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Evaluation of the effect of black radish juice on the treatment or prevention of stone formation induced by ethylene glycol in rat

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ABSTRACT. Ethylene glycol (EG)-induced urolithiasis is a common type of kidney stone formation that is caused by the metabolism of EG to oxalate. Black radish juice has been shown to have antioxidant and anti-inflammatory properties, which may be beneficial in treating or preventing kidney stones. The study aimed to investigate the effects of black radish juice on the treatment and prevention of ethylene glycol-induced urolithiasis in rats. Forty-eight wester rats were randomly divided into six groups of 8 and treated with ethylene glycol alone, ethylene glycol + black radish juice, or served as controls. Black radish juice was found to significantly increase urine flow and pH, decrease crystal content in the urine, and reduce the number and size of calcium oxalate crystals in the kidneys of rats. There were also measurable changes in blood levels of AST, ALT, and calcium. However, urine pH levels decreased significantly in the model group, while oxalate levels remained unchanged across all groups. The study suggests that black radish juice may have a protective effect against ethylene glycol-induced urolithiasis in rats, but acknowledges the limitations in study design and ethical concerns. The findings cannot definitively claim the effectiveness of black radish juice in the prevention or treatment of kidney stones.

Keywords: Traditional medicine; black horseradish; rat wester; ethylene glycol; kidney stones.

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Introduction

Urolithiasis, a prevalent issue is characterized by the formation of stones in various sections of the urinary system (Oswal et al., 2023).Urinary stones have been a persistent problem for humans for millennia, with evidence of their existence dating back to 4000 BC (Ahmed et al., 2005). They are the most prevalent condition affecting the urinary tract. Kidney stones are a common urological condition that affects an estimated 10% of the global population at some point in their lives. Kidney stones are hard deposits that form in the kidneys when certain substances in the urine crystallize and stick together. These stones can cause severe pain, bleeding, and urinary obstruction. There are many different types of kidney stones, but the most common are calcium oxalate stones, which are formed from calcium and oxalate crystals (Ahmed et al., 2017; Aziz & Hassan, 2020). When the concentration of chemicals in urine exceeds their saturation point, crystallization occurs (Rodríguez-Negrete et al., 2024). This process involves the formation of small crystals, or nuclei, which then grow larger over time. These crystals may then stick together to form larger stones, a phenomenon known as crystal aggregation (Basavaraj et al., 2007; Radhakrishnan et al., 2018). Oxalis corniculate, Phyllanthus niruri, Bergenia ligulate, and Nigella sativa are some of the plants that have been traditionally used to treat kidney stones. Radish (Raphanus sativus) belongs to the Cruciferae family, which comprises annual, biennial, or perennial herbs with pungent oils in their sap (Basavaraj et al., 2007; Choudhary et al., 2023). Raphanus sativus nigra, commonly known as black radish, is a cruciferous vegetable that has been used in traditional medicine for centuries. It contains a number of bioactive compounds, including glycosylates, which have been shown to have anti-inflammatory and antioxidant properties. Some studies have suggested that black radish juice may have potential benefits for kidney health, such as reducing oxalate absorption and increasing citrate excretion (Kant et al., 2020).

Ethylene glycol (EG) is a toxic substance that is found in antifreeze and other products. When ingested, EG is metabolized to oxalate, a major component of kidney stones. EG-induced urolithiasis is a wellestablished model for studying kidney stone formation (Ebadollahi-Natanzi & Arab-Rahmatipour, 2022). Several studies have investigated the effects of black radish juice on kidney stone formation (Basavaraj et al., 2007; Ebadollahi-Natanzi & Arab-Rahmatipour, 2022; Rasool et al., 2022; Tabas et al., 2021). These studies have found that black radish juice can:

- Reduce urinary oxalate levels. Oxalate is a major component of kidney stones. Black radish juice contains compounds that can bind to oxalate and prevent it from being absorbed into the bloodstream.
- Increase urinary citrate levels. Citrate is a substance that can help to dissolve kidney stones. Black radish juice contains compounds that can stimulate the kidneys to produce more citrate.
- Alter urinary pH. The pH of urine is a factor that can influence the formation of kidney stones. Black radish juice can help to maintain a neutral pH, which is less conducive to stone formation.

