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Original Article

The effect of aqueous extract of black radish root on urine pH in stone-forming patients: A single blind clinical trial

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Abstract

Background and aims: Given the prevalence of urolithiasis and the popularity of black radish extract for the treatment of this problem, the present study was designed to investigate its effect in alkalizing urine in stone-forming patients referred to Imam Ali clinic in Shahrekord (Iran).

Methods: In this clinical trial, the urine pH was measured along with the 24-hour urine assessment for urine volume and urine citrate, oxalate, calcium, uric acid, and creatinine levels of 20 patients before and after the intervention.

Results: The results showed a significant increase ($P<0.001$) in mean urine pH after the administration of *Raphanus sativus* syrup for 14 days. The mean 24-hour urine volume and citrate level increased significantly ($P<0.05$). In addition, the mean uric acid and oxalate levels decreased significantly ($P<0.05$). Mean changes in 24-hour urine creatinine and calcium levels were not statistically significant after intervention ($P>0.05$).

Conclusion: *Raphanus sativus* extract treatment can increase urine pH, urine citrate level, and urine volume and decreases uric acid and oxalate levels. Therefore, the plant may be used for the treatment and prevention of uric acid and cystine stones.

Keywords: Black radish, Urine pH, Kidney stones

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Introduction

Urolithiasis, referred to as the pathological condition related to the formation of stones in the kidney, bladder, or the urinary tract, has become a main problem globally in recent years (1,2). Kidney stone, for example, is now considered as the third leading urinary tract disease after urinary tract infections and prostate disease. Currently, 1%-15% of people around the world have kidney stones at some stage of their lives (3). The cause of stone formation has not been clearly determined, but various intrinsic and environmental factors are known to be involved. Internal factors include genetics, age, and sex. Climate conditions of growing places, amount of daily consumed water, minerals present in the water and diet are also among the environmental factors contributing to the formation of urinary stones (4). The stones may be subdivided based on their principal material constituent such as calcium, uric acid, struvite and cysteine (5,6). Uric acid stones are formed when the urine pH becomes acidic, showing that the stones most likely dissolve if the urine pH is raised to alkaline state. In addition, cystine stones are of hereditary type, and are characterized by extensive stone formation at an early age. This is a congenital defect that causes large amounts of cystine to enter into the urine, and if not treated properly, it can rapidly damage the kidneys. Early diagnosis and treatment of the kidney and urinary tract stones can prevent the enlargement of the stones and subsequent complications (4,7,8). Due to the stupendous cost of treatment and side effects of urinary tract instrument insertion and surgery, special attention has been paid today to the use of herbal products as supplementary or alternative treatments (9,10). In Iranian traditional medicine, many herbs have been suggested to promote the excretion of kidney stones, and/or prevent their formation. However, the pharmacological mechanism actions of many herbal products have not yet been adequately understood (11). Black radish, with the scientific name *Raphanus sativus* var. *niger* L., belongs to the Cruciferae family. The plant is cultivated in temperate regions, especially those of Asian countries and Iran. The leaves and roots of *R. sativus* are used as anticancer (12). The antibiotic and antimicrobial activities of the roots extract have also been reported (13). The leaf extracts of *R. sativus* have been found to exhibit antiulcer property in the animal models of gastric ulcers (14). Vargas et al showed that the plant aqueous extract markedly prevents the formation of uroliths (15). As stated above, early diagnosis and treatment of kidney and urinary tract stones

can prevent the progression of the disease, recurrence and associated complications. Therapeutic approach such as potassium citrate medications that alkalized the urine pH are currently used especially to treat and prevent recurrence of uric acid and cystine stones. This is however difficult to use it in patients and has several side effects. Given the application of *R. sativus* extract in traditional medicine for kidney stone treatment and previous studies, the present study aims to investigate the effect of *R. sativus* root extract in alkalizing urine, treating kidney stones, and preventing their recurrence in Imam Ali clinic of Shahrekord.

Materials and Methods

Research ethics

This single-blind clinical trial was conducted at Shahrekord University of Medical Sciences in 2018-2019 after obtaining ethical approval for its protocol from the Research and Technology Deputy of the University (IR.SKUMS.REC.1397.180) and registering it in Iranian Registry of Clinical Trials of Iran (identifier: IRCT20190707044130N1; https://www.irct.ir/trial/40702).

