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Original Research / Özgün Araştırma

The Effects of *Viburnum Opulus L.* on Kidneys of Rats with Ethylene Glycol-induced Nephrolithiasis

Etilen Glikolla İndüklenmiş Nefrolitiyazisli Sıçan Böbrekleri Üzerinde *Viburnum Opulus L*'nin Etkileri

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Özet

Amaç: Son yıllarda yapılan çalışmalarda taş oluşumunda oksidatif stres ve serbest oksijen radikallerinin rolü olduğu üzerinde durulmaktadır. *Viburnum Opulus L.* (VO), antioksidan etkinliğiyle bilinen ve Türk geleneksel tıbbında taş düşürmek için suyu hazırlanarak kullanılan bir meyvedir. Bu çalışmanın amacı, etilen glikol (EG) ile indüklenmiş nefrolitiyazisli sıçanlarda VO'nun kalsiyum okzalat (CaOx) kristalizasyonu ve oksidatif stres üzerindeki etkinliğini araştırmaktır.

Gereç ve Yöntemler : 50 adet yetişkin erkek Wistar Hannover türü sıçanlar 5 gruba ayrıldı: Kontrol (Grup 1), EG (Grup 2), EG + 50 mg/kg VO (Grup 3), EG + 100 mg/kg VO (Grup 4), EG + 200 mg/kg VO (Grup 5). 7., 14. ve 28. günlerde 24 saatlik idrar toplandı ve kan örnekleri alındı. 28. günde sıçanlar sakrifiye edildi ve böbrek dokusunda inflamasyon, oksidatif stres ve polarize ışık mikroskobu altında CaOx kristalizasyonu değerlendirildi.

Bulgular: 7., 14. ve 28. günde serumda inflamasyon, akut böbrek hasarı ve oksidatif stres, 28.günde dokuda inflamasyon ve oksidatif stres parametrelerinde Grup 2 (EG) ile Grup 1 (Kontrol) arasında istatistiksel olarak anlamlı farklılık saptandı. Bu parametrelerin Grup 3-5'te Grup 2 (EG)'ye göre iyileşme gösterdiği ve doz arttıkça istatistiksel olarak anlamlılığın arttığı

Abstract

Objective: Recent research has centered on the role of oxidative stress and free oxygen radicals in the formation of stones. *Viburnum opulus L.* (VO) is a fruit species known for its antioxidant activity, and its juice preparation is used in Turkish traditional medicine for stone removal. This study aimed to investigate the effects of VO on calcium oxalate (CaOx) crystallization and oxidative stress in rats with ethylene glycol (EG)-induced nephrolithiasis.

Material and Methods: Fifty adult male Wistar Hannover rats were divided into five groups: control (Group 1), EG (Group 2), EG + 50 mg/kg VO (Group 3), EG + 100 mg/kg VO (Group 4), and EG + 200 mg/kg VO (Group 5). On days 7, 14, and 28, 24-hour urine was collected, and blood samples were taken. On day 28, the rats were sacrificed, and inflammation, oxidative stress, and CaOx crystallization in kidney tissue were evaluated under polarized light microscopy.

Results: A statistically significant difference was found between Group 1 and Group 2 in terms of serum inflammation parameters, acute kidney injury, and oxidative stress evaluated on days 7, 14, and 28, and tissue inflammation and oxidative stress parameters evaluated on day 28. It was observed that these parameters improved in Groups 3-5 compared to Group 2, and the level of statistical significance increased

The study was approved by Bezmialem Vakıf University Animal Experiments Local Ethics Committee (Approval number: 2018/254, Date: 2018/10/30). All research was performed in accordance with relevant guidelines/regulations.

görüldü. 28. günde dokuların histopatolojik değerlendirilmesinde ortalama kristal sayısı Grup 2 (EG)'de Grup 1 (Kontrol)'e göre istatistiksel olarak anlamlı yüksek saptandı. Bu parametrelerin Grup 3-5'te Grup 2'ye göre iyileşme gösterdiği ve Grup 4-5'te istatistiksel olarak anlamlı farklılık olduğu görüldü.

Sonuç: VO'nun EG ile indüklenmiş nefrolitiyazisli sıçanlarda inflamasyon, oksidatif stres, akut böbrek hasarı ve CaOx kristalizasyonunu doz artışıyla doğru orantılı olarak iyileştirdiği saptanmıştır.

Anahtar Kelimeler: *Viburnum Opulus L.*, Böbrek taşı, Nefrolitiyazis, Kalsiyum okzalat, Etilen glikol, İnflamasyon, Oksidatif stres, Akut böbrek hasarı, Kristalizasyon

as the dose increased. In the histopathological evaluation of the tissues on day 28, the mean number of crystals was statistically significantly higher in Group 2 than in Group 1. These parameters improved in Groups 3-5 compared to Group 2, and there was a statistically significant difference when Groups 4 and 5 were compared to Group 2.

Conclusion: It was found that VO improved inflammation, oxidative stress, acute kidney injury, and CaOx crystallization in rats with EG-induced nephrolithiasis in direct proportion to the increase in dose.