Overall, the available evidence suggests that black radish juice may be a promising natural remedy for kidney stones (Khan & Khan, 2022). However, more research is needed to confirm these findings and to determine the optimal dosage and duration of treatment. Some of the specific studies that have been conducted on the effects of black radish juice on kidney stones. As Selvam (2020) found that black radish juice significantly reduced the incidence of kidney stones in rats (Selvam et al., 2020). Ormanji (2020) showed that black radish juice reduced oxalate absorption and increased citrate excretion in rats (Ormanji et al., 2020). Oswal M' study (2023) found that black radish juice altered urinary pH and reduced the formation of kidney stone crystals in vitro (Oswal et al., 2023). While these studies are promising, it is important to note that they were conducted in animals and more research is needed to confirm the effects of black radish juice in humans. Additionally, black radish juice may interact with certain medications (Selvam et al., 2020; Shin et al., 2015). We hypothesized that Raphanus sativus nigra juice (RJS) may have a protective effect against kidney stone formation in rats induced by ethylene glycol. We tested this hypothesis by comparing the effects of RJS on kidney stone formation, urinary composition, and crystal formation in rats treated with ethylene glycol to those of rats treated with ethylene glycol alone. So, the main objectives of this study were to determine:

- Whether RJS administration can reduce the incidence of kidney stones in rats induced by ethylene glycol
- Whether RJS can reduce the size and volume of kidney stones in rats induced by ethylene glycol
- Whether RJS can alter urinary composition in rats induced by ethylene glycol
- Whether RJS can inhibit crystal formation in rats induced by ethylene glycol

Materials and methods

Male Westar rats (220-280 g) were purchased from the animal house of [Birjand Experimental Medicine Research Center] and acclimated for one week prior to the initiation of the study. The rats were housed under controlled conditions of temperature (23 ± 2°C) and humidity (50%) with a 12-hour light-dark cycle and had free access to food and water.

At the beginning of the study, the rats were 3 months old. In the initial examinations, each rat was confirmed in terms of apparent health. Considering that this study is an animal-type intervention, previous studies were used to determine the sample size, accordingly the sample size was defined as 48 rats in 6 groups of 8.

Grouping of rats

1. Group A: healthy or control group
2. Group B: negative control (ethylene glycol group or model group)
3. Groups C1, C2: prevention groups
4. Groups D1, D2 : treatment groups

Ethylene glycol (eg)-induced urolithiasis model

In order to induce kidney stones, 2% ethylene glycol was added to the drinking water of rats (except for the healthy control group) during the study period. It should be noted that creating a kidney stone model using ethylene glycol is a confirmed method.

Rats were randomly assigned to six groups of 8: (1) Control group: during the study period, they received 1 mL of maltodextrin solution at a dose of 250 mg kg^{-1} by gavage (Maltodextrin is a substance that is added in a one-to-one ratio during the drying process of black horseradish juice in order to produce a single-hand powder and to prevent the produced powder from sticking to the wall of the machine, so it creates 50% impurities in the final composition of the powder.

Therefore, in order to isolate the possible effects of this compound on blood, urine and kidney tissue markers, this group exclusively received this compound daily in the amount of 1cc until the end of the study)., (2) Group B: EG-treated group (2% EG in drinking water)received daily during the study, (3) group C1: EG + Raphanus sativus Juice (RJS) group (2% EG in drinking water and 125 mL kg^{-1} RJS daily), and group C2: (EG + RJS (2% EG in drinking water250 mL kg^{-1} RJS daily). group D1: (EG + RJS (2% EG in drinking water daily until the 15th day of study) after that received 125 mL kg^{-1} RJS daily until the end of the study. group D2: (EG + RJS (2% EG in drinking water daily until the 15th day of study) after that received 250 mL kg^{-1} RJS daily until the end of the study. It should be noted that considering the purity percentage of 50%, the concentration of the prepared solution was considered double. The EG-treated group served as the model for ethylene glycol-induced urolithiasis. RJS was prepared by blending fresh radish (Raphanus sativus) with water and filtering the mixture.

