

## Full Length Research Paper

## Antiuro lithic and antihypertensive activities of *Origanum vulgare* on urolithic rats

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*Origanum vulgare* (Oregano) has been used in the fluky medicinal for treating various diseases including urolithiasis and hypertension. This study was designed to investigate the possible antioxidant and antiuro lithic activities of different standardized extracts of Oregano ethanol extract *n*-hexane, ethyl acetate, and aqueous fraction on rats. The dried hydroalcoholic extract of 70% ethyl alcohol Oregano was suspended in water and successively extracted with *n*-hexane and ethyl acetate. Each active extract was screened of phytochemical and standardized spectrophotometrically by estimation of total phenol and total flavonoid content. Antiuro lithic and antioxidant activities were studied on live rat model by oral doses of ethylene glycol and NH<sub>4</sub>Cl. The active extracts of Oregano (20 mg/kg) were given to different groups and one group without extracts was used as control. At the end of the experimental period, blood samples were obtained from studying biochemical parameters and a kidney specimen for histopathology using scanning electron microscope. Ethanol extract, *n*-hexane and aqueous fractions prevented as well as opposed toxic changes, including loss of body weight gain and appetite, raised serum urea, uric acid and creatinine levels, and crystal deposition in the kidneys. These potential urolithic effects of ethylene glycol should be taken into considerations with close monitoring of kidney function tests at frequent intervals. The oregano antioxidant effect might be more effective in the amelioration of ethylene glycol induced kidney injury and urolithiasis.

**Key words:** Anihypertensive, *Origanum vulgare*, urolithiasis, renoprotective, rats.

### INTRODUCTION

Herbs and their active ingredient as sources for new drug discovery and disease treatment have attracted attention in recent years (Butterweck et al., 2009). Their medicinal use has been growing in Arab countries. *Origanum*

*vulgare* is one of the most widely used species of the Lamiacea family and it is a common condiment for various foods and beverages (Kulisic et al., 2004). Oregano is primarily used to treat nausea, but it is also

used as an anti-inflammatory, a pain remedy, a warming remedy and antioxidant herb (Chishti et al., 2013). The main pharmacological effects of *O. vulgaris* are being antioxidant, anti-thrombin and potent antihypertensive through maintaining renal function, creatinine clearance and inhibiting mechanical forces acting on arterial wall so preventing excess hardening and thickening (Goun et al., 2002; Kulisic et al., 2004). Urinary stone formation is one of the early decades known diseases (Atmani et al., 2003). It is one of the most common problems of the urinary tract (Srihari et al., 2008). Oregano is high in antioxidant activity, due to a high content of phenolic acids and flavonoids. It is widely used in the traditional medicine against kidney stones, as a diuretic and antispasmodic. It is also a stimulant, expectorant, antibacterial, anticancer, anti-inflammatory, antioxidant and laxative (Williamson, 2001; Butterweck et al., 2009).

Ethylene glycol has been shown to be toxic to humans. The glycolic acid is the major metabolite of ethylene glycol responsible for renal toxicity that resulted in urolithiasis (Brent, 2001; Divakar et al., 2010; Trasi et al., 2008). This study was designed to study the antihypertensive, renoprotective and antiurolithic effect of different extracts of *O. vulgaris* against ethylene glycol toxicity in experimental rats.

## MATERIALS AND METHODS

### Chemicals and reagents

The following solvents and reagents were purchased from Sigma-Aldrich: ethylene glycol, ammonium chloride, quercetin, gallic acid, folin-ciocalteau reagent, *n*-hexane, ethyl acetate and ethanol 95%.

### Experimental animals

Thirty adult male albino rats, body weight 150 to 250 g were used in the present study. The animals were ensured free from any infection. Rats were housed in stainless steel cages. All animals were weaned and housed at 22 to 25°C, a 12:12-h dark-light cycle (07.00 to 19.00 lights on). Body weights and food consumption were measured (1 day for food consumption and 1 week for weight), following an acclimation period (AOAC, 1994).

### Plant material

*O. vulgare* leaves were bought from Sekam company for medicinal plant (Cairo, Egypt) and authenticated by physical and microscopic examination.

