Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

ASSESSMENT OF THE SYNERGISTIC PREVENTIVE, ROLE OF NIGELLA SATIVA SEED (BLACK SEED) AND PUNICA GRANATUM L. (POMEGRANATE PEELS) AGAINST NEPHROLITHIASIS

Dr. Naima Younus[1], Dr. Sadia Ayub[*2], Saira Masood[3], Syeda Marzia[4], Ayesha Satti[5]

[1, *2,3,4,5]*Department of Biological Sciences, International Islamic University, Islamabad*

naima.younus@iiu.edu.pk[1] , sadia.ayub.vt5108@iiu.edu.pk*[2]
saira.bsbio343@iiu.edu.pk[3] ,marzia.bsbio359@iiu.edu.pk[4],ayesha.bsbio185@iiu.edu.pk [5]

DOI: https://doi.org/10.5281/zenodo.18081233

Keywords
Black Seed; renal disease, pomegranate, uric acid

Article History
Received: 01 November 2025
Accepted: 15 December 2025
Published: 29 December 2025

Corresponding Author: *
Dr. Sadia Ayub

Abstract

Preclinical studies have shown the beneficial effect of black seed (Nigella sativa) and pomegranate peel (Punica granatum L.) in the treatment of kidney stones. Present study was designed to evaluate the preventive effect of black seed and pomegranate peels in male mice against kidney stones. Initially, black seed and pomegranate peel extract was made. Briefly, mice were divided into 5 groups, i.e. group 1(healthy control group), group 2 (negative control group), group 3 (300 mg/kg black seed extract), group 4 (100mg/kg pomegranate peels extract), group 5 (combine concentrations of 300mg/kg black seed and 100mg/kg pomegranate peel extract). Mice were induced kidney stones by 1% ethylene glycol in drinking water. All these substances were given to mice from first day of experiment to last day of experiment i.e. 28th day. In the end, the biochemical analysis for serum parameters (sodium, potassium, magnesium, phosphorous, calcium, uric acid, bicarbonate and chloride) and urinary parameters (calcium and uric acid) was done for kidney stones. The results indicated that serum level of sodium decreased significantly (P<0.0001) in group 3, when compared with negative control group 2. Similarly, highest value of serum magnesium and serum bicarbonate level was seen in group 3, showing that it is effective for preventing kidney stones. The group 4 also showed declined level of serum and urinary parameters, indicating their preventive effect against kidney stone formation. Above all, the combination of black seed and pomegranate peels was most effective for kidney stones such as, the serum potassium, phosphorous, calcium, uric acid and chloride showed the lowest value in combination group, when compared with negative control group. Likewise, the urinary parameter analysis for calcium and uric acid of 14th and 28th day of experiment showed lowest value in combination group of black seed and pomegranate peel group. The overall results of the study indicated that the combined effect of black seed and pomegranate peel is productive to cure kidney stones. These two natural products are viable option for kidney stone prevention and treatment and hence, can be novel medication design in future.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Introduction:

Kidney stones are hard deposits of minerals and salts mostly of calcium and less uric acid or cystine. Small-sized kidney stones can pass out through the urinary tract without causing problems but larger-sized stones can get stuck in the ureter and cause severe pain. (Walter, 2022). The global prevalence of kidney stones with regional variation has rates of 9.3% in North America, 3.96% in South America, 4.7–6.8% in Europe, and 1.9–17.6% in Asia. (Aiumtrakul et al., 2024). Several factors causing kidney stones include genetic history, age, gender, certain disorders, dehydration, diet, stress, unhygienic water, obesity, certain medications and climatic conditions, etc. (Rashidi, Sazegar, Baghdadabad, & Naghsh, 2022).

Kidney stones can be treated using various methods including extracorporeal shock wave lithotripsy (ESWL), percutaneous nephrolithotomy (PCNL), and open surgery however they may lead to side effects such as infection, hematoma, and adjacent organ damage. (Jiang et al., 2023). Due to the harmful effects and high cost of current treatments herbal treatments are preferred because of their wide availability, lower price, and less toxic effects.

Black seed (*Nigella sativa*) also known as black cumin and kalonji is a plant from *the Ranunculaceae* family which is grown and cultivated worldwide and has been widely used for centuries due to exerting a range of therapeutic benefits. It is a spice plant that is widely used for the prevention and treatment of many ailments worldwide.(Tavakkoli, Mahdian, Razavi, & Hosseinzadeh, 2017).

Nigella sativa is a flowering herb, which grows to 20–30 cm tall and has linear lanceolate leaves. The flowers have 5-10 petals and are usually yellow, white, pink, pale blue, or pale purple in color. The fruit of the plant is a large and inflated capsule composed of 3-7 united follicles, each containing numerous seeds. The black-colored seeds are flattened, oblong, and angular, funnel-shaped, with the length of 0.2 cm and 0.1 cm wide. (Forouzanfar, Bazzaz, & Hosseinzadeh, 2014). Many active compounds have been identified and reported in black seed including unsaturated fatty acids such as arachidonic, eicosadienoic, linoleic, linolenic, oleic, palmitoleic, palmitic, stearic, and myristic acid, and saturated fatty acids such as thymoquinone (TQ), thymohydroquinone (THQ), thymoquinone, thymol, carvacrol, α and β-pinene, d-limonene, dcitronellol, *p*-cymene *p*-cymene, carvacrol, t-anethole, 4-terpineol and longifoline. It also contains alkaloids and saponins. Thymoquinone is the most important active compound of black seed. The nutritional compositions of *N. sativa* are vitamins, carbohydrates, fats, proteins, and minerals such as Fe, Ca, K, Zn, P, and Cu. (Forouzanfar et al., 2014). Historically, black seed has been considered a diuretic agent. Many clinical studies have shown that this herb helps in the prevention and treatment of renal diseases including renal stones and renal damage e.g.; Ahmed and Abd El-Mottaleb (2013) have reported that *N. sativa* oil has renal protective effects against acetaminophen-induced renal injury in rats. Also, Hadjzadeh et al. (2011) have reported that black seed oil inhibited collagen deposition and the severity of fibrosis in a bromobenzene-induced hepato-renal injury model. Another study by Ardakani Movaghati et al (2019) on *N. sativa* L. found that aqueous-ethanolic extract of *N. sativa* L. significantly reduced the number and size of the kidney calcium oxalate deposits than did the ethylene glycol group in male Wistar rats. Studies have reported that black seed acts as an analgesic, anthelmintic, and antipyretic. Phenolics and flavonoids in *N. sativa* promote sweating that's helps in reducing fever and also helps in toxin removal. It also helps in relieving bloating and removing extra water that the body doesn't need. Thymoquinone of black seeds promotes urine formation which helps the kidneys to stop sodium reabsorption. (Huchchannanavar, Yogesh, & Prashant, 2019). The antioxidant activity of Black seed helps to prevent kidney damage caused by ischemia by reducing apoptosis in renal epithelial tubular cells. (Mousavi & Mohajeri, 2014). Our kidneys are mostly exposed to many xenobiotics which include drugs, food additives, environmental chemicals, etc. that can cause damage to our kidneys. Research has revealed that exposure to xenobiotics which include therapeutic drugs, heavy metals, pesticides, and other chemicals that used to exist in the environment cause kidney injury in experimental animals, however when they were treated using black seed and TQ the kidney damage