Keywords: *Viburnum opulus L.*, Kidney stone, Nephrolithiasis, Calcium oxalate, Ethylene glycol, Inflammation, Oxidative stress, Acute kidney injury, Crystallization

INTRODUCTION

Urinary stone disease is seen common around the world, it is reported at a rate of 7-13% in North America, 5-9% in Europe, and 1-5% in Asia (1). In Türkiye, located in the endemic stone belt, two epidemiological studies on urinary stone disease have reported its prevalence to be 14.8% and 11.1%, respectively (2,3).

It is considered that the neutralization of oxidative stress through antioxidants may be beneficial for renal function and reduce the recurrence of kidney stones. In recent years, the efficacy of various antioxidants has been investigated in rats with experimentally induced calcium oxalate (CaOx) nephrolithiasis. Many antioxidants, such as green tea (4), pomegranate juice (5), and saffron (6), have been shown to have a protective effect on these rats. *Viburnum opulus L.* (VO), commonly known as the European cranberry bush, is a fruit species with known antioxidant activity (7). The juice prepared from VO fruit in Central Anatolia is used in Turkish traditional medicine for stone removal (8).

This study aimed to investigate the effects of VO on CaOx crystallization and oxidative stress in rats with ethylene glycol (EG)-induced CaOx nephrolithiasis.

MATERIALS AND METHODS

Extract Preparation

The fruit of VO was collected from Kayseri province. For extraction, dried VO fruit was ground into powder, and 100 g of powder was mixed with 1,000 mL of cold distilled water for 24 hours. The resulting maceration extract was lyophilized by evaporation. The antioxidant profiles of the prepared extracts were photometrically measured based on total phenol, total flavonoid, total antioxidant levels and cupric-reducing antioxidant capacity (CUPRAC) (Figure 1).

Animals

Fifthy adult, 12-week-old male Wistar Hannover rats, weighing approximately 350-400 g, were obtained from the Experimental Animals Laboratory of Bezmialem Vakif University. The rats were kept in rooms with a temperature of 22–23 °C under a 12-hour light and 12-hour dark cycle. The animals were fed a standard rat chow diet, and water was provided *ad libitum*.

Experimental Design

The rats were randomly divided into five groups and placed in metabolic cages three days prior to the experiments to acclimate them to the environment. Group 1 (control) was only given drinking water, Group 2 (EG) was given 0.75% EG in drinking water, Group 3 (EG + low-dose VO) was given 50 mg/kg of VO by oral gavage with 0.75% EG in drinking water, Group 4 (EG + medium-dose VO) was given 100 mg/kg of VO by oral gavage with 0.75% EG in drinking water, and Group 5 (EG + high-dose VO) was given 200 mg/kg of VO by oral gavage with 0.75% EG in

drinking water. These procedures were followed for 28 days. One rat in Group 2 was excluded from the study due to insufficient nutrition and significantly lower weight compared to the remaining rats.

On days 7, 14, and 28, the rats were placed in metabolic cages, and their 24-hour urine samples and blood samples were taken. The urine samples were stored at -80 °C until analysis. The blood samples were taken into gel biochemistry tubes. After waiting for 15 minutes for coagulation, the blood samples were centrifuged at 2,500 xg for 10 minutes to separate the serum. The separated sera were placed in Eppendorf tubes and stored at -80 °C until analysis. After day 28, the rats were sacrificed under general anesthesia. One of the kidneys was fixed with a 10% neutral buffered formaldehyde solution for histopathological examinations. For biochemical examination, the other kidney was homogenized in 1 ml of phosphate-buffered saline (PBS) in a homogenizer, centrifuged at 10,000 xg at +4 °C for 30 minutes, and the supernatants were separated. After protein determination using the Bradford method, the samples were stored at -80 °C until analysis.