### Preparation of extracts for biological studies

Five hundred grams of leaves of the plant material was cleaned of

foreign material, ground and kept soaked for three days in the aqueous-ethanol (30:70) with occasional shaking, at room temperature. It was covered with aluminum foiled paper and put in a dark place after twice filtration using a muslin cloth. The extract changed to a thick pasty mass called ethanol crude extract (68 g), yielding approximately 13%. The concentrated ethanolic extract (65 g) was then suspended in distilled water (300 ml) and filtered. The water soluble portion was defeated with *n*-hexane (3 × 500 ml), the combined *n*-hexane fraction was concentrated under vacuum at 30°C to dryness (27 g). The defeated fraction was partitioned several times with ethyl acetate (3 × 500 ml). The combined ethyl acetate fraction was concentrated under vacuum at 40°C to dryness (18 g). The remaining aqueous extract was subjected to freeze drying to afford a residue (20 g). One gram of each extract (ethanol extracts, ethyl acetate fraction, and *n*-hexane fraction was dissolved or suspended in 250 ml distilled water separately to give different extracts of concentration of 4 mg/ml.

### Estimation of total phenol content

Total phenolic content of each extract was determined by Folin-Ciocalteau reagent method as described by Singleton and Rossi (1965), using gallic acid as standard.

### Estimation of total flavonoid content

The total flavonoid content was determined using the aluminum chloride calorimetric method as described by Chang et al. (2002) using quercetin as standard.

### Experimental design

Thirty rats were divided into 5 equal groups (each 6 rat). Group 1 received oral feeding of normal saline by stomach tube (5 ml/kg), for 21 days. Groups 2 to 5, were injected twice with one day intervals ethylene glycol (EG; 0.75%) and ammonium chloride (AC; 1%) treatment for 21 days (Bashir and Gilani, 2011). Group 2 is the control positive group and received oral feeding of EG (0.75%) alone. Groups 3 to 5 were treated orally with ethanolic extract, aqueous and *n*-hexane fractions at a dose of 20 mg/kg, body weight, respectively for 21 days.

### Measurement of blood pressure and heart rate

At the beginning and at the end of the study, blood pressure (mmHg) and heart rate were recorded in pre-warming animals using the tail-cuff plethysmographic non-invasive method (Letica LE 5100, pan-lab, Barcelona, Spain).

### Sampling

#### Blood samples

At the end of the experiment, all living rats were anesthetized, abdominal incised and blood was collected from the hepatic portal vein in non-heparinized microtubes used for serum biochemical Analysis.

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### Antioxidant and renal markers analysis

The antioxidant markers, reduced glutathione (GSH), superoxide dismutase (SOD), total antioxidant (TAC), and malondialdehyde (MDA), were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits, Sandwich Immunoassay (Cayman Chemical Co. USA). Plates were read on a computerized automated microplate ELISA reader at 450 nm and a correction wavelength of 550 nm. Creatinine, urea, uric acid, calcium, phosphorous, albumin and total protein were estimated spectrophotometrically (Human Diagnostic Co. Germany) according to the manufacturer's instructions of the enclosed pamphlet (BM Co. Germany, 5010).

### Histological studies

Rats were humanly sacrificed under ether anesthesia and kidney specimens were collected for histopathological studies. Following laparotomy, kidney specimens were divided into two halves, one for scanning electron microscope for ultra-structure examinations according to Fan et al. (1999) and the other part fixed at 10% neutral buffered formalin for light histopathological examination.

### Statistical analysis

The data were analyzed using Statistical Package for Social Science (SPSS) 20 for Window. Data are expressed as the mean  $\pm$  standard error (SE); analysis of variance (ANOVA) were performed by different treated group followed by least significant difference (LSD), and the differences were considered significant at  $P \leq 0.05$ .

## RESULTS

### Morphological appearance

The survival rate in negative control and *O. vulgaris* extracts treated group was 100%; moreover, the *O. vulgaris* extracts ameliorate apathetic and lazy appearance of ethylene glycol toxicity in rats, while the survival rate on ethylene glycol treated group alone as 66.6%.

