The antiurolithic activity of *Origanum vulgare* on rats treated with ethylene glycol and ammonium chloride: Possible pharmaco-biochemical and ultrastructure effects

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**Abstract**

**Background:** *Origanum vulgare* (OV) Linn is one of the conventional remedies for urolithiasis. Hence, we tested the potential antiurolithic effect of OV active extract, in order to rationalize its medicinal use.

**Materials and methods:** The in vivo study was of male Westar rats receiving lithogenic treatment consisting of two 0.75% ethylene glycol injections with a 1 day interval and then in drinking water given for 3 weeks along with ammonium chloride (NH₄Cl) from the 2nd day to the 7th day.

**Results:** The active ethanolic extract of OV treatment (20 mg/kg) reversed toxic changes including loss of body weight gain and appetite, raised serum urea and creatinine levels, and raised blood pressure compared to controls.

**Conclusions:** The acquired data thus suggested that OV showed antiurolithic effects against renal calcium oxalate crystal deposits by combined mechanisms acting on multiple sites through hypoxaliuric, hypocalciuric, and antioxidant effects.

**Keywords:** Ammonium chloride; Ethylene glycol; *Origanum vulgare*; Pharmacobiochemical effects

1. Introduction

Oregano is light green with a strong aromatic odor. Oregano with a pleasantly bitter taste goes well with tomato sauces, pizza, and vegetables.[1] Some researches showed that oregano improves growth in growth-retarded animals and has nonspecific immunostimulatory effects on porcine immune cells.[2] *Origanum vulgare* (*OV*) is a member of the Lamiaceae family, and is known as Wild Marjoram or Winter Sweet, and Mirzaniosh in Pakistan.[3] It is found in Asia, North America, and Europe.[4] The major use of OV is in diuretic, lithotriptic, and antispasmodic traditional medicines. It is also used as a stimulant, antibacterial, anti-inflammatory, expectorant, anticancer, laxative, and antioxidant.[5]

The study of urinary stones, urolithiasis, is ancient. Archaeologists found many samples that contain evidence that humans have been suffering for centuries from bladder stones, and even mummies have shown bladder stones.[6] It is a general problem of the urinary tract through a lifespan hazard of 2%–5% in Asia, 8%–15% in America and Europe, and about 20% in the Middle East. With about 10%–23% yearly, it has a great return ratio of 50% in 5–10 years and 75% in 20 years. Once you get it, its relapse rate is raised and the recurrence interval is lessened.[7]

Its occurrence is increasing and the rate of onset is decreasing, which may be because of new lifestyles, climate, and diet.[8] Modern methods developed for better therapy of urinary bladder stones such as ureterostomy, and percutaneous nephrolithotomy have improved training but bladder stone reappearance still has not dropped. Extracorporeal shock-wave lithotripsy is more effective for calcium oxalate (*CaOx*) dehydrate and uric acid stones than for *CaOx* monohydrate and cystine stones.[9] With high cost and a high chance of recurrence, these methods result in side effects such as renal injury, pancreatitis, extracorporeal shock-wave lithotripsy persuasive hypertension, severe hematuria, renal damage, steinstrasse (several little stone obstructing the ureter), dirt, and remaining wreckages for a possible new stone realization. Large perfusion of the collecting system may also occur as a complication, in addition to ureteral injury, urosepsis, and extravasations of urine and late flow.[10]

A narrow choice of pharmacological agents includes citrate and thiazide diuretics, with limited efficacy and low tolerability. As there is small choice of pharmacotherapy, an interest in herbal medicine has been increasingly developed for the treatment of urolithiasis. Herbal remedies include a number of constituents working in a number of different pathways to produce effects required for urolithiasis, such as diuretic, antispasmodic, and pain release.[11]

However, we aimed to elucidate the antiurolithic activity of OV extracts potentially mediated via inhibition of CaOx precipitation, antioxidant effects, renal epithelial cell protection,
and antispasmodic activities, in order to rationalize its medicinal use in urolithiasis.