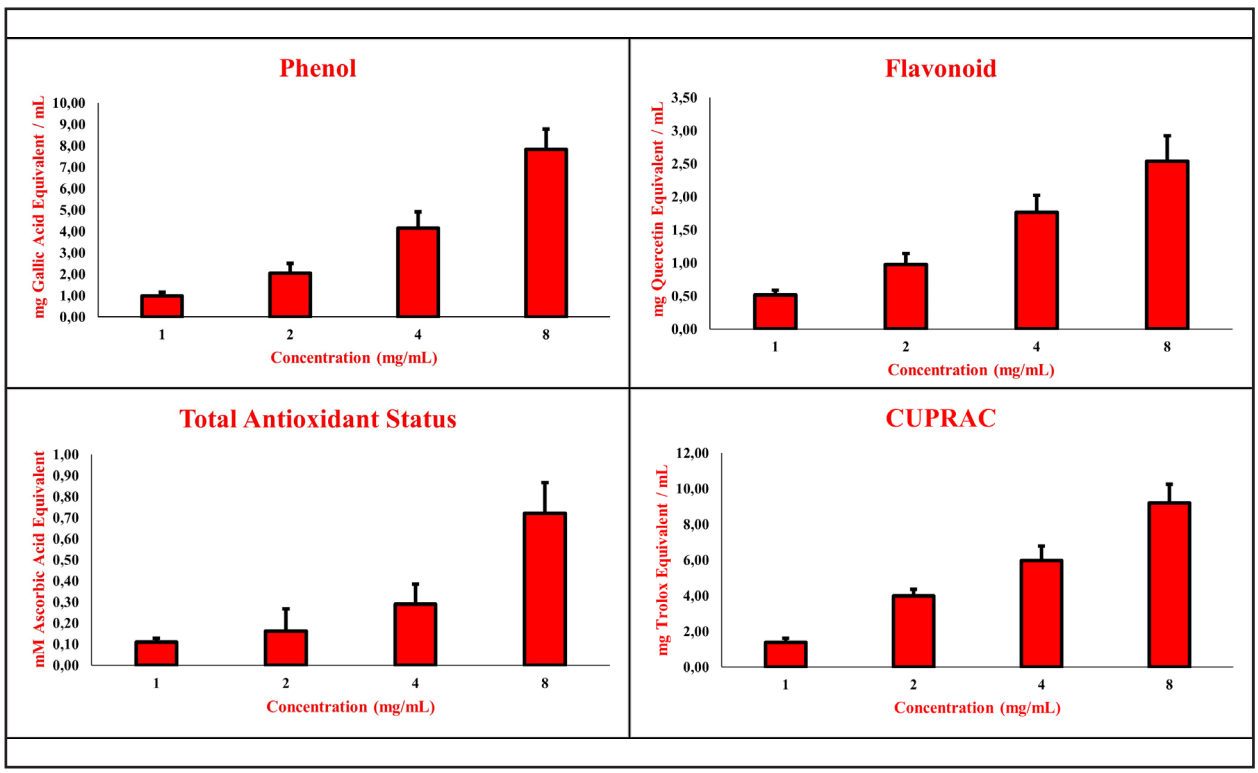

Figure 1. Antioxidant profiles of the prepared extracts

Serum Analyses

Serum urea, creatinine, sodium, and potassium values were measured in blood samples taken on days 7, 14, and 28 using an autoanalyzer (Abbott Architech ci16200). In addition, the blood samples taken on day 28 were used to photometrically determine serum cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), serum tumor necrosis factor-alpha (TNF-α), interleukin 1-beta (IL-1β), IL-6, total oxidant status (TOS), total antioxidant status (TAS), total thiol, and native thiol values, using commercial enzyme-linked immunosorbent assay (ELISA) kits. The oxidative stress index (OSI) was obtained by mathematical calculation (OSI = TOS / TAS).

Urine Analyses

Urine volume, pH, creatinine, total protein, calcium, and leukocyte count, and the presence of CaOx crystals were evaluated from the 24-hour urine samples collected on days 7, 14, and 28 using an autoanalyzer (Dirui, H800).

Tissue Analyses

After making protein measurements of homogenized kidney tissues, commercial rat TNFα, IL-1β, IL-6, TOS, and TAS ELISA kits were measured photometrically, and the results per mg protein were recorded. OSI was found by mathematical calculation (OSI = TOS / TAS).

Histopathological Examination

Kidney specimens were divided into two at the hilus plane through a transverse coronal complete incision. After 24 hours of 10% buffered formaldehyde fixation, they were taken into routine pathology tissue processing. The tissues were dehydrated with increasing alcohol levels and finally cleared with xylene. After processing, the tissues of both kidney halves were embedded in paraffin blocks. For a routine hematoxylin examination, both anterior and posterior sections were taken using four micrometer-thick sections, as two sections per slide. CaOx crystals were determined as transparent crystals in the renal tubular and collecting system lumens and examined under polarized light. Each tissue pair was examined for the crystals' density, size, and localization (cortical or medullar). The number and density of CaOx crystals were counted, separately for the cortex and the medulla, based on observation birefringence under polarized light in five adjacent high-magnification fields where these crystals were most dense (Figure 2).

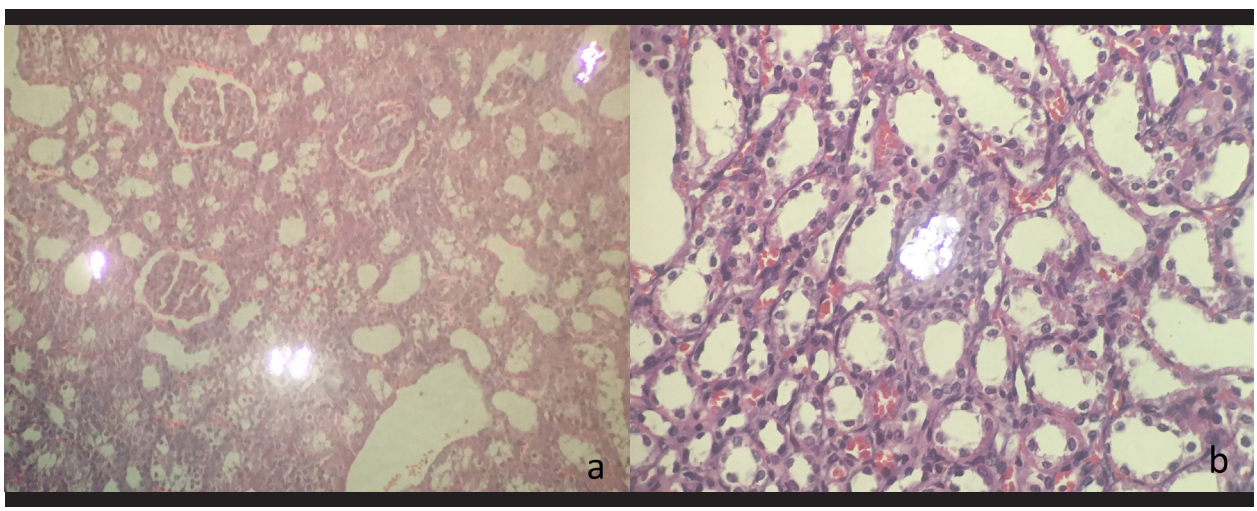

Figure 2. Histopathological examination of the kidney: the appearance of CaOx crystals a) in the cortex and b) in the medulla