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

improved.(Hannan et al., 2021). A study comparing *Nigella sativa* and tamsulosin effect on kidney stones shows that both reduced the number, and size of urinary stones and pain intensity during their passage without a significant difference. However, *Nigella sativa* showed more efficacies in the treatment of stones. (Shakeri, Mehrabi, & Paymard, 2021).

According to Hadjzadeh et.al (2007), ethanolic extract of NS seeds reduces both the number and size of calcium oxalate deposits in rats with ethylene glycol-induced kidney stones. The rats that received black seed extract from the first to the end of the experiment (preventive group) showed lower urinary oxalate levels and fewer crystal deposits compared to the group of rats that received black seed extract from the 14th to the last day of the experiment (treatment group)(Hadjzadeh, Khoei, Hadjzadeh, & Parizady, 2007).

Al-Attaret.al (2010) reported that Sprague Dawley Rats Exposed to Diazinon, cause significant hematological and biochemical alterations, indicating damage to the blood, liver, kidneys, and heart. However, after they were given black seed extract it showed much improvement due to their antioxidant and anti-per oxidative properties indicating beneficial effect of *Nigella sativa* seeds as treatment against hematotoxicity, hepatoxicity, nephrotoxicity, and cardiotoxicity caused by diazinon and it also has the potential to act against organophosphates and other environmental pollutants.(Al-Attar & Al-Taisan, 2010).

Pomegranate *(Punica granatum L.),* belongs to Lythraceae family, that is native fruit in Central Asia and commonly found in Middle East, Iran, Turkmenistan and northern India.

P. granatum is a fruit-bearing a small tree that grows up to 501507 m. Its red or white seeds are small, slightly flattened, elliptical, and hard.(Mohammadi, Boghrati, Emami, & Akaberi, 2023).

Extracts of all parts of pomegranate fruit exhibit therapeutic properties and target a range of diseases including cancer, cardiovascular disorders, diabetes, male infertility, Alzheimer's disease, aging, and AIDS. (Varicella et al., 2018). Pomegranate peels are identified by pericarp which maintains an orange and greenish color when ripe. The peel covers the arils which are divided by a light membrane inside the peel. The pomegranate peels make up 43% of the whole fruit. (Maphetu, Unuofin, Masuku, Olisah, & Lebelo, 2022). They are rich in polyphenols, flavonoids, tannins, and antioxidants. They have gained attention in recent years due to their numerous beneficial properties and potential applications due to having phenolics (punicalagin and ellagic acids), urolithins, and polysaccharides. A number of studies indicated that it has antioxidant, antiinflammatory, antihyperlipidemic, antimicrobial, antiosteoporosis, and anticancer properties.(Arra, Pasupula, & Anandam, 2024).

Pomegranate have been used for regulating urine discharge and the burning sensation of urine; its seed oil, juice, flowers, and peel are used for protection against nephrotoxicity, and the extracts for renal failure and renal arteries. The anti-hypercalciuria and anti-urolithiasis effects of this plant attracted considerable attention toward pomegranate for use in the prevention of renal calculus formation. Its therapeutically beneficial phytochemicals are responsible for muscle relaxation in the urinary and biliary tract; consequently, stones can be easily removed from the kidney. (Nirumand et al., 2018). A study in barium-chloride-induced rats found that dietary pomegranate peels had beneficial effects on nephrotoxicity. The results demonstrated that pomegranate peels mitigate oxidative damage restore enzymatic antioxidants caused by barium and improve renal function. (Elwej et al., 2016)

A study performed in invitro condition demonstrates that per polyphenol-rich extracts (Exs), anthocyanin fractions (AFrs), and non-anthocyanin fractions (NFrs) derived from pomegranate peel extract exhibit anti-crystallization activity against calcium oxalate and brushite crystals. These compounds inhibit crystal growth by adsorbing onto crystal surfaces, blocking active sites, and reducing crystal size and aggregation. NFrs, which are rich in gallates, tannins, and phenolic acids, demonstrated strong inhibition, likely due to their ability to donate protons and complex with $Ca2^{+}$. AFrs, were less effective, but still contributed to inhibition, in pomegranate-derived fractions. Polyphenols have the potential to inhibit the crystallization of calcium oxalate due to

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

their ability to form anions that adsorb onto crystal surfaces and disrupt crystallization. The study highlights that both anthocyanins and non-anthocyanins inhibit stone formation in two ways, either interfering with the crystallization process or binding with renal proteins to prevent crystal growth. This dual role of polyphenols suggests that pomegranate could have potential applications in the prevention or treatment of renal stones and their formation. (Nazir et al., 2025).

Another study suggested that pomegranate peel extract PPE protected cadmium-induced albino mice from nephrotoxicity. PPE administration showed a decreased level of urea, creatinine, and blood urea nitrogen (BUN), reduced the level of serum malondialdehyde (MDA) as a lipid peroxidation marker, and enhanced the detoxification process in mice. (El-Daly, 2016).

A study on renal failure in adenine-induced male rats suggests that pomegranate peel extract is a potent nephroprotective agent in chronic renal failure rats. The renoprotective effect of pomegranate extracts can be due to their high phenolic content and due to their antioxidant, anti-inflammatory properties, and different signaling pathways. (El-Habibi, 2013).

Keeping in view the preventive effect of black seed and pomegranate peel extract main objective of this study was to investigate the effect of black seed and pomegranate peel and their combined effect against kidney stone formation.

Materials and Method:

The aim of this study was to evaluate the efficiency of black seed and pomegranate peel against stone formation in kidneys in mice. For this purpose, ethylene glycol and black seed and pomegranate peel extract were given throughout the study.