### Preliminary phytochemical screening

Preliminary phytochemical screening showed that ethanolic extract, carbohydrates and/or glycosides, flavonoids, sterols and/or triterpenes and tannins were detected while alkaloids and saponins were few as a percentage. *N*-hexane fraction, volatile oil non polar (+++) and lipids were present, while carbohydrates and/or glycosides, flavonoids, sterols and/or triterpenes, tannins, alkaloids and saponins were absent in this extract. Ethyl acetate fraction, carbohydrates and/or glycosides, flavonoids aglycones, sterols and/or triterpenes were detected, while tannins, alkaloids and saponins were absent. Aqueous extract, carbohydrates and/or glycosides, flavonoid glycosides and tannins were

detected, while alkaloids, sterols and/or triterpenes and saponins were absent. The phenols and flavonoid content were higher in the ethanol extract when compared with the aqueous extract, *N*-hexane and ethyl acetate fractions (Table 1).

### Body weight

The final body weight in the ethylene glycol treated group and *O. vulgaris* extracts treated group were significantly decreased when compared with the negative control group and non significant differences between all *O. vulgaris* extracts treated group (Table 2).

### Heart rate and blood pressure

The heart rates at the 3rd week post treatment were significantly increased in the ethylene glycol treated group when compared with the control group as well as ethanol and aqueous treated groups with ethylene glycol (Table 3). Systolic blood pressure was significantly increased in ethylene glycol treated group when compared with the control and ethanol treated group (Table 4). Diastolic blood pressure in ethylene treated group was significantly increased when comparison with other treated group as shown in Table 5.

### Oxidative stress and antioxidant

Levels of the antioxidant markers, GSH, TAC and SOD have significantly decreased in the ethylene glycol treated group, while MDA has significantly increased when compared with the negative control group. On the other hand, treatment with *O. vulgaris* ethanol extract caused a significant increase in GSH, SOD and TAC as compared to the positive control group. In the aqueous and *N*-hexane treated groups, GSH and SOD were elevated when compared with positive control group. On the other hand, in the ethanolic treated group, the MDA was non significantly changed, in comparison with the negative control group as displayed in Table 6.

### Renal markers

Table 7 shows the result of the effect of the ethylene glycol and *O. vulgaris* extracts on kidney function of rats. The ethylene glycol treated rats showed a significant increase in blood urea, creatinine, uric acid, and phosphorous, while calcium, albumin and total protein were significantly decreased when comparison with the negative control group. *O. vulgaris* extract has reduced the concentrations of uric acid, phosphorous, creatinine,

**Table 1.** Total phenols and flavonoid content of different extracts of *Origanum vulgare*.

Extract	Phenol content mg/g GAE	Flavonoid content mg/g QE
Ethanol	124.57 ± 0.75	63.08 ± 0.02
N- hexane	12.30 ± 4.36	0.77 ± 0.06
Ethyl acetate	55.19 ± 1.46	16.43 ± 0.11
Aqueous	51.41 ± 3.75	44.62 ± 0.02

**Table 2.** Initial and final body weight (mean±SE) of rats treated with ethylene glycol and *Origanum vulgare* extracts.

Group	Negative	Positive	Ethanol	Aqueous	N-Hexane
Initial weight	234 ± 16.67	234 ± 13.3	229 ± 13.3	222 ± 2.4	223 ± 11.38
1st week	252 ± 17.29	240 ± 14.05	233 ± 13.1	231 ± 2.4	229 ± 0.92
2nd week	266 ± 16.63	242 ± 14.05	237 ± 13.6	241 ± 25.73	235 ± 9.83
3rd week	282 ± 17.64 <sup>a</sup>	244 ± 13.9 <sup>b</sup>	248 ± 13 <sup>b</sup>	249 ± 6.09 <sup>b</sup>	243 ± 2 <sup>b</sup>

The different latter in the same row is significantly different at P<0.05

**Table 3.** Initial and final heart rate (means±SE) of all rats.

Group	Control	Ethylene glycol	Ethanol	Aqueous	N-Hexane
Initial (HR)	345±3.9	353±5.3	341±7.5	351±6.94	347±5.17
1st week	364±4.1	373±7.6	365±7.1	361±4.6	356 ± 9.57
2nd week	359±6.9 <sup>b</sup>	398±8.9 <sup>a</sup>	366±7.5 <sup>b</sup>	382±6.9 <sup>c</sup>	384±9.12 <sup>c</sup>
3rd week	361±8.3 <sup>b</sup>	396 ± 5.8 <sup>a</sup>	365±5.5 <sup>b</sup>	371 ± 6.2 <sup>b</sup>	385± 7.4 <sup>c</sup>

Means in the same row not followed by the same letter differ significantly (P<0.05).

and urea when comparison with the ethylene glycol rats. Also, total protein, albumin and calcium were improved towards negative control group. Moreover, the ethanolic extract is higher in renal protective when comparison with the aqueous and hexane extracts.