Statistical Analysis

Categorical data were expressed as numbers and rates. Data for continuous variables were shown using mean and standard deviation values. The normality of the distributions for continuous variables was determined using the Shapiro-Wilk test. The comparison of mean values between the two groups was undertaken with the independent-samples t-test for normally distributed data and the Mann-Whitney U test for the data without normal distribution. The frequencies of categorical variables were compared using the Pearson chi-square test.

RESULTS

Serum Biochemical Parameters

Serum urea, creatinine, sodium, and potassium values were found to be statistically significantly higher in Group 2 (EG) than in Group 1 (control) on days 7, 14, and 28. It was determined that these values improved in Groups 3-5 (EG + low-, medium-, and high-dose VO, respectively) compared to Group 2. This improvement was not statistically significant only for the potassium value measured in Group 3. The creatinine and sodium values in Group 5 approached the level of Group 1, with no statistically significant difference found between these two groups. The data on serum biochemical parameters are shown in Table 1.

Urine Parameters

There was no statistically significant difference between the groups in terms of pH and urine volume measured from 24-hour urine samples taken on days 7, 14, and 28. The creatinine, calcium, total protein, and leukocyte values were statistically significantly higher in Group 2 than in Group 1. These values were determined to improve in Groups 3-5 compared to Group 2. Urine CaOx crystals were observed in all rats in Group 2 on days 7, 14, and 28, while they were present in all rats in Groups 3-5 only on day 7, with the percentage of CaOx crystals being statistically significantly lower in Groups 3-5 than in Group 2 on days 14 and 28. The data on 24-hour urine parameters are given in Table 2.

Table 1. Comparison of serum biochemical parameters

	Urea (mg/dL)	Creatinine (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)
Group 1 (control)				
Day 7	26.9 ± 4.2	0.5 ± 0.06	139.7 ± 7	4.9 ± 0.5
Day 14	27.5 ± 2.8	0.6 ± 0.06	139.8 ± 5	5 ± 0.4
Day 28	26.2 ± 1.3	0.6 ± 0.04	141.2 ± 6.3	5.1 ± 0.4
Group 2 (EG)				
Day 7	57.6 ± 3.7**	0.7 ± 0.07**	178.4 ± 6.8**	7.3 ± 0.1**
Day 14	61.2 ± 3.6**	0.7 ± 0.06**	186.8 ± 7.5**	7.5 ± 0.2**
Day 28	65.8 ± 4.9**	0.7 ± 0.06**	191.8 ± 6.4**	7.8 ± 0.2**
Group 3 (EG + 50 mg/kg VO)				
Day 7	51.4 ± 5**,++	0.6 ± 0.05*,+	168.3 ± 9.8**,+	7 ± 0.4**
Day 14	55.1 ± 4**,++	0.7 ± 0.04**,+	176 ± 10.4**,+	7.4 ± 0.5**
Day 28	59.6 ± 3.6**,++	0.7 ± 0.05**,+	183.1 ± 8.9**,+	7.6 ± 0.4**
Group 4 (EG + 100 mg/kg VO)				
Day 7	46 ± 4.2**,++	0.6 ± 0.05++	158.8 ± 12.2**,++	6.4 ± 0.1**,++
Day 14	49.7 ± 3.1**,++	0.6 ± 0.05++	163.6 ± 12.2**,++	6.6 ± 0.2**,++
Day 28	52.6 ± 3**,++	0.6 ± 0.05*,++	168.4 ± 18.3**,++	6.8 ± 0.1**,++
Group 5 (EG + 200 mg/kg VO)				
Day 7	42.2 ± 2.7**,++	0.5 ± 0.02++	143.5 ± 15.2++	6.1 ± 0.1**,++
Day 14	45.2 ± 2.7**,++	0.6 ± 0.03++	148.3 ± 14.8++	6.6 ± 0.2**,++
Day 28	47.9 ± 2.3**,++	0.6 ± 0.04++	151.1 ± 14.3++	6.7 ± 0.2**,++

Significant difference compared to Group 1: *p < 0.05, **p < 0.01
Significant difference compared to Group 2: +p < 0.05, ++p < 0.01
EG: ethylene glycol, VO: *Viburnum opulus L.*