Herbal ingredients, extraction method and standard syrup formulation

The root samples of *R. sativus* obtained from local market in Shahrekord were processed after the identity of the plant was confirmed by an expert botanist (Shirmardi, Hamzeh Ali, Ph.D., Research Center of Agriculture and Natural Resources, P.O. Box 415, Shahrekord, Iran); and the voucher specimen (no.: 1016) was registered at the Medicinal Plants Research Center (no. SKUMS-1016) for it. The powdered dried samples were macerated with 70% ethanol for 72 hours, and the resulting extract was filtered and then concentrated using rotary evaporator. The extract was then freeze-dried from which a 1.4% formulation extract was prepared as per a procedure previously described (15). A syrup was also prepared as per the method used in the United States Pharmacopeia (USP). To this end, 1.4 g of extract, 9 g of glycerin and 10 g of sorbitol were mixed with 50 mL of autoclaved water using a shaker. 0.01 g of edible sodium benzoate, as a preservative, and 5-6 drops of flavoring and 100 g of honey was added and its volume increased to 100 mL with addition of water. Finally, the prepared syrup was packaged in special jars and kept in the refrigerator until the day of the test.

Exclusion criteria

The exclusion criteria for patients were renal colic development during the study, intolerance to the studied extract, lack of having appropriate renal function, and pregnancy and lactation during the study.

Inclusion criteria

Samples of this study included 20 stone-forming men and women with a history of uric acid or cystine stones and at least one episode of kidney stone excretion in the last 3 years, who currently had no kidney and ureteral stones and had satisfactory kidney function, and referred to the Imam Ali clinic for follow-up of kidney and urinary tract conditions.

Intervention and evaluation of effectiveness

At baseline, data were collected to evaluate urinary tract and to ensure the absence of kidney stones and evaluate appropriate renal functions. Regarding diet, consumption of vegetable and water was recorded and medicinal plants were not used. The pre-intervention tests included renal and urinary ultrasound, urine pH measurement using Litmus and 24-hour urine sample preparation for the investigation of urine volume and citrate, oxalate, calcium, uric acid and creatinine levels. Then, patients were advised to use 15 mL of *R. sativus* extract syrup three times daily during each meal for 14 days. The urine pH was measured on days 7 and 14 of syrup treatment the syrup treatment. In the latter case, 24-hour urine samples were collected for measurement of urine volume and urine citrate, oxalate, calcium, uric acid and creatinine levels. The effect of *R. sativus* extract on factors relevant to the treatment and prevention of kidney stones were recorded.

Data analysis

Data were analyzed using SPSS 18 by descriptive statistics including frequency, percentage, mean and standard deviation as well as inferential statistics including paired *t* test. Significance level (*P* value) was considered at the level of $P < 0.05$.

Results

In the present study, which aimed to investigate the therapeutic effect of R. sativus extract in alkalizing urine, treating kidney stones, and preventing their recurrence in Imam Ali clinic in Shahrekord from March 2018 to September 2019, 20 patients who had a history of uric acid or cystine stones were treated with 15 mL R. sativus syrup three times a day for 14 days. The mean age of patients was 41.7 ± 6.72 (range: 19-55) years. Fourteen (70%) patients were male and 6 (30%) were female.

Comparison of mean (±standard deviation) urine pH before intervention, and one and two weeks after intervention

The results of the paired *t* test regarding radish syrup consumption for urine pH are shown in Tables 1, 2 and 3. According to Table 1, the urine pH increased by 0.475 on the 7th day after treatment with the extract and the data was statistically significant ($P < 0.001$) when compared to the pH on the first day of treatment. Moreover, Tables 1 and 2 show the changes in urine pH following treatment with of *R. sativus* extract in the first and second weeks of treatment. In this case, the urine pH increased by 0.625 on the 14th day when compared with the initial pH ($P < 0.001$). The results regarding urine pH changes

before and after the intervention are shown in Table 3. Post-intervention urine pH increased by 1.100 compared ($P < 0.001$) to pre-intervention urine pH.

