV-visible spectroscopy showed that the silver nanoparticles had an absorption peak at 450 nm (8, 10).

Preparation of Edible Extract from the Extract

40, 80, and 120 mg of dried extract were dissolved individually in 10 mL of physiological serum. The oral solution was prepared at doses of 40, 80, and 120 mg/kg. Animals were fed 2 mL of each solution per 200 g of mouse weight.

Study design

In this study, 20 adult mice were randomly divided into 3 experimental groups and 1 control group. The experimental groups received silver nanoparticles produced by *Kelussia* extract at concentrations of 40, 80, and 120 mg/kg orally for 6 days. And on the seventh day, the mice were given blood and renal function tests were performed. All ethical protocols regarding laboratory animals were followed, there were no casualties during the injections, and the rats were delivered to the university animal hospital after bleeding.

Control Group

Animals in this group did not receive the extract, but were given 2 mL per 200 g body weight of normal saline for 6 days.

Treatment Group 1

Animals in this group received daily between 10 am and 11 am 2 mL of silver nanoparticles produced by the extract of *Kelussia* medicinal plant 40 mg/kg 200 g of body weight orally for 6 days.

Treatment Group 2

Animals in this group received 2 mL daily of silver nanoparticles produced by 80 mg/kg *Kelussia* extract. per 200 g of body weight orally for 6 days.

Treatment Group 3

Animals in this group received 2 mL daily of silver nanoparticles produced by the extract of the medicinal plant *Kelussia* 120 mg/kg. Orally for 200 grams of body weight received within 6 days.

One day after the last dose of silver nanoparticle extracts produced by the extract of *Kelussia* and normal saline, the food was withdrawn. Ethyl ether was used to anesthetize the rats. After anesthesia, blood samples were taken from the heart, and blood samples were centrifuged at 3000 rpm for 10 minutes to remove serum for evaluation of renal function factors. Total protein, bilirubin, albumin, urea, and creatinine were measured by Biotechnica, Italy, automated analyzer model 3000 BT using Pars Test enzymatic detection kits.

Statistical Analysis

Data were collected using SPSS 21 software and statistical analysis was performed using t-test. Results are presented as mean and standard deviation (mean±SEM).

Results

Total protein

Oral administration of silver nanoparticles produced by the extract of *Kelussia* medicinal plant at doses of 40.80 and 120 mg/kg showed no significant difference with the control

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group (Table 1).

Albumin

Oral administration of silver nanoparticles produced by *Kelussia* extract at 40.80 and 120 mg/kg showed no significant difference with the control group.

Creatinine (Cr)

Oral administration of silver nanoparticles produced by the extract of *Kelussia* medicinal plant at doses of 40.80 and 120 mg/kg showed no significant difference with the control group.

Urea (Urea)

Oral administration of *Kelussia* extract at doses of 120 and 80 mg/kg significantly reduced urea compared to the control group (P<0.05).

Bilirubin (BUN)

Oral administration of *Kelussia* extract at 80 mg/kg dose significantly reduced bilirubin compared to the control group (P<0.05) (Table 1).

Table 1. Effect of silver nanoparticles produced by the extracts from *Kelussia* on kidney function

Compare groups	Average body weight	Cr (mg/dL)	BUN (mg/dL)	Urea (mg/dL)	Albumin (g/dL)	Total Proteins (g/dL)
Control group	197.03	0.560±0.033	0.33 ± 0.008	27.2916±0.94	4.1541±0.08	6.0208 ±0.08
40 mg/kg	196.27	0.541±0.291	0.32 ± 0.008	26.014±0.61	4.3277±0.09	6.1458±0.12
80 mg/kg	195.71	0.538±0.029	0.31 ± 0.02*	23.332±0.83*	4.1250±0.18	6.1208±0.09
120 mg/kg	197.88	0.550±0.025	0.32 ± 0.01	22.83±0.51*	4.1583±0.04	6.145±0.08

-The values are "Mean ± SEM". The presence (*) in the table indicates a significant difference (P <0.05) compared to the control group

Discussion

Results of this study showed that administration of silver nanoparticles produced by *Kelussia* extract had no significant effect on renal factors of total protein, albumin and creatinine. It was more. On the other hand, according to the results of this study, silver nanoparticles produced by *Kelussia* extract had a significant effect on urea and bilirubin levels.

Urea

In this study, a significant decrease in urea levels was observed at 120 and 80 mg/kg dose of extract. Study results Tanumand et al. (2018) entitled The effect of alcoholic extract of aloe vera gel (Aloe barbadencens) on the surface of urea serum, uric acid and creatinine and kidney structure in adult mice treated with ethidium showed that treatment with a dose of 1200 mg/kg aloe vera gel significantly reduced urea, uric acid and serum creatinine (P<0.05) (13). Which is consistent with our results. Also consistent with studies on the effects of plant extracts on renal function such as the results of Samavati Sharif et al. (2012) (14) Sadeghi et al. (2018) (15) and Modares et al. (2006) (16) results but

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with the results of Zare et al. (2012) (17) was not aligned. Silver nanoparticles produced by the extract of *Kelussiamedicinal* plant seem to have an effect on the terminal area of the urinary collecting duct, known as the urea reabsorption zone. And reduced urea reabsorption in this area (16). The antioxidant and anticancer effects of plant extracts are directly related to the phytochemicals and secondary metabolites of the extract (11). *Kelussiacontains* a substance called coumarins that prevents cell damage by free radicals, and contains essential oil that dilates the kidneys so that uric acid is better excreted (10).On the other hand, *Kelussia* leaves and stems contain phenolic compounds (11), and the reduction of urea at different doses compared to the control group showed that the extract at the above doses had no toxic effect on the kidney of rats, and probably had high antioxidant power.

Bilirubin

In this study, a significant decrease in bilirubin levels was observed at a dose of 80 mg/kg. In a study, Modares et al. investigated the effect of hydroalcoholic extract of ginger on blood urea nitrogen (BUN) and creatinine in rats, the results showed that ginger treatment at doses of 10, 20 and 40 mg/kg significantly decreased the mean BUN concentration in all experimental groups compared to the control group (16). This is consistent with our study. It seems that a decrease in serum BUN levels may be due to an increase in glomerular filtration performance (18). Glomerular filtration rate (GFR) is the most favorable indicator of renal function (13). *Kelussia*, on the other hand, contains phenolic compounds (11) and therefore has strong antioxidant properties (16) and reduces BUN. The lack of increased bilirubin at different doses compared to the control group indicates that the extracts of these plants at the mentioned doses have no toxic effect on rats (19).

Study weaknesses

Histology is the study of the microscopic structure of biological material and the ways in which individual components are structurally and functionally related, unfortunately, histology is not possible.

Since the toxicity of silver nanoparticles has been reported orally in many scientific papers and is slightly different from our results, it may be due to the short duration of oral administration (6 days) and the ratio of 4 to 1 primary extract (200 mL) to 50 mL distilled water dissolved in silver nitrate.

However, it is recommended that in future studies the effects of essential oils, total alkaloids, flavonoids and other secondary metabolites of *Kelussia* be studied separately in combination with histological studies to determine which secondary metabolites have the greatest effect.

Conclusion

Synthesis of silver nanoparticles solution with *Kelussia* extract have no negative effect on kidney function.

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Conflict of Interest

There is no conflict of interest.

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