Table 2. Comparison of urine parameters

	Creatinine	Total protein	Calcium	Leukocyte	CaOx
	mg/day	mg/day	mg/day	cells/μL	%
Group 1 (control)					
Day 7	1241.6 ± 131	140.5 ± 17.2	161,9 ± 36,5	0±0	0 (0)
Day 14	1259.7 ± 121.4	150.2 ± 11.5	171 ± 35,5	0±0	0 (0)
Day 28	1231.1 ± 93.5	152.2 ± 10.3	169,3 ± 34,4	0±0	0 (0)
Group 2 (EG)					
Day 7	2147.2 ± 245.3**	241.4 ± 13.7**	254,9 ± 43,6**	106.6 ± 27.5**	9 (100)**
Day 14	2444.3 ± 273.3**	272.1 ± 17.4**	286,8 ± 39,6**	125 ± 0**	9 (100)**
Day 28	2754.1 ± 120.9**	312.1 ± 14.6**	341,3 ± 23,6**	125 ± 0**	9 (100)**
Group 3 (EG + 50 mg/kg VO)					
Day 7	1899.2 ± 137.8**,+	236.8 ± 15.4**	244,5 ± 17,7**	86.5 ± 26.5**	10 (100)**
Day 14	2159.5 ± 168.5**,+	242.32 ± 19.8**,++	265,2 ± 25,4**	92 ± 28.4**,++	5 (50)+
Day 28	2307.9 ± 151**,++	247.9 ± 20.4**,++	270,2 ± 22,9**,++	97.5 ± 28.9**,+	4 (40)*,++
Group 4 (EG + 100 mg/kg VO)					
Day 7	1684.5 ± 175.8**,++	228 ± 15.2**	241,3 ± 34,3**	81 ± 23.1**,+	10 (100)**
Day 14	1906.7 ± 161.2**,++	235.9 ± 7**,++	253,5 ± 47,6**	86.5 ± 26.5**,++	2 (20)++
Day 28	2080.2 ± 109.7**,++	241.1 ± 8.5**,++	266,2 ± 47,3**,++	92 ± 28.4**,++	3 (30)++
Group 5 (EG + 200 mg/kg VO)					
Day 7	1633.9 ± 149.4**,++	215 ± 9.9**,++	225,3 ± 16**	70 ± 0.0**,++	10 (100)**
Day 14	1724.8 ± 168.3**,++	219 ± 9.4**,++	243,8 ± 16,4**,+	81 ± 23.1**,++	5 (50)**,+
Day 28	1860 ± 106.4**,++	222.1 ± 13.3**,++	255,4 ± 25,6**,++	81 ± 23.1**,++	2 (20)++

Significant difference compared to Group 1: *p < 0.05, **p < 0.01
Significant difference compared to Group 2: +p < 0.05, ++p < 0.01
EG: ethylene glycol, VO: *Viburnum opulus L.*,CaOx: calcium oxalate

Oxidative Stress Parameters

On days 7, 14, and 28, the serum TOS and OSI values were statistically significantly higher, and the TAS, total thiol and native thiol values were statistically significantly lower in Group 2 than in Group 1. It was determined that the TOS and OSI values of Groups 3-5 statistically significantly decreased compared to those of Group 2. Statistically significantly higher TAS values were detected in Groups 3 and 4 on days 14 and 28 and in Groups 5 on days 7, 14, and 28. Although the total thiol and native thiol values increased in Groups 3-5 compared to Group 2, statistically significantly higher levels were found only in Group 5 on days 7, 14, and 28. Table 3 presents the serum values of oxidative stress parameters.

On day 28, the tissue TOS and OSI values were statistically significantly higher, and the TAS value was statistically significantly lower in Group 2 than in Group 1. Although an improvement in these values was observed in Group 3 compared to Group 2, there was no statistically significant difference. However, in Groups 4 and 5, the TOS and OSI values were statistically significantly lower, and the TAS value was statistically significantly higher when compared to Group 2. The values of oxidative stress parameters evaluated in kidney tissue are given in Table 4.

Table 3. Comparison of serum oxidative stress parameters

	TOS	TAS	OSI	Total thiol	Native thiol
	(μmol H_2O_2/L)	(mM AAE)	(AU)	(μM)	(μM)
Group 1 (control)					
Day 7	8.8 ± 2.2	1.2 ± 0.1	7.3 ± 1.8	500.7 ± 0.8	469.5 ± 84.4
Day 14	8.1 ± 2	1.2 ± 0.1	6.7 ± 1.8	499.4 ± 79.2	461.4 ± 79.2
Day 28	8.5 ± 1.4	1.1 ± 0.1	7.3 ± 1.9	522.9 ± 59.8	482.9 ± 58.2
Group 2 (EG)					
Day 7	20.5 ± 2.9**	0.4 ± 0.1**	49.4 ± 15.3**	355.4 ± 29.4**	293.5 ± 19.1**
Day 14	23.1 ± 3.5**	0.3 ± 0.09**	71.8 ± 17.8**	260.4 ± 55**	159.8 ± 17.7**
Day 28	25.5 ± 1.5**	0.2 ± 0.05**	101 ± 24.4**	202.4 ± 40.9**	102.2 ± 8.3**
Group 3 (EG + 50 mg/kg VO)					
Day 7	16.3 ± 3.6**,++	0.4 ± 0.10**	34.4 ± 10.7**,+	367.5 ± 70.07**	308.8 ± 60.5**
Day 14	17.5 ± 1.6**,++	0.4 ± 0.06**,+	42.6 ± 7.5**,++	268.4 ± 32.5**	192.5 ± 48.5**
Day 28	18.1 ± 1.4**,++	0.3 ± 0.04**,++	49.9 ± 7.1**,++	234.9 ± 52.2**	183.4 ± 13.6**,++
Group 4 (EG + 100 mg/kg VO)					
Day 7	14.9 ± 2.3**,++	0.5 ± 0.04**	29.1 ± 5.7**,++	400.5 ± 65.8*	355.1 ± 42**,++
Day 14	15.9 ± 1.7**,++	0.4 ± 0.05**,+	35.8 ± 3.6**,++	301.5 ± 87.4**	204 ± 76.1**
Day 28	16.3 ± 2.5**,++	0.3 ± 0.06**,++	44.2 ± 11**,++	300.5 ± 50**,++	195.5 ± 45.9**,++
Group 5 (EG + 200 mg/kg VO)					
Day 7	13.1 ± 1.9**,++	0.5 ± 0.1**,++	23.2 ± 4.6**,++	435.9 ± 75.7++	395.1 ± 94.4++
Day 14	14 ± 1.3**,++	0.5 ± 0.05**,++	26.8 ± 3.7**,++	363.7 ± 96.9**,++	223.4 ± 90.4**,+
Day 28	15.1 ± 1**,++	0.4 ± 0.04**,++	35.8 ± 4.1**,++	340.8 ± 60.1**,++	207.7 ± 45.5**,++