Comparison of mean (± standard deviation) urine volume before and after intervention

As it shows in Table 3, comparison of the results regarding urine volume before and after the intervention by paired *t* test, showed that the mean urine volume after the intervention significantly increased compared to that before the intervention ($P < 0.05$).

Comparison of mean (± standard deviation) urine citrate level before and after intervention

Comparison of the results regarding urine citrate levels before and after the intervention using paired *t* test, showed that the mean urine citrate level after the intervention significantly increased compared to that before the intervention (353.8065 ± 195.78, 353.8650 ± 11.395, $P < 0.05$) (Table 4).

Comparison of mean (± standard deviation) urine oxalate level before and after intervention

The mean urine oxalate levels before and after the intervention were compared using paired *t* test: the mean oxalate level after the intervention significantly decreased when compared to that before the intervention (28.104 ± 17.159, 19.104 ± 11.395, $P < 0.05$) (Table 4).

Table 1. Urine pH levels before and one week after intervention

	Mean	Standard deviation	*P* value
Pre-intervention urine pH	5.300	54.0	<0.001
pH urine one week post-intervention	5.775	73.0	

Table 2. Urine pH levels one and two weeks after intervention

	Mean	Standard deviation	*P* value
Urine pH one-week post-intervention	5.775	73.0	<0.001
Urine pH two weeks post-intervention	6.400	50.0	

Table 3. Urine pH levels before and after intervention

	Mean	Standard deviation	*P* value
Pre-intervention urine pH	5.300	54.0	<0.001
Post-intervention urine pH	6.400	50.0	

Comparison of mean (± standard deviation) urine calcium level before and after intervention

The results regarding mean 24-hour urine calcium levels in patients before and after the intervention are compared using paired *t* test. According to these results, the mean calcium level after the intervention increased to 2.135 mg which was not statistically significant (175.607 ± 82.27, 177.742 ± 104.29, $P > 0.05$) (Table 4).

Comparison of mean (± standard deviation) uric acid level before and after intervention

The mean uric acid level before and after the intervention was compared using paired *t* test. According to these results, the mean uric acid level after the intervention increased to 92.955mg that was statistically significant (459.697 ± 190.063, 366.742 ± 144.493, $P < 0.05$) (Table 4).

Comparison of mean (± standard deviation) urine creatinine level before and after intervention

The mean 24-hour urine creatinine level was measured before and after the intervention. The mean creatinine level decreased to 46.185 mg after the intervention but the change was not statistically significant (1122.70 ± 237.11, 1076.51 ± 234.97, $P > 0.05$) (Table 4).

Discussion

In recent years, special attention has been given to medicinal plants because they are rich sources for the identification of complementary drugs (16). In this regard, numerous studies have shown the promising results regarding the use of various medicinal plants for the treatment of renal stones (17). However, the action mechanisms of many plants remain to be explained (11). In developed countries, the incidence of uric acid stones, which accounts for 10% of all types of kidney stones, has steadily increased in recent years. Risk factors for uric acid formation include low urine pH, increased uric acid levels, and decreased urine volume. Formation of cystine stones is associated with excessive urinary excretion of cystine. The cause of this stone formation is a type of genetic disease in which renal reabsorption of the cysteine amino acid occurs and thus it accumulates in the urine (18). Given that some salts crystallize in acidic than alkaline medium, urine alkalization in patients with uric acid and cystine stones is recommended for treatment and prevention of stone recurrence because they crystallize in

Table 4. Urine citrate, oxalic acid, calcium, uric acid (mg/24 h) and creatinine levels before and after intervention

Levels of	Pre-intervention		Post-intervention		*P* value
	Mean (mg/24 h)	Standard deviation	Mean (mg/24 h)	Standard deviation	
Citrate	353.8065	195.78	353.8650	11.395	<0.05
Oxalic acid	28.104	17.159	19.104	11.395	<0.05
Calcium	175.607	82.27	177.742	104.29	>0.05
Uric acid	459.697	190.063	366.742	144.493	<0.05
Creatinine	1122.70	237.11	1076.51	234.97	>0.05