Extract of black seed:

Black seeds (*Nigella sativa*) were purchased from local market in Islamabad to make its extract. Briefly, black seeds were grounded in grinder to make powder. Afterwards, 200 g of powder was mixed with 500ml solvent containing 80% ethanol and 20% distilled water for 4 days. Later on, it was placed in shaking incubator at 25°C with 200rpm for 20 minutes and then baked at 45°C in dry oven for 40 minutes. The solvent containing extract from black seed was first separated by using tea strainer and then via filter paper. The filtrate was placed in shaking incubator for 20 minutes at 37°C with to separate ethanol from the extract and then stored in refrigerator at 4°C until use.

Extract of pomegranate peel:

Pomegranates were also purchased from local market, Islamabad. Pomegranate peels were separated, washed and then left for shade dry for 8 days after that it was dried in dry oven at 70°C for approximately 3 hours. The dried peels were crushed into small pieces with pestle and mortar and then completely crushed into powder by using grinder. To make extract of pomegranate peels, briefly, 20g of pomegranate peel powder was mixed in 100ml solvent i.e. 70% ethanol and 30% distilled water. Then the solution was incubated at 37°C for 24 hours in a shaking incubator with 110rpm and then filtered with filter paper and filtrate was stored at 4°C in the refrigerator until used.

Mice grouping:

Total 25 mice with weight 35g-40g were taken from National Institute of Health (NIH), Islamabad and they were given standard feed obtained from NIH containing all essential ingredients which they needed during whole experiment and kept them at controlled condition.

Table 1: Mice Feed Composition:

Ingredients	Quantity per kg
Wheat flour	30%
Fish meal	15%
Skim milk powder	25%
Vegetable oil	5%
Molasses	1%

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Wheat grain	23%

Mice were divided into 5 groups, 5 mice in each group. Mice of each group were housed in a separate cage.

Healthy control group (1):1% distilled water added to the drinking water.

Negative control group (2): 1% ethylene glycol added to the drinking water.

Intervention group (3): 300mg/kg of black seed extract and 1% ethylene glycol added into the 100ml drinking water.

Intervention group (4):100mg/kg pomegranate peel extract and 1% ethylene glycol added into the 100ml drinking water.

Intervention group (5):300mg/kg black seed, 100mg/kg pomegranate peel extract and1% ethylene glycol added into the 100ml drinking water.

Throughout the trial, 1% ethylene glycol was added to the drinking water of all groups except the healthy control group to induce kidney stones in mice. Proper hygiene conditions were maintained during the experiment by spreading sawdust on the floor of cages and cleaning the cages every after 2 days.

Sample analysis:

This study was ended in 28 days. During this time, mice were given free access to water and food and their clinical symptoms were observed through urine and serum test.

The 24 hour urine samples were collected on 14th and 28th days of the experiment from each group while each were kept in a metabolic cage. The samples were sent to the medical diagnostic laboratory for biochemical analysis for the level of uric acid and calcium in the urine.

After sedating the mice with chloroform, blood samples were collected from each group collectively at the end of experiment on 28th day from their hearts using a syringe. After that, the samples were sent to the medical diagnostic laboratory to determine the level of sodium, potassium, calcium, magnesium, phosphorus, chloride, uric acid and bicarbonate in the blood. Biochemical analysis were done by using Microlab 300 LX semi-automated Biochemistry analyzer (ELITechGroup 8.V, Netherlands) based on Beer Lambert law.

Results:

The prevalence of black seed and pomegranate against kidney stones is shown in Table 2, Table 3 and Table 4. The results were analyzed using one-way ANOVA followed by LSD.

Results of serum parameters in mice:

The pairwise comparison of groups indicated significant difference among all experimental groups i.e., serum sodium (n=3, SEM=1.4383, P=0.0012, F=10.84) indicating significantly highest value in negative control group (149 mg/dl) and lowest value in black seed group (139.5 mg/dl). Similarly pairwise comparison of serum potassium (n=3, SEM=0.2981, P=0.001, F=27.15) indicated significantly highest value in negative control group (7.76 mg/dl) and lowest value in combination group of black seed and pomegranate peel (3.9mg/dl). Additionally the pairwise comparison indicated significant difference among all experimental groups (n=3, SEM=0.2039, P=0.001, F=27.4) with higher serum magnesium value in black seed group (3.9 mg/dl) and lowest value in negative control group (1.1 mg/dl). Similarly, pairwise comparison of serum calcium (n=3, SEM=0.1738, P=0.001, F=93.96) indicated significantly highest value in negative control group (11 mg/dl) and lowest value in combination group of black seed and pomegranate peel (7.1 mg/dl).

The pairwise comparison of serum chloride (n=3, SEM=1.8506, P=0.0263, F=4.39) indicated significantly highest value in negative control group (106.5 mg/dl) and lowest value in combination group of black seed and pomegranate peel (97.5 mg/dl). Similarly, the pairwise comparison of serum bicarbonate (n=3, SEM=0.9127, P=0.1504, F=2.14) indicated significantly highest value in

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

black seed group (25.5mg/dl) and lowest value in negative control group (19.5 mg/dl). The pairwise comparison of serum phosphorous (n=3, SEM=0.185, P=0.0003, F=15.17) indicated significantly highest value in negative control group (5.5 mg/dl) and lowest value in combination group of black seed and pomegranate peel (3.4 mg/dl). Similarly, the pairwise comparison of serum uric acid (n=3, SEM=0.2692, P=0.001, F=60.95) indicated significantly highest value in negative control group (8.8 mg/dl) and lowest value in combination group of black seed and pomegranate peel (4.5 mg/dl).

Results of urinary parameters in mice:

The pairwise comparison of urinary uric acid (n=3, SEM=0.2692, P=0.001, F=36.24) indicated significantly highest value in negative control group (7.8mg/dl) and lowest value in combination group of black seed and pomegranate peel (4.1 mg/dl) after 14th day of experiment. Similarly, the pairwise comparison of urinary calcium (n=3, SEM=0.3048, P=0.0014, F=10.34) indicated significantly highest value in negative control group (10.8 mg/dl) and lowest value in combination group of black seed and pomegranate peel (8.4mg/dl) after 14th day of experiment. The pairwise comparison of urinary uric acid (n=3, SEM=0.2595,P=0.001, F=54.9) indicated significantly highest value in negative control group (8.9 mg/dl) and lowest value in combination group of black seed and pomegranate peel (3.7 mg/dl) after 28th day of experiment. Similarly, the pairwise comparison of urinary calcium (n=3, SEM=0.2933,P=0.0004, F=14.26) indicated significantly highest value in negative control group (11.2mg/dl) and lowest value in combination group of black seed and pomegranate peel (8.2 mg/dl)after 28th day of experiment.

Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Table 2: Effect of black seed, pomegranate peel and combination on serum parameters

Experimental Groups	Parameters							
	Sodium	Potassium	Magnesium	Calcium	Chloride	Bicarbonates	Phosphorus	Uric acid
PCG	135^{c}	3.7^{c}	3.5 ^{a}	7.4^{c}	97^{b}	22.5^{ab}	3.5 ^{c}	3.6^{c}
NCG	149^{a}	7.76^{a}	1.1^{b}	11^{a}	106.5^{a}	19.5^{b}	5.5^{a}	8.8^{a}
BS	139.5^{bc}	5.5^{b}	3.9^{a}	7.5 ^{c}	98.7^{b}	25.5^{a}	4.3^{b}	6.6^{b}
PM	144.5^{a}	4.5^{bc}	3.5^{a}	9.7^{b}	99.5^{b}	23.2^{ab}	4.1^{b}	6.7^{b}
BS+PM	140.2^{ab}	3.9 ^{c}	3.3^{a}	7.1 ^{c}	97.5^{b}	22.4^{ab}	3.4 ^{c}	4.5 ^{c}
SEM	1.4383	0.2981	0.2039	0.1738	1.8506	0.9127	0.185	0.2692
P-value	0.0012	0.001	0.001	0.001	0.0263	0.1504	0.0003	0.001
F-value	10.84	27.15	27.4	93.96	4.39	2.14	15.17	60.95

Results were analyzed by One-way ANOVA followed by LSD (n=3) with different letters along the different rows indicated significant differences among experimental groups (P<0.05)
Positive control group (PCG), Negative control group (NCG), Black seed group (BS), Pomegranate group (PM), Black seed plus Pomegranate group (BS+PM)

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Table 3: Effect of black seed, pomegranate peel and combination on urinary parameters of 14th day of experiment

Experimental Groups	Parameters	
	Calcium	Uric acid
PCG	8.1 [c]	3.6 [c]
NCG	10.8[a]	7.8[a]
BS	8.8 [c]	6.6[b]
PM	9.1[b]	4.7[c]
BS+PM	8.4 [c]	4.1[c]
SEM	0.3048	0.2692
P-value	0.0014	0.001
F-value	10.34	36.24

Results were analyzed by One way ANOVA followed by LSD (n=3) with different letters along the different rows indicated significant differences among experimental groups (P<0.05)
Positive control group (PCG), Negative control group (NCG), Black seed group (BS), Pomegranate group (PM), Black seed plus Pomegranate group (BS+PM)

Table 4: Effect of black seed, pomegranate peel and combination on urinary parameters of 28th day of experiment

Experimental Groups	Parameters	
	Calcium	Uric acid
PCG	8.9 [c]	5.8 [b]
NCG	11.2 [a]	8.9 [a]
BS	9.8 [b]	5.6 [b]
PM	8.4 [c]	4.4 [c]
BS+PM	8.2 [c]	3.7 [c]
SEM	0.2933	0.2595
P-value	0.0004	0.001
F-value	14.26	54.9

Results were analyzed by One way ANOVA followed by LSD (n=3) with different letters along the different rows indicated significant differences among experimental groups (P<0.05)
Positive control group (PCG), Negative control group (NCG), Black seed group (BS), Pomegranate group (PM), Black seed plus Pomegranate group (BS+PM)

Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

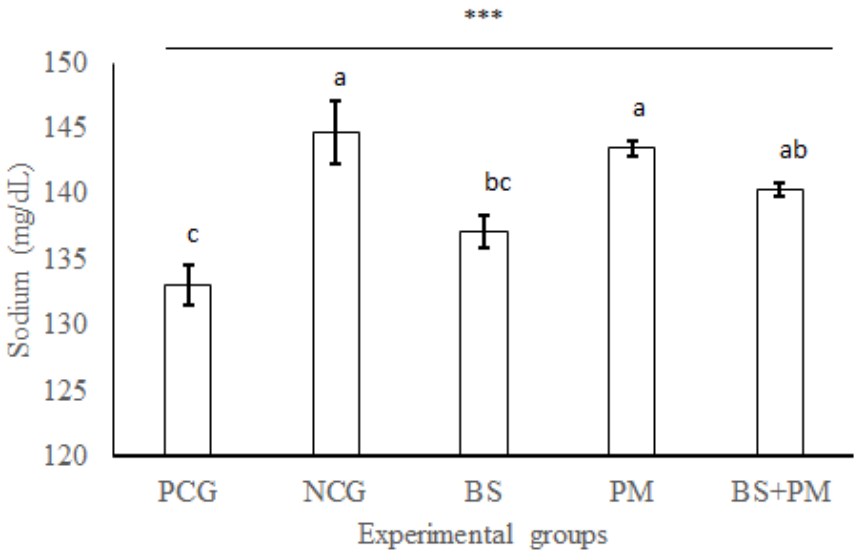

Figure 01: Effect of black seed, pomegranate peel and combination on serum sodium level

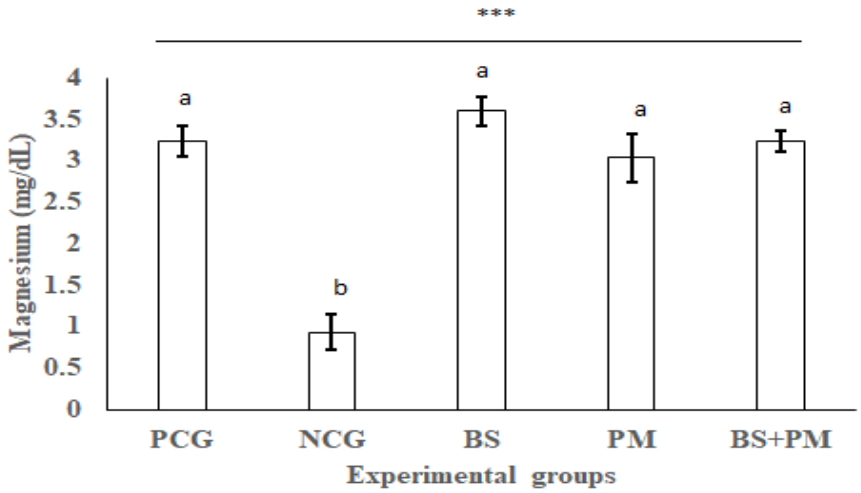

Figure 02: Effect of black seed, pomegranate peel and combination on serum magnesium level