2. Materials and methods

2.1. Animals and experimental protocol

Four-month-old male albino rats procured from the animal house from Umm Al Qura University, College of Medicine weighing 150–250g were used in the initial ages of the study. Thirty male adult Wistar rats were used in the following research. These rats were taken care of according the requirements for the upkeep and use of trial creatures of Umm Al-Qura University, KSA, and the trials were approved following the guidance from Control and Supervision of Experiments on Animals (CPCSEA) and ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. Rats were fed according to Food (#5322) Purina Expert Rascal Measure ad libitum, and were individually caged in stainless steel cages with wire-mesh floors. Due to being on food restriction while traveling, rats were gradually fed and ad libitum feeding was completed to stop them from overindulging, which may cause mucoid enteropathy. For the entire study period (3 weeks), rats were housed at 22–25°C temperature on a 12:12-h dark–light cycle and fed on bit food and running water ad libitum. The rat’s weight and food consumption were noted at regular intervals during the study (1 day for nourishment ingestion and 7 days for mass). The total number of rats included in our study were 30, classified into 2 main groups; Group 1 (6 rats) and Group 2 (24 rats). Later, group 2 were divided into 4 subgroups (6 rats for each). OV different extracts were applied only on b,c,d subgroups. The following parameters were noted for all the groups at the start and end: blood compression, body weight, and serum concentrations of creatinine.

2.2. Plant extracts preparation

Leaves of OV were purchased from Sekam (Cairo, Egypt) and identified by a taxonomist. The leaves were cleaned of adulterants and soaked for three days in aqueous-ethanol (30:70) with occasional shaking at room temperature. Filtration was carried out with muslin cloth and then it passed through filter paper of Whatman qualitative grade 1. This process occurred two times and after this procedure all the filtrates that were obtained were mixed and concentrated on a rotary evaporator equipped with a B-700 recirculation chiller and a 461-model water bath at a temperature 65°C under a vacuum. Then a thick pasty mass was obtained from the extract known as the crude extract (OV). The yield was 12% that was reconstituted in distilled water and was used in the later research.

2.3. Phytochemical screening

OV was tested to obtain altered phytochemical collections such as saponins, alkaloids, coumarins, sterols, terpenes, tannins, and flavonoids by employing different extraction approaches such as: A. Ethanolic, B. Aqueous, C. Ethyl acetate, and D: Hexane.[12]

2.4. Study of the classical animal model of urolithiasis

All the animals were divided according to body weight of 5 animals each in 10 groups. Antiurolithic was studied with the help of classical CaOx urolithiasis. The renal CaOx deposit induction was attained by supplying them with 0.75% ethylene glycol (EG) two doses in a day, having a break of 1 day, and after by mixing it in the drinking water for 21 days with the addition of ammonium chloride (NH₄Cl) from the 2nd to the 7th day.[13]

2.5. Measurement of blood stress and heart rate

From the start to end, the animal’s blood pressure (mm Hg) and heart rate were recorded with the help of the tail-cuff plethysmographic noninvasive method. Blood pressure (mm Hg) and heart rates were noted several times for every rat and four heart readings and blood pressure medians where the difference was 10mmHg, were averaged. The four median reading average was used as mean blood pressure (mm Hg) and heart rate.

2.6. Design of the work

For 21 days, the normal negative control group 1 (6 rats), oral nourishment with usual salt via a belly tube (5 mL/kg). The rest of the groups received two 0.75% EG injections, with 1 day break, then 0.75% EG and 1% ammonium chloride for 2–7 days. Animals in group 2 (24 rats) were matched and divided into 4 subgroups:

(a) The control positive (untreated) group was fed orally EG (0.75%) by stomach tube (1 mL/kg) for 14 days.
(b) The treated group of extract A was fed orally EG (0.75%) for 14 days and also extract A in a dose of 20 mg/kg (5 mL/kg).
(c) The treated group of extract B was fed EG (0.75%) for 14 days and also extract B in a dose of 20 mg/kg (5 mL/kg).
(d) The treated group of extract C was fed EG (0.75%) for 14 days and extract C in a dose of 10 mg/kg (5 mL/kg).

At the end of the research (21 days), rats were allowed to fast overnight for chemical study of their urine and serum. Rats who appeared lethargic or those who lost a considerable amount of mass were omitted from the study, whereas the remaining rats were continuously monitored for weight and activity throughout the study. Their water intake was noted during 24h periods and urine samples were taken at the end of 21 days treatment. Under the influence of anesthesia blood samples from their hepatic portal veins were collected in dry centrifuge tubes to assess their blood picture and serum separation to determine serum creatinine and blood urea nitrogen. The blood obtained was centrifuged for 10 min at 3000 rpm for serum separation, at 18°C for analysis. Organs were then obtained, washed with saline, dried with filter paper, weighed, and then stored in a freezer for further analysis.