Significant difference compared to Group 1: *p < 0.05, **p < 0.01
Significant difference compared to Group 2: +p < 0.05, ++p < 0.01
EG: ethylene glycol, VO: *Viburnum opulus L.*, TOS: total oxidant status, TAS: total antioxidant status, OSI: oxidative stress index, AAE: ascorbic acid equivalent

Table 4. Comparison of oxidative stress parameters measured in kidney tissue

	TOS	TAS	OSI
	(μmol H_2O_2/L)	(mM AAE)	(AU)
Group 1 (control)	7.2 ± 1.2	0.4 ± 0.08	17.6 ± 3.9
Group 2 (EG)	15.3 ± 2.4**	0.1 ± 0.05**	84.8 ± 23.9**
Group 3 (EG + 50 mg/kg VO)	13.4 ± 2.8**	0.2 ± 0.04**	60.7 ± 9.8**
Group 4 (EG + 100 mg/kg VO)	11 ± 2.8**,++	0.2 ± 0.04**,+	47.8 ± 21.4**,++
Group 5 (EG + 200 mg/kg VO)	8.9 ± 3.3++	0.3 ± 0.06**,++	28.8 ± 11.8*,++

Significant difference compared to Group 1: *p < 0.05, **p < 0.01
Significant difference compared to Group 2: +p < 0.05, ++p < 0.01
EG: ethylene glycol, VO: *Viburnum opulus L.*, TOS: total oxidant status, TAS: total antioxidant status, OSI: oxidative stress index, AAE: ascorbic acid equivalent

Inflammation Parameters

The serum and tissue inflammation parameters are given in Table 5. On days 7, 14, and 28, the serum IL-1β, IL-6, and TNFα values were statistically significantly higher in Group 2 than in Group 1. These values were observed to improve in Groups 3-5 compared to Group 2. This improvement was not statistically significant only for the IL-1β value of Group 3 measured on day 7. On day 28, the tissue IL-1β, IL-6, and TNFα values were statistically significantly higher in Group 2 than in Group 1. It was observed that these values improved in Groups 3-5 compared to Group 2. This improvement was not statistically significant only for the TNFα value of Group 3.

Acute Kidney Injury Parameters

On days 7, 14, and 28, the serum NGAL and cystatin C values were found to be statistically significantly higher in Group 2 than in Group 1. These values decreased in Groups 3-5, being statistically significantly lower than those in Group 2. Table 6 presents the serum values of acute kidney injury parameters.

Table 5. Comparison of serum and tissue inflammation parameters

	IL-1β (pg/mL)	IL-6 (ng/L)	TNFα (ng/L)
Group 1 (control)			
Day 7	206.4 ± 26.6	2.3 ± 0.7	72.4 ± 18.8
Day 14	219.7 ± 36.8	2.2 ± 0.3	71.2 ± 13.4
Day 28	292.9 ± 34.3	2.4 ± 0.6	71.8 ± 10.3
Group 2 (EG)			
Day 7	342.4 ± 37.4**	9.1 ± 1.8**	171.2 ± 10.4**
Day 14	432.1 ± 38.6**	11.6 ± 2.8**	194.3 ± 11**
Day 28	515.3 ± 36.8**	13.7 ± 2.7**	221.8 ± 20.3**
Group 3 (EG + 50 mg/kg VO)			
Day 7	323.2 ± 44.7**	7.4 ± 1**,+	153 ± 16**,+
Day 14	373.7 ± 37.5**,++	9.3 ± 1.3**,++	165.5 ± 15.9**,++
Day 28	421.5 ± 40.1**,++	10.6 ± 1.2**,++	183.2 ± 13.8**,++
Group 4 (EG + 100 mg/kg VO)			
Day 7	293.6 ± 27.7**,++	6.3 ± 0.9**,++	138.7 ± 31**,++
Day 14	332.9 ± 29.1**,++	7.8 ± 0.7**,++	156.7 ± 32.4**,+
Day 28	385.8 ± 41.1**,++	9.1 ± 0.9**,++	176.3 ± 28.7**,++
Group 5 (EG + 200 mg/kg VO)			
Day 7	216.5 ± 30.1++	4.8 ± 0.6**,++	121.5 ± 10.6**,++
Day 14	264.9 ± 30.4**,++	5.6 ± 0.6**,++	135.5 ± 13.1**,++
Day 28	307.9 ± 25.1**,++	6.3 ± 1.5**,++	145.9 ± 13.9**,++
Tissue			
Group 1 (control)	586.6 ± 100	8.3 ± 0.9	353 ± 70.1
Group 2 (EG)	739.3 ± 87.9**	28 ± 3.7**	503.3 ± 76.4**
Group 3 (EG + 50 mg/kg VO)	561.6 ± 87.5++	23.7 ± 2.4**,++	455.3 ± 76.8**
Group 4 (EG + 100 mg/kg VO)	498.3 ± 79.5*,++	20.6 ± 2.1**,++	424.2 ± 86.9**,+
Group 5 (EG + 200 mg/kg VO)	388.4 ± 49.1**,++	16.6 ± 5.2**,++	356 ± 32.8++