Frontier in Medical & Health Research
ISSN: (e) 3007-1607 (p) 3007-1593

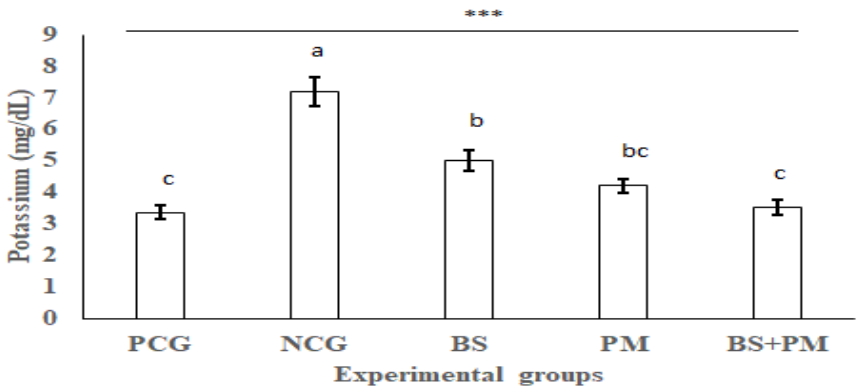

Figure 03: Effect of black seed, pomegranate peel and combination on serum potassium level

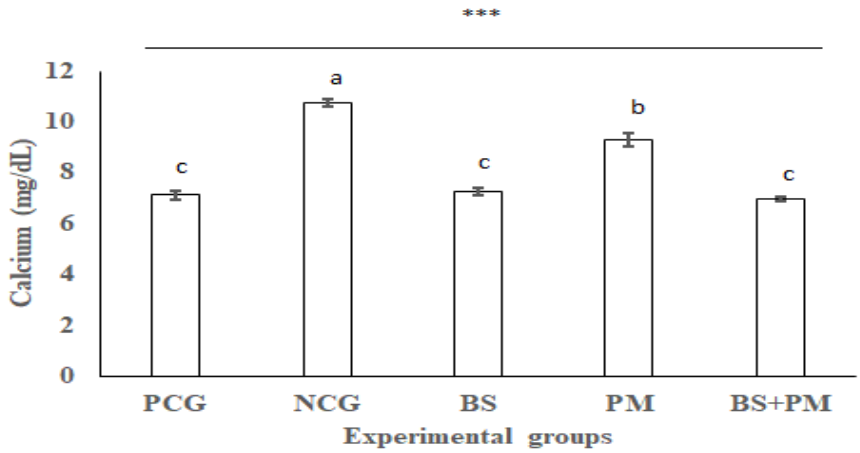

Figure 04: Effect of black seed, pomegranate peel and combination on serum calcium level

Frontier in Medical & Health Research
ISSN: (e) 3007-1607 (p) 3007-1593

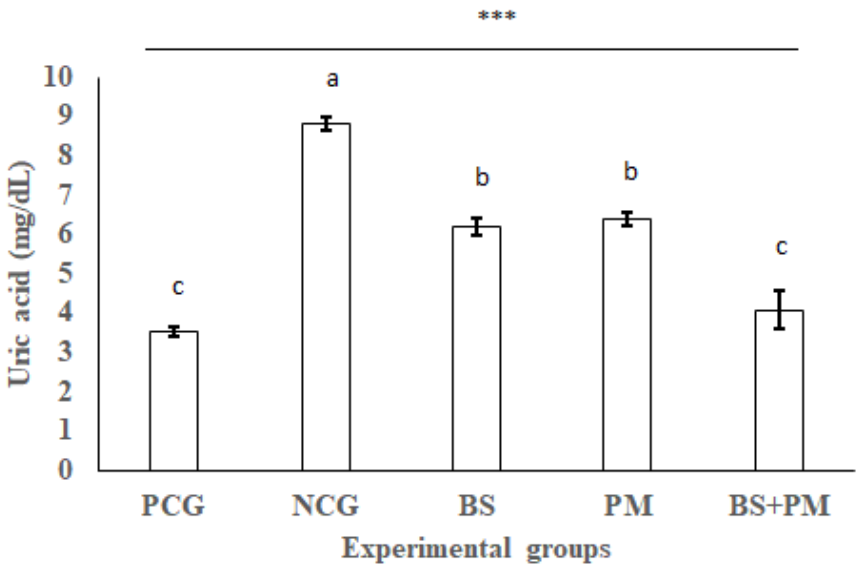

Figure 05: Effect of black seed, pomegranate peel and combination on serum uric acid level

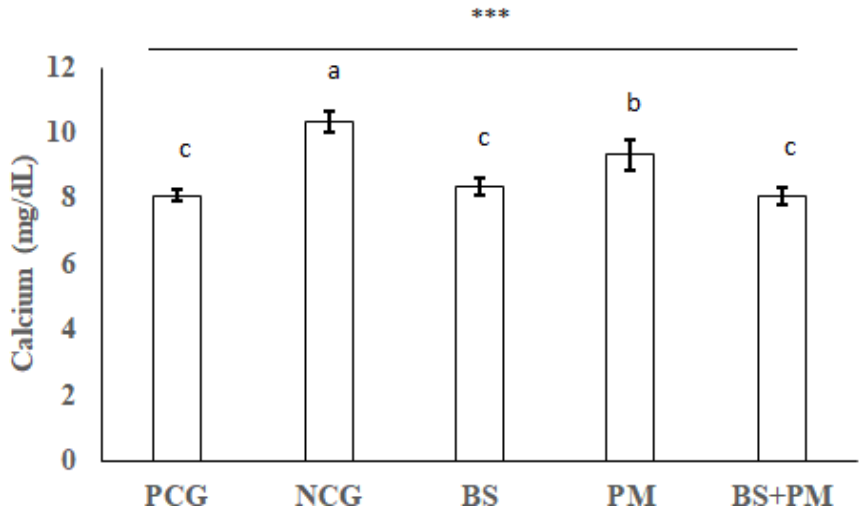

Figure 06: Effect of black seed, pomegranate peel and combination on urinary calcium level on 14th day of experiment