2.7. Chemical and biochemical analysis

2.7.1. Determination of moisture and ash. Moisture, protein content, the total nitrogen, fat, and ash contents were determined according to methods described by the A.O.A.C.[14] Carbohydrate were estimated by difference as follows: carbohydrate = 100- (% protein +% fat +% moisture +% ash).

2.7.2. Blood biochemistry estimation. At the start of the study blood samples from tail veins were collected. After 3 weeks, at the end of the research, from the vena cava after sacrifice and anesthesia in nonheparinized microtubes. Then these samples were centrifuged to obtain their sera and then sera samples were divided into different microtubes for analysis of blood chemistry using the ARCHITECT c4000 clinical chemistry autoanalyzer. Enzymatic colorimetric assay kits from Bio Vision, Inc., CA were used to estimate serum blood levels of glucose, total cholesterol, triglycerides, HDL-cholesterol, creatinine, urea, and uric acid. The VLDL and LDL determination were carried out according to the Lee and Nieman method, as follows: VLDL (mg/dL) = Triglycerides/5, LDL (mg/dL)= Total cholesterol – HDL – VLDL.[15]
2.7.3. Lecithin-heparin. For the procedure of the complete blood count, the samples were divided into different microtubes. By using an hemocytometer, the number of crystals per mm³ was calculated under a light microscope.

2.8. Data investigation
Data are expressed as mean±standard error (SEM) and the median effective concentration (EC₅₀ value) with 95% confidence intervals. All statistical evaluations among the groups were made by the student's t-test using SPSS version 22 software package. Cyclic Redundancy Checks were analyzed by GraphPad Software.

3. Results
For 14 consecutive days the initial and final body weight of treated rats and normal control rats with (untreated) urolithiasis were recorded, and then they were treated with various extracts of OV. The results are shown as mean±SE and summarized in Table 1. P value equals to <0.05 and NS value equals to nonsignificant contrasted with initial values according to the Student t-test. Body weight gain for the Hexane treatments showed a mild significant increase as compared with the positive control (p<0.05).

During the 2 weeks, the initial and final systolic and diastolic blood pressure of treated rats and normal control rats with untreated urolithiasis were recorded, and then they were treated or not treated with OV (20mg/kg/day), as shown in Tables 2 and 3, respectively.

The initial and final heart rate and complete blood count of treated rats and normal control rats with untreated urolithiasis, and then they were treated or not treated with OV (20mg/kg/day) are presented in Tables 4 and 5, respectively.

The current study disclosed the growth performance in terms of food intake and food efficiency. It is note-worthy that food intake of all treatments showed no-significant difference as compared with the negative control. Rats that received ethanolic extract of OV had higher values of food intake, whereas rats of the positive control had the lowest values of food intake 30.04±1.42 and 26.86±1.34/day, respectively. In addition, the food efficiency ratio showed a significant difference (p<0.01) among rats that received ethanolic extract of OV as shown in Table 6.

### Table 1
The initial and final body weight of rats during the 2 weeks of study.

| Group            | Negative mean ± SD | Positive mean ± SD | Ethanol mean ± SD | Aqueous mean ± SD | Hexane mean ± SD |
|------------------|--------------------|--------------------|-------------------|-------------------|------------------|
| Initial weight   | 234±16.67          | 234±13.30          | 209±13.93         | 223±24.50         | 193±11.38        |
| End of 1st week  | 252±17.29          | 240±14.05          | 212±13.51         | 231±24.00         | 198±0.92         |
| End of 2nd week  | 266±16.63          | 242±14.05          | 217±13.96         | 241±25.73         | 204±9.83         |
| Final weight     | 282±17.64          | 244±13.90          | 228±13.70         | 249±26.09         | 210±1.61         |

SD = standard deviation.
* Differences are significant at p≤0.05.

### Table 2
The initial and final systolic pressure of rats during the 2 weeks of study.

| Group            | Negative mean ± SD | Positive mean ± SD | Ethanol mean ± SD | Aqueous mean ± SD | Hexane mean ± SD |
|------------------|--------------------|--------------------|-------------------|-------------------|------------------|
| Initial systolic | 120±1.51           | 124±2.50           | 121±3.51          | 105±4.06          | 125±3.04         |
| End of 1st week  | 123±1.26***        | 157±1.18           | 157±1.25***       | 160±4.15          | 161±3.65         |
| End of 2nd week  | 122±1.67***        | 173±4.88           | 145±2.47***       | 150±3.9***        | 145±4.22***      |
| Final systolic   | 125±1.70***        | 181±5.39           | 134±4.39***       | 142±5.51***       | 127±4.09***      |

SD = standard deviation.
*** Differences are highly significant at p<0.01.
** Differences are very highly significant at p<0.01.