Significant difference compared to Group 1: *p < 0.05, **p < 0.01
Significant difference compared to Group 2: +p < 0.05, ++p < 0.01
EG: ethylene glycol, VO: *Viburnum opulus* L., IL: interleukin, TNF: tumor necrosis factor

Table 6. Comparison of serum acute kidney injury parameters

	NGAL (ng/mL)	Cystatin C (ng/mL)
Group 1 (control)		
Day 7	14.7 ± 2.2	5 ±1.2
Day 14	15.6 ± 3.3	7.7 ± 1.1
Day 28	14.6 ± 3.9	8.9 ± 1.2
Group 2 (EG)		
Day 7	69.2 ± 8.7**	36 ± 5.8**
Day 14	75 ± 7.9**	46.2 ± 5.1**
Day 28	79.4 ± 6.9**	51.6 ± 4.3**
Group 3 (EG + 50 mg/kg VO)		
Day 7	62.2 ± 2.1**,+	30.3 ± 3.3**,+
Day 14	65.7 ± 2.4**,++	37.8 ± 3.5**,++
Day 28	68.2 ± 4.6**,++	41.4 ± 2.6**,++
Group 4 (EG + 100 mg/kg VO)		
Day 7	57.2 ± 4.2**,++	26.3 ± 3.3**,++
Day 14	60.3 ± 2.3**,++	32.7 ± 2.6**,++
Day 28	63.2 ± 1.6**,++	35.3 ± 2.3**,++
Group 5 (EG + 200 mg/kg VO)		
Day 7	53.2 ± 5.5**,++	22 ± 3.4**,++
Day 14	56.3 ± 2.6**,++	27 ± 2**,++
Day 28	58.6 ± 3.4**,++	29.2 ± 2.2**,++

Significant difference compared to Group 1: *p < 0.05, **p < 0.01
Significant difference compared to Group 2: +p < 0.05, ++p < 0.01
EG: ethylene glycol, VO: *Viburnum opulus* L., NGAL: neutrophil gelatinase-associated lipocalin

Histopathological Parameters

On day 28, the percentage of crystallization was 0% in Group 1, 66.7% in Group 2, 50% in Group 3, 30% in Group 4, and 20% in Group 5. A statistically significant increase was found in the mean number of crystals in Group 2 compared to Group 1. The mean number of crystals was found to decrease in Groups 3-5 compared to Group 2, and the total number of crystals in Groups 4 and 5 was statistically significantly lower than in Group 2.

DISCUSSION

In experimental studies, CaOx kidney stones are formed in rats using various agents, such as sodium oxalate, ammonium oxalate, hydroxy-L-proline, EG, and glycolic acid, which are often combined with vitamin D, a magnesium-poor diet, or ammonium chloride. Applying approximately 0.75% EG to rats for approximately 12 days results in persistent crystalluria, and the application of approximately three weeks of this agent results in kidney crystallization (9). In the current study, CaOx crystals were present in the urine samples of all rats in Group 2 (EG) on days 7, 14, and 28, and this was statistically significantly higher than in Group 1. In addition, CaOx crystal formation was observed in 66.7% (6/9) of the rats in Group 2 on day 28. The mean number of crystals was statistically significantly higher in Group 2 than in Group 1.

The Viburnum genus, belonging to the Caprifoliaceae family, includes more than 230 species spread from South America to Southeast Asia, with most being endemic (10). VO, commonly known as the European cranberry bush, has red and oval fruit. It ripens in August-September and remains throughout the winter. The fruit is rarely used as food due to its bitter taste (11) but it is utilized in natural remedies for various diseases, such as circulatory, respiratory, digestive, and urinary system disorders (12). It has been shown that VO contains high amounts of total phenolics, ascorbic acid, flavonoids, and anthocyanins and has antioxidant activity (7,13). Prior to the experiment, we also evaluated the antioxidant profiles of VO extracts photometrically based on total phenol, total flavonoid, total antioxidant levels and CUPRAC. We found that these extracts had sufficient antioxidant activity.