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

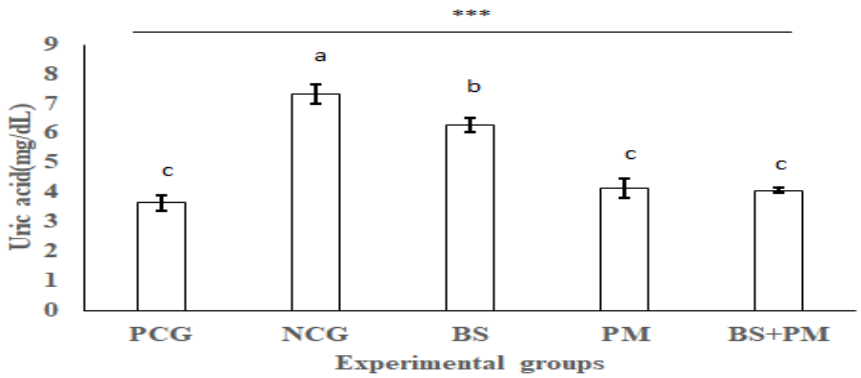

Figure 07: Effect of black seed, pomegranate peel and combination on urinary uric acid level on 14th day of experiment

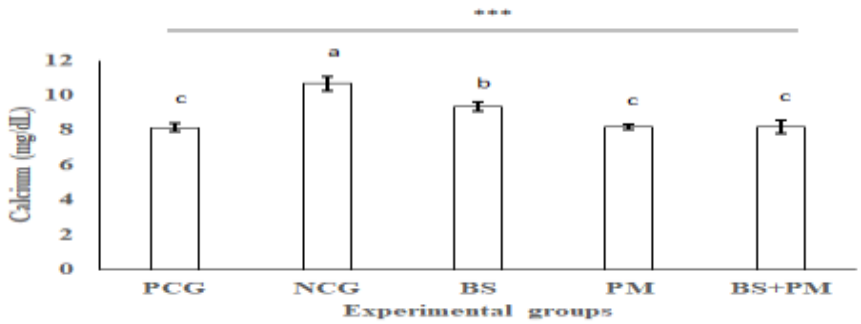

Figure 08: Effect of black seed, pomegranate peel and combination on urinary calcium level on 28th day of experiment

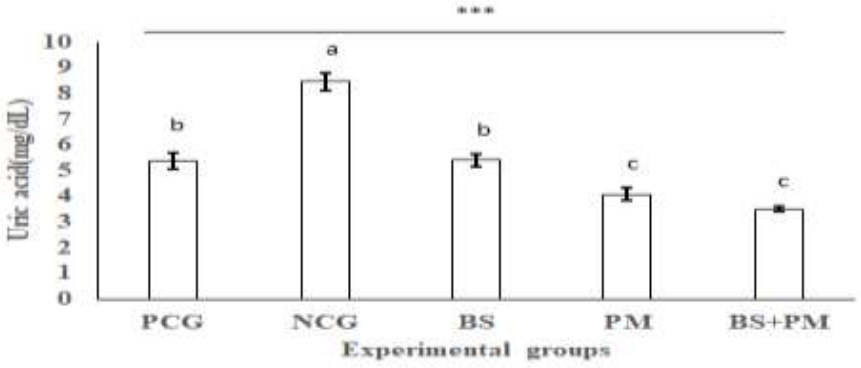

Figure 09: Effect of black seed, pomegranate peel and combination on urinary uric acid level on 28th day of experiment

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Discussion

The prevalence and incidence of kidney stones are increasing across the world due to the involvement of several genetic and environmental factors. (Romero, Akpinar, & Assimos, 2010) and has increased in Pakistan in the last 5 decades. (Hussain, Somro, Abidi, & Rizvi, 2024). Different plants were found to possess anti-urolithic properties among them black seed studied by different scientists.(Shahsavari, 2021). The present study was designed to investigate the preventive effect of black seed extract and pomegranate peel extracts against kidney stone formation by evaluation of the serum sodium, potassium, magnesium, calcium, chloride, bicarbonates, phosphorus, serum uric acid, urinary calcium, and urinary uric acid. Results indicated the ethanolic extract of black seed and pomegranate peels showing their preventive effect on stone formation in the kidney of mice.

In our study, ethylene glycol was used to induce kidney stones. The negative group showed the highest level of serum and urinary parameters from all the treatment groups. In this study, serum sodium levels showed decreased values in all treatment groups with the lowest value in the black seed group when compared with the negative control group having the highest value. Pomegranate individually and when given with a combination of black seed showed decreased values, indicating black seed effectiveness for the treatment of kidney stones. The level of sodium increases in kidney stone disease. (Rashidi et al., 2022).In a previous study, Rashidi et.al (2022), reported that black seed individually and in combination with honey reduced blood sodium levels even though its level raised in the negative control group since the black seed is effective for renal toxicity. (Rashidi, Sazegar, Baghdadabad, & Naghsh, 2022).

Serum potassium level showed a decline in the black seed and pomegranate peel group, while the lowest value was in the combination group of black seed and pomegranate peel, showing that the combination group is more effective. The highest value was seen in the negative group, as potassium level increases in kidney stones. (Rashidi et al., 2022). Rashidi et.al (2022) reported that serum potassium levels decreased when treated with a combination of black seed and honey. (Rashidi et al., 2022)

In our study, the blood level of calcium decreased more in the combination group of black seed and pomegranate peel, having the lowest value than when given individually. Serum uric acid level had the lowest value in the combination group more than other treatment groups, when compared with the negative control group. In a study, Rathod et. al (2012) reported that *Punica granatum* chloroform extract decreased the level of serum uric acid in ethylene glycol-induced urolithiasis in rats as the study showed the decreased level was due to the antioxidant-rich property of *Punica granatum*. (Rathod et al., 2012).

The results of our experiment indicated lowest value of serum bicarbonate level in the negative group indicating kidney stones, while the highest level was found in the black seed group. The pomegranate peel group and combination group also resulted in an increased level of bicarbonate. Similarly results were reported by a cohort study conducted by Tangri et.al (2023) on patients reported that the risk of kidney stones increases with low bicarbonate levels in the blood.(Tangri et al., 2023). Furthermore, in our study, the level of phosphorous in blood had the lowest value in the combination group and also showed a decline in level when given individually black seed and pomegranate peel.