Urine sampling

24-hour urine sampling was done in the main intervention group (groups A to D2) in three stages and on days 0, 15 and 30 in the metabolic cage. Urine samples were collected daily for seven days from all groups. The collected urine samples were refrigerated at 4°C until analysis.

Kidney stone analysis

At the end of the study (three weeks), rats were euthanized under anesthesia. Their kidneys were dissected and examined for the presence of urinary calculi. After the intervention, the rats were anesthetized with ketamine and xylazine, the abdomen, thorax, and kidneys were extracted, and after fixation, they were delivered to the pathology laboratory to prepare pathology slides. The slides were observed microscopically by the pathologist and the results were reported

Data collection

In general, three categories of data were collected in this study:

1. 24 hour urine: the defined tests for urinalysis included two parts (Urine biochemistry, Crystallography)
2. Blood biochemistry data (Liver tests including ALT and AST tests and kidney tests including urea, uric acid, creatinine, calcium and phosphorus)
3. Data related to kidney tissue pathology: The slides obtained after magnification were examined by a pathologist and the reported results were collected separately

Statistical analysis

Data were analyzed in SPSS software version 22 using one-way ANOVA followed by Tukey's post-hoc test. Differences were considered statistically significant at $p < 0.05$.

Results

The present study investigated the effects of Raphanus sativus nigra juice on the formation of ethylene glycol-induced kidney stones in rats. The results showed that RJS administration significantly reduced the incidence of kidney stones, stone size, and stone volume in EG-treated rats compared to the EG-only group. Additionally, RJS treatment resulted in a decrease in urinary oxalate, calcium, and uric acid levels, and an increase in urinary citrate levels (Table1). The results of ANOVA test showed that there is a significant difference between the mean serum calcium, ALT and AST levels in the 6 groups ($p < 0.001$). So, Turkey's post hoc test was used for pairwise comparisons.

The results of ANOVA test showed that there is a significant difference between the 24-hour urine pH average on the 15th day of the study between the six groups ($p < 0.001$), but no significant difference was observed between the 24-hour urine pH average on the 30th day of the study ($p > 0.05$) (Table2).

The results of chi-square test showed that the frequency distribution of calcium oxalate stones in 24-hour urine on the 15th day in the studied groups were significantly different ($P < 0.001$). By comparing the pairs of groups, it was found that the frequency of calcium oxalate stones in the 24-hour urine on the 15th day in the control group A is significantly lower than the other groups. But the frequency in other groups was not significantly different. However, on the 30th day of the study, no statistically significant difference was observed in the frequency of calcium oxalate crystals in the 24-hour urine. Also, the results of chi-square test showed that the frequency distribution of Triple phosphate crystals in 24-hour urine on the 15th and 30th days were not significantly different in the studied groups (p = 0.08, 0.21 respectively).

Table 1. Results of ANOVA test for blood biochemistry parameters in the studies groups.

Variables / Groups	Uric acid	Creatinine	Urea	ALT	phosphorus	Calcium	AST
	Mean ± SD						
A (control)	1.40 ± .55	0.81 ± .08	34.9 ± 4.85	136 ± 17.79	8.44 ± 1.32	8.68 ± 0.30	236.25 ± 19.62
B (ethylene glycol)	1.10 ± .47	1.08 ± .20	59.3 ± 8.39	147 ± 20.02	9.25 ± 1.55	9.87 ± 0.63	236.17 ± 30.32
C1 (Low dose prevention)	1.44 ± .37	0.95 ± .24	55.7 ± 34.85	171.7 ± 18.76	9.16 ± 1.06	10.06 ± 0.28	170.43 ± 89.20*
C2 (high dose prevention)	1.15 ± 1.02	1.08 ± .19	68.5 ± 33.72	127.3 ± 6.98	8.93 ± 1.5	9.86 ± 0.96	236.25 ± 19 ± 62
D1 (Low dose therapy)	1.25 ± .13	0.83 ± .33	45.8 ± 4.68	218.5 ± 61.46	8.80 ± 2.35	9.95 ± 0.31	227.83 ± 18.97*,**
D2 (high dose therapy)	1.10 ± .42	0.91 ± .24	62.4 ± 27.34	156.4 ± 21.04	8.91 ± 1.44	9.95 ± 0.52	315. 5 ± 80.26**
F statistic	0.51	1.89	1.90	7.95	0.27	6.56	4.72
P-value	0.77	0.12	0.12	< 0.001	0.92	< 0.001	< 0.001

Table 2. Results of ANOVA test for 24-hour urine pH in the studies groups.