The use of VO for stone removal in Turkish traditional medicine has paved the way for clinical studies. Tuglu et al. stated that VO could be substituted for potassium citrate in patients with mild or moderate hypocitraturic stones (14). Kızılay et al. found that VO facilitated the removal of stones smaller than 10 mm (15). In an animal study investigating the effects of different extracts of VO fruit on urolithiasis, İlhan et al. found that lyophilized VO juice had a preventive effect in rats with sodium oxalate-induced urolithiasis (16). In the current study, urinary CaOx crystals were found to be statistically significantly lower in Groups 3-5 (EG + low-, medium-, and high-dose VO, respectively) on days 14 and 28 compared to Group 2. Although the mean number of crystals in kidney tissue was lower in Group 3 than in Group 2, there was no statistically significant difference between the two groups. However, statistically significant differences were observed in comparing Groups 4 and 5 with Group 2. The main difference is our study from İlhan et al.'s study that (16) our evaluation of the effects of different doses of VO. We determined that the curative effect of VO on oxidative stress, inflammation, and acute kidney injury increased with increasing doses. Concerning crystallization, more improvement was observed in Groups 4 and 5 than in Group 3.

Modern medical treatments to prevent the formation of kidney stones have centered on preventing supersaturation (17). However, although supersaturation is required to initiate this process, it does not always lead to the formation of CaOx stones (18,19). In many individuals, crystal aggregation and retention do not occur as a result of supersaturation, and crystals are excreted through urine before stone formation. In other words, renal cells respond to increased supersaturation. This response can be physiological or pathological. During this process, crystallization inhibitors play a crucial role in preventing the formation of stones, and damage to inhibitor-forming cells may lead to insufficient or ineffective inhibitor production. Free oxygen radicals seem to be responsible for damage to these cells; therefore, neutralization of free oxygen radicals and inhibition of oxidative stress can prevent urinary stone formation (18). In our study, we aimed to neutralize oxidative stress with VO. Consistent with similar studies, we detected oxidative stress most in Group 2, which was given EG, and observed that the VO used in Groups 3-5 improved oxidative stress in direct proportion to the application dose. Although oxidative stress parameters evaluated in kidney tissue showed an improvement in Group 3 compared to Group 2 on day 28, no statistically significant difference was found. However, there was a statistically significant difference between Groups 2 and Groups 4 and 5.

Human, animal, and cell culture studies have clearly revealed the relationship between CaOx accumulation and renal epithelial damage (20–23). Baggio et al. reported that renal enzymes, such as gamma-glutamyl-transpeptidase, angiotensin 1-converting enzyme, β-galactosidase, and N-acetyl-β-glucosaminidase, which indicate renal cell damage, were higher than normal in the urine samples of patients with idiopathic CaOx stones (21). Boonla et al. found that 8-hydroxydeoxyguanosine, which is used as a marker of oxidative DNA damage, was higher in patients with nephrolithiasis than in healthy individuals (22). Zuo et al. determined that the renal

and urinary excretion of kidney injury molecule-1, an essential marker of renal damage, was significantly increased in rats with hydroxy-1-proline-induced hyperoxaluria (23). Similarly, in our study, we found that the creatinine and total protein values measured from urine samples on days 7, 14, and 28 were higher in Group 2 than in Group 1, while these values indicated an improvement in Group 3-5. In addition, according to our evaluation of serum cystatin C and NGAL, which are important biomarkers of acute kidney injury (24), the values of these parameters on days 7, 14, and 28 indicated a statistically significant increase in Group 2 when compared to Group 1. There was a statistically significant improvement in Groups 3-5.

Human, animal, and cell culture studies also indicate that urinary stone formation elicits an inflammatory response (23,25–27). Boonla et al. found that low-grade inflammation occurred in patients with nephrolithiasis. In addition, the authors noted that the mRNA expressions of monocyte chemoattractant protein-1 and IL-6 were significantly higher in those with nephrolithiasis presenting with impaired renal function, which they attributed to renal damage (26). Mushtaq et al. detected increased excretion of anti-inflammatory proteins, such as anti-calgranulin, α-defensin, and myeloperoxidase, produced by neutrophils in response to inflammation in the urine samples of patients with stones (27). In the current study, the highest values of inflammation parameters were observed in Group 2, and VO improved inflammation in direct proportion to the dose applied. In contrast, Altun et al. investigated the anti-inflammatory activity of VO at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg and found that VO did not show anti-inflammatory activity at these doses (28). In our study, VO may have shown an indirect anti-inflammatory effect by improving oxidative stress and renal epithelial damage.

This study has certain limitations. First, although most idiopathic stones are formed by binding to subepithelial calcium phosphate deposits on renal papillary surfaces, called Randall's plaques (29), none of the models developed to elicit kidney stone pathogenesis are identical to the idiopathic stone formation process or provide the formation of stones that attach to Randall's plaques on the papillary surface. Instead, the crystals that form are intraluminal and resemble Randall's plugs (30). Second, our findings were not supported by immunohistochemical methods. The lower number of CaOx crystals than expected in light of similar previous studies constitutes one of the limitations of the study. Despite these limitations, our study is valuable since it is, to the best of our knowledge, the first to test the effects of different doses of VO on nephrolithiasis and evaluate oxidative stress, acute kidney injury, and inflammation, which are three essential factors in the pathogenesis of stone formation.

CONCLUSION

VO antioxidant activity can reduce CaOx crystallization and stone formation by improving oxidative stress, acute kidney injury, and inflammation in rat kidneys with EG-induced nephrolithiasis, and this effect is proportional to the dose of VO. These findings must be supported by human studies to produce more credible results.

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