### Table 3
The initial and final diastolic pressure of rats during the 2 weeks of study.

| Group            | Negative mean ± SD | Positive mean ± SD | Ethanol mean ± SD | Aqueous mean ± SD | Hexane mean ± SD |
|------------------|--------------------|--------------------|-------------------|-------------------|------------------|
| Diastolic        | 76.00±1.39***      | 78.17±2.10         | 86.5±2.2***       | 69.7±2.6*         | 82.59±2.00       |
| End of 1st week  | 76.59±0.92***      | 102.8±4.21         | 112.7±5.3         | 108±2.92          | 117.5±2.94***    |
| End of 2nd week  | 76.83±2.1***       | 109.0±3.81         | 101.8±4.4         | 104±2.62          | 105.35±4.43      |
| Final diastolic  | 77.3±1.63***       | 123.0±7.47         | 91.8±2.4***       | 99.2±2.2***       | 93.17±3.43***    |

SD = standard deviation.
* Differences are significant at p≤0.05.
** Differences are highly significant at p<0.01.
*** Differences are very highly significant at p<0.001.
Final heart rate 3.91 ± 0.53
End of 1st week 3.74 ± 0.75
End of 2nd week 4.04 ± 0.63
Final heart rate 3.91 ± 0.32

Food ef M% 1.3 ± 0.04
Blood cells; SD

Group Negative mean ± SD Positive mean ± SD Ethanol mean ± SD Aqueous mean ± SD Hexane mean ± SD

| Group            | Initial heart rate | End of 1st week | End of 2nd week | Final heart rate |
|------------------|--------------------|-----------------|-----------------|-----------------|
|                  | 3.65 ± 0.39        | 3.93 ± 0.25     | 3.61 ± 0.79     | 3.61 ± 1.14     |
|                  | 3.74 ± 0.40        | 3.61 ± 0.63     | 3.51 ± 1.01     | 3.51 ± 1.43     |
|                  | 4.02 ± 0.85        | 3.88 ± 1.26     | 3.32 ± 1.03     | 3.32 ± 1.27     |
|                  | 3.91 ± 0.32        | 3.82 ± 1.58     | 3.64 ± 2.04     | 3.56 ± 1.96     |

SD = standard deviation.

Differences are significant at p < 0.05.

Differences are highly significant at p < 0.01.

Differences are very highly significant at p < 0.001.

| Group            | Mean ± SD Ethylene glycol | Mean ± SD Ethanol | Mean ± SD Aqueous | Mean ± SD Hexane |
|------------------|---------------------------|-------------------|-------------------|-----------------|
| Monocytes        | 0.32 ± 0.15               | 0.59 ± 0.27       | 0.05 ± 0.04       | 2.69 ± 1.7      |
| Lymph/μL         | 75.4 ± 6.69               | 51.89 ± 9.22      | 73.2 ± 2.43       | 63.86 ± 0.98    |
| MNL μL           | 1.3 ± 0.72                | 8.90 ± 1.63       | 1.63 ± 0.61       | 7.83 ± 2.29     |
| Neutrophils/μL   | 19.08 ± 3.86              | 51.88 ± 7.23      | 21.52 ± 1.27     | 28.1 ± 6.41    |
| RBCs μL/μL       | 8.22 ± 0.65               | 7.83 ± 0.77       | 8.89 ± 0.37       | 7.67 ± 1.36     |
| Hb μL/μL         | 15.67 ± 0.616             | 14.03 ± 0.36      | 14.02 ± 0.58      | 14.98 ± 0.51    |
| HCT              | 48.98 ± 2.52              | 50.7 ± 1.82       | 48.08 ± 1.76      | 51.23 ± 2.21    |
| MCV μL/μL        | 56.67 ± 1.71              | 46.67 ± 1.46      | 53.83 ± 1.35      | 53.17 ± 1.17    |
| MCH μL/μL        | 19.15 ± 1.13              | 15.42 ± 0.16      | 16.53 ± 0.56      | 16.17 ± 0.18    |
| MCHC μL/μL       | 35.37 ± 1.51              | 29.17 ± 0.21      | 30.27 ± 1.1       | 29.4 ± 0.73     |
| Platelets μL/μL  | 101 ± 1.2                 | 134 ± 97.53       | 109 ± 77.2        | 122 ± 1.3       |

Osc = complete blood count; Hb = hemoglobin; HCT = hematocrit; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cells; SD = standard deviation.