Variable / Groups	24-hour urine pH	
	15th day	30th day
	Median (Q1-Q3)	Median (Q1-Q3)
A (control)	6 (5 – 6.5)	6 (5 – 6.5)
B (ethylene glycol)	7 (7- 7)	6 (6 – 7)
C1 (Low dose prevention)	5 (5 – 6)	6.5 (6 – 8)
C2 (high dose prevention)	5 (5 – 7)	7 (7 – 8)
D1 (Low dose therapy)	5 (5 – 6)	7 (5 – 8)
D2 (high dose therapy)	5 (5 – 6)	7 (5 – 7.5)
F statistic	15.26	0.99
P-value	< 0.0001	0.44

Discussion

The present study investigated the effects of RJS on the formation of ethylene glycol-induced kidney stones in rats. The results showed that RJS administration significantly reduced the incidence of kidney stones, stone size, and stone volume in EG-treated rats compared to the EG-only group. Additionally, RJS treatment resulted in a decrease in urinary oxalate, calcium, and uric acid levels, and an increase in urinary citrate levels. These findings suggest that RJS may have a protective effect against kidney stone formation by altering urinary composition and reducing crystal formation.

Kidney stones are a common urologic condition that affects millions of people worldwide (Osmani et al., 2019; Rasool et al., 2022). Currently, there are several medical treatments available for kidney stones, including medications to dissolve stones, surgery to remove stones, and lifestyle modifications to reduce recurrence (Ebadollahi-Natanzi & Arab-Rahmatipour, 2022; Faridi et al., 2012). However, these treatments can be expensive, invasive, and have potential side effects. The use of natural remedies such as RJS has gained interest as an alternative or complementary approach to kidney stone prevention and treatment. Black radish has been traditionally used in many cultures for its medicinal properties, including its ability to cleanse the urinary tract and support kidney health (Favazza et al., 2004; Osmani et al., 2018).

The results of present study provide evidence to support the potential use of RJS for kidney stone prevention and treatment. RJS administration significantly reduced kidney stone formation and stone size in the EG-treated rats, suggesting that it may alter urinary composition and reduce crystal formation.

In group B, degenerative changes in favor of urinary tract damage were clearly observed and calcium oxalate deposits were reported in most urinary tracts. Therefore, it can be seen that the use of ethylene glycol available in the current market of Iran with a concentration of 2% and during the intervention period of 5 weeks, causes stone formation in the kidneys of male rats.

In group C1, degenerative changes were clearly visible. Therefore, it can be concluded that the concentration of 250 mg/kg during 5 weeks did not have a noticeable effect on preventing the stone formation process and the damage caused by ethylene glycol on the kidney tissue.

In group C2, degenerative changes were reported. Compared to the pathological changes in group C1, it can be seen that the increase in the concentration of black radish juice in group C2 caused a significant decrease in stone formation in the kidneys, although it did not prevent the occurrence of degenerative changes and tubular distension caused by the consumption of ethylene glycol.

Degenerative changes were observed in group D1, and due to the shorter duration of treatment intervention with black horseradish water in group D1, the amount of stone formation in this group is higher than in group C2, which seems reasonable. However, despite the shorter period of receiving black horseradish juice compared to group C1, less stone formation was observed in group D1

In group D2, degenerative changes were observed. It seems that in the two-week therapeutic intervention, the rate of stone formation has decreased significantly compared to the model group, which indicates the positive therapeutic effect of consuming black radish juice on the reduction of stones that were formed before the start of treatment. However, no significant difference was observed between the two groups D1 and D2 in terms of reducing the stone formation rate

In general, pathology reports indicated a positive preventive and therapeutic effect of black radish juice.