The highest value of serum magnesium was found in the black seed group, while the second highest value was seen in the pomegranate group. The combination group also had a high value when compared with the negative control group having the lowest value i.e. 1.1 mg/dl. Rashidi et.al (2022) suggested that when the black seed is given at a dose of 250 mg/kg the level of blood magnesium level, indicating the prevention of kidney stone formation.(Rashidi et al., 2022). Additionally, declined serum chloride level was seen in all treatment groups, with the lowest value in the combination of black seed and pomegranate peel group, and the highest level in the negative control group. The level of chloride is high in kidney stones.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

In kidney stones, urinary calcium levels are usually high urinary calcium excretion can be caused by increased gastrointestinal absorption, increased bone resorption, increased tubular reabsorption, hormonal imbalance, and genetic factors. (Dawson & Tomson, 2012). In our study, the level of urinary calcium was reduced in the intervention groups compared to the negative control group indicating the preventive effect of black seed and pomegranate peels against kidney stones formation. The combination of black seed and pomegranate peels resulted in the lowest quantity of urine calcium i.e. 8.4 mg/dl on the 14th day and 8.2 mg/dl on the last day of the experiment.In the study of Hadjzadeh et.al, the ethanolic extract of black seed resulted in a 56% reduction of the number of oxalate crystals in a preventive group compared to the only ethylene glycol-induced group which supports our results. (Hadjzadeh, Khoei, Hadjzadeh, & Parizady, 2007).

Uric acid is also associated with kidney stones and its level is high in kidney stones. In our study the uric acid in urine is highest in the negative control group and lower in the intervention groups. In the combination of black seed and pomegranate peels, the quantity is lowest i.e. 4.1mg/dl on the 14th and 3.7mg/dl on the 28th of the experiment. Halabe et.al (1994) found that an increased concentration of uric acid in the urine causes uric acid stone formation which is also known as Hyperuricosuria. (Halabe & Sperling, 1994).

Conclusion:

Natural remedies have always been admired as some medicines possess side effects. Our study revealed that by studying urinary and serum parameters, combination of black seed and pomegranate peel was evidently efficient for kidney stones.Hence, curative properties of black seed and pomegranate peel would make them foremost preference for treatment and prevention of kidney stones.This herbal treatment can be used as novel medication design in future and play effective role in renal stone dissolution.

References

Abd-Elkareem, Mahmoud, Soliman, Mahmoud, Abd El-Rahman, Mokhless AM, & Abou Khalil, Nasser.

S. (2022). Effect of Nigella sativa L. seed on the kidney of monosodium glutamate challenged rats.

Frontiers in pharmacology, 13, 789988.

Aiumtrakul, Noppawit, Thongprayoon, Charat, Suppadungsuk, Supawadee, Krisanapan, Pajaree,

Pinthusopon, Preyarat, Mao, Michael A, .Miao, Jing. (2024). Global trends in kidney stone awareness: a Time Series Analysis from 2004–2023. *Clinics and Practice, 14*(3), 915-927.

Al-Attar, Atef M, & Al-Taisan, WAA. (2010). Preventive effects of black seed (Nigella sativa) extract on Sprague Dawley rats exposed to diazinon. *Australian Journal of Basic and Applied Sciences, 4*(5), 957-968.

Ardakani Movaghati, Mohammad Reza, Yousefi, Mahdi, Saghebi, Seyed Ahmad, Sadeghi Vazin,

Mahmoud, Iraji, Aida, & Mosavat, Seyed Hamdollah. (2019). Efficacy of black seed (Nigella

sativa L.) on kidney stone dissolution: A randomized, double-blind, placebo-controlled, clinical trial. *Phytotherapy Research, 33*(5), 1404-1412.

Arra, Keerthana, Pasupula, Rajeshwari, & Anandam, Singireddy. (2024). In Vivo Assessment of Punica granatum Leaf Extract: Anti-Urolithiatic and Nephroprotective Effects. *Natural Product Sciences, 30*(2), 80-92.

Beigi, Faramarz. Efficacy of black seed (Nigella sativa L.) and honey on stone excretion and metabolic disorders in patients with kidney stones: A non-randomized.

Curhan, Gary C, Willett, Walter C, Rimm, Eric B, & Stampfer, Meir J. (1993). A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *New England journal of medicine, 328*(12), 833-838.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting the risk for kidney stones in women. *Annals of internal medicine, 126*(7), 497-504.

Dawson, Charlotte H, & Tomson, Charles RV. (2012). Kidney stone disease: pathophysiology, investigation and medical treatment. *Clinical medicine, 12*(5), 467.

Dollah, Mohammad Aziz, Parhizkar, Saadat, & Izwan, Mohammad. (2013). Effect of Nigella sativa on the kidney function in rats. *Avicenna journal of phytomedicine, 3*(2), 152.

El-Daly, Amal A. (2016). Pomegranate peel Extract Protects Cadmium-induced nephrotoxicity in albino mice. *Journal of Bioscience and Applied Research, 2*(6), 362-375.

El-Demerdash, Fatma M, Al Mhanna, Ansam B, El-Sayed, Raghda A, Mohamed, Tarek M, & Salem,

Maha M. (2025). Use of Nigella sativa silver nanocomposite as an alternative therapy against thioacetamide nephrotoxicity. *Genes & Nutrition, 20*(1), 6.

El-Habibi, EM. (2013). Renoprotective effects of Punica granatum (pomegranate) against adenineinduced chronic renal failure in male rats. *Life Sci J, 10*(4), 2059-2069.

Elwej, Awatef, Ghorbel, Imen, Marrekchi, Rim, Boudawara, Ons, Jamoussi, Kamel, Boudawara, Tahia, Sefi, Mediha. (2016). Improvement of kidney redox states contributes to the beneficial effects of dietary pomegranate peel against barium chloride-induced nephrotoxicity in adult rats. *Archives of physiology and biochemistry, 122*(3), 130-140.

Forouzanfar, Fatemeh, Bazzaz, Bibi Sedigheh Fazly, & Hosseinzadeh, Hossein. (2014). Black cumin (Nigella sativa) and its constituent (thymoquinone): a review on antimicrobial effects. *Iranian journal of basic medical sciences, 17*(12), 929.

Hadjzadeh, MA, Khoei, Alireza, Hadjzadeh, Zahra, & Parizady, Mohammadreza. (2007). Ethanolic extract of nigella sativa L seeds on ethylene glycol-induced kidney calculi in rats. *Urology journal, 4*(2), 86-90.

Hannan, Md Abdul, Zahan, Md Sarwar, Sarker, Partha Protim, Moni, Akhi, Ha, Hunjoo, & Uddin, Md

Jamal. (2021). Protective effects of black cumin (Nigella sativa) and its bioactive constituent, thymoquinone against kidney injury: An aspect on pharmacological insights. *International Journal of Molecular Sciences, 22*(16), 9078.

Halabe, Aaron, & Sperling, Oded. (1994). Uric acid nephrolithiasis. Mineral and electrolyte metabolism, 20(6), 424-431.