Differences are highly significant at p < 0.01.

Food intake and food efficiency ratio of rats.

| Group            | Control (−) mean ± SD | E. glycol mean ± SD | Ethanolic mean ± SD | Aqueous mean ± SD | Hexane mean ± SD |
|------------------|-----------------------|---------------------|---------------------|-------------------|------------------|
| Food intake      | 32.3 ± 2.3            | 26.86 ± 1.34        | 30.1 ± 1.41**       | 27 ± 3.2          | 28.3 ± 5         |
| Food efficiency ratio | 0.04 ± 0.003         | −0.05 ± 0.002       | −0.03 ± 0.001**     | −0.1 ± 0.005      | 0.1 ± 0.001**    |

SD = standard deviation.

Differences are significant at p < 0.05.

Differences are highly significant at p < 0.01.

Fasting serum uric acid, creatinine, and urea levels for all treated rats were measured and summarized in Table 7. It is clear that serum uric acid, creatinine, and urea in all toxic rats showed a nonsignificant increase as compared with the negative control as shown in Table 7.

Histochemical light microscopic findings include:

(a) Control group: As shown in Figure 1, a transverse and oblique sections of normal renal convoluted tubules with prominent healthy blood vessels.

(b) Untreated group: In contrast to the control group, the lumen of the aorta adsorbed more debris and red blood cells than that of the controls. The surface endothelium was coarse,
wrinkled, and protuberant and lacked an elastic layer. The tunica media was thick, whereas the tunica adventitia was thick and fibrose (Fig. 2).

(c) Treated group (After 3 weeks of treatment): Rats had diminished smooth muscle cell mass, normal lumen diameter and media thickness, and no further alterations in the media/lumen ratio, whereas a part of the aortic wall was regular but the other part was irregular as in Figures 3 and 4.

4. Discussion and conclusion

We investigated whether OV supplementation can prevent EG toxicity and life risk outcomes. The information obtained demonstrated that OV unquestionably forestalls the fatal consequence of EG noxiousness and secures the rat’s aorta, spleen, cerebrum, and bone marrow from its destructive effects. A recent report mentioned that reactive oxygen species (ROS) involved in the development of renal insufi ciency and that OV might block or diminish its lethal damage. [16] Noticeably, excessive influx of polymorphonuclear cells associated with glomerulonephritis tend to release H2O2 that leads to exaggerated ROS production and tissue injury. [17] However, a low level of ROS is an indicator for better mitigation of acute glomerulonephritis. [18]

Considering the possible roles of OV in urolithiasis, we assessed its antiurolithic impact in in vivo rodent models and discovered that CaOx stones might cause tissue injury due to ROS induction. These results were the same as Aihara K et al., and Escobar C et al. findings. [19,20]

The cytoprotective impact of the plant was affirmed when pretreatment of the typical aorta epithelial cells with OV fundamentally prolonged the survival rate. This explains its anti-cancer therapeutic effects. [21] Administration of alpha-adrenoreceptor antagonists or Ca2+ channel blockers improve the rate of removal of these crystals and decrease colic occurrence. [22] Plant extracts may show various multiple therapeutic approaches presumably by virtue of having a blend of phytochemicals. Furthermore, synergistic and/or side-effect neutralizing effect of plants may exist. [1]

The antiurolithic impact of OV was assessed on the most generally applied EG-initiated model for urolithiasis, [23] and male Wistar rodents, which create changes in urine electrolytes and CaOx super-immersion because of their more prominent effect on EG toxicity. [1]

A study by Vanachayangkul et al., revealed that administration of EG prompted extraordinary weight loss and eventually rat death. [24] A further preventive study, explained that administration of both EG and Ammonium Chloride was behind the
augmented crystalluria due to hyperoxaluria, increase of water intake and urine output, and finally renal damage.\cite{Eknoyan2004} Our study showed a critical increment in serum levels of creatinine, blood urea nitrogen, and total protein in the lithogenic group compared to the normal one, which was reversed by the OV treatment. Consistent with past reports, hyperoxaluria increased oxalate and decreased Ca\textsuperscript{2+} discharge in the lithogenic group.\cite{Wiessner2004,Al-Majed2004} Another evidence is that hyperoxaluria, a major risk factor for CaOx-mediated nephrolithiasis through production of superoxide and hydroxyl free radicals that create an oxidative stress environment that leads to disruption of membrane integrity and cell death.\cite{ElSawy2004} Wiessner et al., reported that CaOx crystal adherence resulted in renal tubules retention.\cite{Wiessner2004} This suggest that the inhibitory effect of OV on CaOx deposition comes from its antioxidant activity.