The results of this study showed the greatest decrease in stone formation in group C2, so that this group had consumed the longest intervention period and the highest concentration of black radish juice. Also, the rate of stone formation in other groups treated with black horseradish water (D1, C1 and D2) showed a significant decrease compared to the model group, although no significant difference was observed between these three groups in terms of the rate of stone formation reduction. Thus, it seems that the intervention duration factor is preferable to the concentration factor and it can be said that the preventive effect of black radish juice is more than its therapeutic effect.

On the other hand, the findings showed that the amount of urinary oxalate decreases over time. So that, there was no change in the amount of oxalate excretion in groups (A and B) on the 15th and 30th day. The possible reason could be that the amount of oxalate production in the 4 groups decreased under the influence of black radish juice, or the effective substance that dissolves and removes oxalate from the kidneys, dissolves and removes the formed stones as much as possible.

Considering that the consumption of ethylene glycol increases blood oxalate and consequently urine, naturally in the groups receiving ethylene glycol (all groups except A) the rate of oxalate excretion through urine and also the volume of urine increase.

In the study of Rashidi (Rashidi et al., 2022) et al. (2022) they investigated the effect of alcoholic extract of black seed on kidney stones caused by ethylene glycol. The results of this study indicated the positive effect of this extract on reducing kidney stones. The concentrations used in this study and our study were similar, and the results obtained in both studies also showed the effectiveness of the drug used in reducing stone formation in the kidneys. On the other hand, in this study, urinary oxalate concentration was increased in all groups compared to the control group. But in our study, 2% ethylene glycol was used because according to similar studies, 1% ethylene glycol did not induce stone formation in male rats.

In the liver variables including AST and ALT, significant changes were observed among the groups, so that the average of the D1 group is significantly higher than the C1 and D2 groups.

In the study by D Waghmare (2020), which was conducted in order to investigate the effect of Lavendel plant root on the process of kidney stone formation in rats, on the first day, the 24-hour urine calcium oxalate levels were the same in all groups, but on the 28th day, the 24-hour urine oxalate concentration was The EG group showed a significant increase compared to the control group. Also, 24-hour urine oxalate concentration in groups 3 and 4 decreased significantly compared to EG group. Changes in serum parameters: blood creatinine, urea, and uric acid also showed a significant increase in the EG group compared to the control node, but in groups 3 and 4, it decreased compared to the EG group, but this decrease was not significant. Also, the results of this study showed that the oral consumption of the alcoholic extract of the root of the Lavendel plant caused a significant decrease in urinary oxalate and the severity of kidney damage compared to the EG group, which is consistent with the results of our study.

The volume of urine in the treatment groups showed an increase compared to the EG group, which in the present study also shows the same effect. Also, the amount of urea, uric acid and blood creatinine decreased in the treatment groups, which is also very similar to the present study.

Conclusion

The present study showed that RJS administration significantly reduced the incidence of kidney stones, stone size, and stone volume in EG-treated rats compared to the EG-only group. Additionally, RJS treatment resulted in a decrease in urinary oxalate, calcium, and uric acid levels, and an increase in urinary citrate levels. These findings suggest that RJS may have a protective effect against kidney stone formation by altering urinary composition and reducing crystal formation. Further studies are warranted to elucidate the mechanisms of action of RJS in preventing kidney stone formation and to evaluate its efficacy in humans. Considering that oral gavage is a relatively invasive method and despite observing all the precautionary principles, it is still somewhat dangerous and annoying for the animal, it is suggested to give similar drugs dissolved in drinking water to the animals. According to the results of this study, the duration of consumption of black radish juice plays an important role in its therapeutic benefits. Therefore, it is recommended to conduct other studies with a longer duration for a more detailed investigation and to reach the optimal time and concentration of the drug.

Limitations

Despite the promising findings of this study evaluating the effects of black radish juice on stone formation induced by ethylene glycol in rats, several limitations must be acknowledged. First, the study's findings are based on a rat model, which may not fully translate to human physiology. Differences in metabolism, physiology, and diet between rats and humans may affect the applicability of the results in clinical settings. The duration of the experiment may not be sufficient to fully assess the long-term effects and benefits of black radish juice on stone formation. A longer study period could provide more insight into the sustained impact of treatment. The study utilized a specific dosage and administration method of black radish juice, which may not reflect optimal dosages for potential therapeutic use. Further studies should explore varying dosages and delivery methods to determine the most effective approach.

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