acidic medium (19). In the present study, to the best of our knowledge for the first time, the effects of *R. sativus* root in reducing urine pH were investigated in patients with kidney stones who had a history of uric acid and cystine stones and no kidney stones at enrollment. The result showed that, *R. sativus* treatment caused a significant increase in urine pH in patients with stone-forming kidneys who had a history of excretion of uric acid or cystine stones but had no stones during the study. In addition, after intervention the mean urine volume and citrate level increased significantly after 24 hours. Moreover, the mean uric acid and oxalate levels decreased significantly. The mean 24-hour urine creatinine in these patients decreased after the intervention but the change was not statistically significant. Moreover, 24-hour urine calcium levels in these patients increased that was not statistically significant. In the study of Kessler et al, the effects of black currant (*Ribes nigrum)*, blueberry (*Cornus mas*) and plum (*Prunus domestica*) extracts on kidney stones was investigated in 12 healthy men aged 18-38 years. For this purpose, 24-hour urine samples were collected in three stages. Th juice of *R. nigrum* increased urine pH and excreted citric acid. However, after consuming *C. mas* juice, urine pH and citric acid excretion decreased slightly. Excretion of oxalic acid also increased substantially after administration of both extracts. As well, the juice of *P. domestica* had no significant effect on urine composition. The results of that study showed that *R. nigrum* juice could be effective for the treatment of uric acid stones due to its alkalizing properties, and because *C. mas* juice acidifies urine, it could be useful for treating brush and struvite stones as well as urinary tract infection (20). Hönow et al conducted a study on nine healthy women to investigate the effects of grapefruit (*Citrus* × *paradise*), apple (*Malus domestica*) and orange (*Citrus* × *sinensis*) syrup on changes in urine and risk of formation of urinary stone crystals. That study showed that all three syrups increased urine pH but only *C. paradisi* decreased the crystallization of calcium oxalate stones (21). In the study of Vargas et al, the effect of aqueous extract of black radish on the treatment of urolithiasis was investigated in rats. In that study, the diuretic and anti-urinary stone formation of aqueous extract of black radish was investigated and a dose-dependent decrease in the size of the stones was observed after administration of 40, 70 and 140 mg/kg of the extract. The aqueous extract at 140 mg/kg caused a 90.78% decrease in stone deposition and maximum urine volume (93.9%) compared to the control group, and also a decrease in stone weight and an increase in urine volume. The results of that study showed that the plant extract significantly prevented the formation of urinary tract stones in a dose-dependent manner and studies are being conducted to identify active principles of the plants (15). The results of the present study are consistent with these findings. With respect to the results, it can be argued that the use of *R. sativus* extract can be effective for the prevention and treatment of uric acid and cystine kidney stones. However, it is recommended that the effect of *R. sativus* extract in individuals with other conditions and with longer duration of treatment need to be investigated. Mazdak et al conducted a study to investigate the effect of black radish on urine pH in Isfahan. In that study, 30 healthy individuals who had no history of kidney stones in their first-degree relatives, did not take any particular medication, and were not on any specific diet, were studied for 4 days. During the first 2 days, black radish extract was not given and on the following two days, 30 mL of black radish extract in 100 mL was administered. Among the eight pairs of pH meter data, only two pairs of data had a *P* value less than 0.05 and for the rest, the *P* value was greater than 0.05, which indicates a statistically significant difference in urine pH before and after black radish treatment. In that study, the effect of black radish in decreasing the urine pH of healthy individuals was not demonstrated. Although the diuretic effect of black radish in rats has been demonstrated, it is necessary to replicate that study with other human populations (22). In our study, the duration and amount of *R. sativus* extract treatment was longer and the participants were selected from patients with history of uric acid and cystine acid who often had acidic urine; and therefore the reasons for the response observed for *R. sativus* extract in our study can be the standard and the 14-day duration of extract treatment and the selection of individuals with a history of renal stones.

Conclusion

The results of this study showed that consumption of *R. sativus* extract could increase urine pH. It also increased urine citrate level and urine volume and decreased urine uric acid and oxalate levels. Therefore, the plant may be helpful for the treatment and prevention of stones of the urinary tract, especially where uric acid and cystine stones are implicated. Additional studies will help to establish the potential application of the plant in urolithiasis as a dietary supplement.

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Conflict of Interest Disclosures

The authors declare that they have no competing interests.

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