In our study, OV resulted in an immediate recuperation in the treated group compared with the untreated one, which was remarkably manifested by weight gain, significant drop in urinary oxalate, renal crystal deposition, and improvement in renal functions compared to the lithogenic group. Furthermore, phytochemical analysis showed the existence of multiple gradients of saponins, alkaldoids, coumarins, sterol, terpenes, flavonoids, and tannins associated with various activities. Flavonoids are considered to be antispasmodic, Ca\textsuperscript{2+} channel blocker,\cite{Semwal2004} antioxidant,\cite{Al-Ghrani2004} and diuretic.\cite{Saponins2004} Saponins acts as an anti-precipitating agent by preventing either accumulation of mucoproteins or any precipitation promoters.\cite{Saposins2004} Thus, these activities are extraordinary beneficial in addition to their antiuricotoxic activity.

Interestingly, our hematological findings revealed a profound reduction in WBCs, RBCs, and platelets counts with EG overdose with significant reduction in total hemoglobin and hematocrit levels, whereas the monocyte count exceptionally increased. A recent Italian study on mice reached the same conclusion that released toxic adducts have devastating effects on bone marrow DNA.\cite{Usmanghani2004} But, our OV treated rats did not show any of these manifestations. An involuntary convulsion was noticed in rats treated by EG and dead ones, but not in other groups treated by EG and concomitantly treated with OV extracts. This shows the neuroprotective role of OV against EG-induced neuronal injury in rats. This clinical manifestation was further confirmed by our ultrastructural findings. In same way, others documented that among EG toxicity effects there was hepatonecephalopathy and brain damage.\cite{Eknoyan2004,Al-Majed2004,Al-Ghrani2004} Additionally, Al-Majed et al. reported another neuroprotective effect of OV on neuronal injury that causes forebrain ischemia and chronic toluene exposure.\cite{Al-Majed2008}

There is a paucity of data about histopathological abnormalities after using OV extracts on blood vessels of experimental animals. Various reports proposed that vascular endothelial dysfunction associated with EG might elevate lipoprotein levels in the intima media,\cite{Wagner2004} exposure to ROS with persistent aging and hypertension leading to more advanced pathological changes characterized by prominent villi and granular structures.\cite{Wagner2004} Our current study showed significantly raised blood pressure and heart rate in the EG treated group and without any significant changes in both OV treated and control groups, whereas the OV treated group exhibited a more significant subsidence of systolic blood pressure than the other groups as in the Aguila et al. study.\cite{Aguila2004} Systolic blood pressure less than 130 mmHg is a requisite for delayed progression of renal disease in hypertension and metabolic toxicity mentioned by Eddouks et al.\cite{Eddouks2004} This fact was confirmed (1) histopathologically by reduction in size and number of glomeruli, glomeruli basement membrane thickness, and glomerulosclerosis, and (2) serum metabolites including significant rises of creatinine, urea, and uric acid levels in the EG treated group. However, serum creatinine levels increased more in the untreated group than in the EG treated group, suggesting the antioxidant properties of OV.

Our data thus suggests that OV exhibits antiurolithic effects against renal CaOx crystal deposition by combined mechanisms acting on multiple sites through CaOx inhibition, and hypocalciuric and antioxidant effects. Research shows that in hypertensive patients, aortic endothelial cells have subcellular cuts leading to arterial dysfunction. Ultimately, the most interesting finding is that OV supplementation used as a herbal medicine has formidable antihypertensive effects and ameliorates hypertensive-related biochemical alternations and aortic vascular wounds in rats.

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### Statement of ethics

Our animal study was approved for upkeep and usage of trial creatures committee of Umm Al-Qura University, The Kingdom of Saudi Arabia, and the trials following the guidance from Control and Supervision of Experiments on Animals (CPCSEA) and ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

### Conflict of interest statement

No conflict of interest has been declared by the author.

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None.

### Author contributions

NAE contributed to the design and implementation of the research and OFM contributed to the analysis of the results and to the writing of the manuscript.

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