Huchchannanavar, Shilpa, Yogesh, LN, & Prashant, SM. (2019). The black seed Nigella sativa: A wonder seed. *Int. J. Chem. Stud, 7*(3), 1320-1324.

Jiang, Chen, Wang, Lingyun, Wang, Yang, Xu, Rongjia, Yang, Hongtao, & Peng, Jibin. (2023).

Therapeutic effects of Chinese herbal medicines for treatment of urolithiasis: a review. *Chinese herbal medicines, 15*(4), 526-532.

Maphetu, Nhlanhla, Unuofin, Jeremiah Oshiomame, Masuku, Nelisiwe Prenate, Olisah, Chijioke, & Lebelo, Sogolo Lucky. (2022). Medicinal uses, pharmacological activities, phytochemistry, and the molecular mechanisms of Punica granatum L.(pomegranate) plant extracts: A review. *Biomedicine & Pharmacotherapy, 153*, 113256.

Mohammadi, Mehran, Boghrati, Zahra, Emami, Seyed Ahmad, & Akaberi, Maryam. (2023).

Pomegranate: A review of the heavenly healer's past, present, and future. *Iranian Journal of Basic Medical Sciences, 26*(11), 1245.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Mousavi, Ghafour, & Mohajeri, Daryoush. (2014). Effect of ground black seeds (Nigella sativa L.) on renal tubular cell apoptosis induced by ischemia/reperfusion injury in the rats. *Iranian Journal of Basic Medical Sciences, 17*(12), 1032.

Nazir, Mudassir, Haq, Muhammad Abdul, Sherazi, Syeda Moahida Batool, Naz, Shahina, Mobin, Lubna, & Tsoupras, Alexandros. (2025). Effects of Polyphenolic Extracts From Sumac, Pomegranate

Peel, Indian Almond Leaves, Falsa, and Banana Bracts on Calcium Oxalate and Brushite

Crystallization In Vitro. *Chemistry & Biodiversity*, e202500023.

Nirumand, Mina Cheraghi, Hajialyani, Marziyeh, Rahimi, Roja, Farzaei, Mohammad Hosein, Zingue, Stéphane, Nabavi, Seyed Mohammad, & Bishayee, Anupam. (2018). Dietary plants for the prevention and management of kidney stones: preclinical and clinical evidence and molecular mechanisms. *International journal of molecular sciences, 19*(3), 765.

Ragheb, Ahmed, Attia, Ahmed, Eldin, Walid Shehab, Elbarbry, Fawzy, Gazarin, Sana, & Shoker, Ahmed. (2009). The protective effect of thymoquinone, an anti-oxidant and anti-inflammatory agent, against renal injury: a review. *Saudi Journal of Kidney Diseases and Transplantation, 20*(5), 741-752.

Rashidi, Bentolhoda, Sazegar, Hosein, Baghdadabad, Leila Zareian, & Naghsh, Nooshin. (2022). Effects of Hydroalcoholic Extract of Black Seed (Nigella Sativa) and Honey on Changes in Serum, Urinary, and Kidney Tissue Factors in Kidney Stones of Male Mice.

Shakeri, Nahid, Mehrabi, Sadrollah, & Paymard, Akvan. (2021). Comparison efficacy of oral Nigella sativa seeds and tamsulosin on pain relief and passage of 4 to 10 mm stones of kidney and ureter; a randomized clinical trial. *Journal of Nephropharmacology, 11*(1), e8-e8.

Tavakkoli, Alireza, Mahdian, Vahid, Razavi, Bibi Marjan, & Hosseinzadeh, Hossein. (2017). Review on clinical trials of black seed (Nigella sativa) and its active constituent, thymoquinone. *Journal of pharmacopuncture, 20*(3), 179.

Taylor, Eric N, Stampfer, Meir J, & Curhan, Gary C. (2005). Obesity, weight gain, and the risk of kidney stones. *Jama, 293*(4), 455-462.

Walter, Kristin. (2022). Kidney stones. *Jama, 328*(9), 898-898.

Dawson, Charlotte H, & Tomson, Charles RV. (2012). Kidney stone disease: pathophysiology, investigation and medical treatment. *Clinical medicine, 12*(5), 467.

Hadjzadeh, MA, Khoei, Alireza, Hadjzadeh, Zahra, & Parizady, Mohammadreza. (2007). Ethanolic extract of nigella sativa L seeds on ethylene glycol-induced kidney calculi in rats. *Urology journal, 4*(2), 86-90.

Halabe, Aaron, & Sperling, Oded. (1994). Uric acid nephrolithiasis. *Mineral and electrolyte metabolism, 20*(6), 424-431.

Hussain, Manzoor, Somro, Abdul Saboor, Abidi, Syed Saeed, & Rizvi, Syed Adeeb Hassan. (2024). Stepping Stones In Prevention of Kidney Stone Disease In Pakistan. *Pakistan Journal of Kidney Diseases, 8*(2), 2-10.

Rashidi, Bentolhoda, Sazegar, Hosein, Baghdadabad, Leila Zareian, & Naghsh, Nooshin. (2022). Effects of Hydroalcoholic Extract of Black Seed (Nigella Sativa) and Honey on Changes in Serum,

Urinary, and Kidney Tissue Factors in Kidney Stones of Male Mice.

Rathod, NR, Biswas, Dipak, Chitme, HR, Ratna, Sanjeev, Muchandi, IS, & Chandra, Ramesh. (2012).

Anti-urolithiatic effects of Punica granatum in male rats. *Journal of ethnopharmacology, 140*(2), 234-238.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Romero, Victoriano, Akpinar, Haluk, & Assimos, Dean G. (2010). Kidney stones: a global picture of prevalence, incidence, and associated risk factors. *Reviews in urology, 12*(2-3), e86.

Shahsavari, Somayeh. (2021). An overview of the most important medicinal plants used in iranian traditional medicine for the treatment of kidney stones: A mini-review article. *Plant Biotechnology Persa, 3*(1), 37-38.

Tangri, Navdeep, Mathur, Vandana, Reaven, Nancy L, Funk, Susan E, Whitlock, Reid H, Wesson, Donald E, & Bushinsky, David A. (2023). Association of serum bicarbonate with the development of kidney stones in patients with chronic kidney disease: a retrospective cohort study. *Clinical Kidney Journal, 16*(7), 1113-1121.

Varicola, KARUNA SREE, Siddiqua, AA, Dintyala, KEERTHI, & Ventrapati, GANDHI. (2018). Fruit peel extracts with potential for dissolving simulated renal stones in in vitro conditions. *Int J Pharm Sci, 10*(11), 71-74.