### Histopathological examination

Microscopically, kidneys of rat from Group 1 (negative control) showed the normal histology of renal parenchyma (Figures 1 and 7). However, kidney sections from rats in Group 2 (positive control) revealed shrinkage glomerulus with crystals in duct (Figure 2), thickened fibrosed blood vessels, inflammatory infiltrates (Figure 3) and by electron microscope showing disrupted urothelium. Ductal openings are crystalline obstructed. Covering epithelium sloughed at ductal opening (Figure 8), while in ethanol treated groups, light microscope showing apparent normal renal parenchyma with no histopathological changes (Figure 4). Conversely, few proteinaceous casts in the lumen, injury in some renal tubules and granularity of the cytoplasm of some renal

tubules was noticed in kidneys of rat from Groups 4 and 5 (Figures 5 and 6), respectively. The electron microscope showing urothelium has been reconstituted. Ductal openings are again identifiable. The epithelium appears to be hanging through a ductal opening. At some places, urothelium appears to be migrating laterally (Figures 9 to 11).

### DISCUSSION

This study was designed to investigate the effect of standardized *O. vulgare* ethanol, n-hexane and aqueous extracts to prevent the development of ethylene glycol urolithiasis. These extracts were standardized to total phenol and total flavonoid contents (Table 1). Similarly, phenols, flavonoid, and saponin, have been identified in the phytochemical screen of genus *Origanum* (Chishti et al., 2013)

In the current study, animal and cellular assays have revealed that calcium oxalate crystals cause injury to kidney cells caused by crystallization and crystal deposition in the kidney by promoting crystal nucleation,

**Table 4.** Initial and final systolic blood pressure (mean±SE) of all rats.

Group	Control	Ethylene glycol	Ethanol	Aqueous	N-Hexane
Initial systolic	120 ± 4.51	124 ± 2.50	121 ± 3.51	118 ± 4.06	125 ± 3.04
1st week	123 ± 1.26 <sup>b</sup>	157 ± 1.18 <sup>a</sup>	127 ± 1.25 <sup>b</sup>	120 ± 4.15 <sup>b</sup>	121 ± 3.65 <sup>b</sup>
2nd week	122 ± 4.67 <sup>c</sup>	173 ± 4.88 <sup>a</sup>	125 ± 5.47 <sup>c</sup>	150 ± 3.9 <sup>b</sup>	145 ± 4.22 <sup>b</sup>
3rd week	125 ± 5.7 <sup>c</sup>	181 ± 5.39 <sup>a</sup>	129 ± 6.4 <sup>c</sup>	142 ± 5.5 <sup>b</sup>	147 ± 4.1 <sup>b</sup>

Means in the same row not followed by the same letter differ significantly (P<0.05).

**Table 5.** Initial and final diastolic blood pressure (mean±SE) of all rats.

Group	Control	Ethylene glycol	Ethanol	Aqueous	N-Hexane
Initial Diastolic	76.1±1.39	78.17 ± 2.10	76.5 ± 2.2	79.7± 2.6	78.50± 2.0
1st week	76.5 ± 2.92 <sup>b</sup>	102.8± 4.21 <sup>a</sup>	82.7 ± 5.3 <sup>b</sup>	104 ± 4.92 <sup>b</sup>	107.5± 5.94 <sup>b</sup>
2 <sup>nd</sup> week	76.8 ± 2.1 <sup>c</sup>	109 ± 3.81 <sup>a</sup>	78.8± 4.46 <sup>c</sup>	88.4± 4.62 <sup>b</sup>	85.3± 4.45 <sup>b</sup>
3 <sup>rd</sup> week	77.3 ± 4.6 <sup>b</sup>	123 ± 7.47 <sup>a</sup>	81.8 ± 4.4 <sup>b</sup>	79.9 ± 5.2 <sup>b</sup>	83.2± 5.4 <sup>b</sup>

Means in the same row not followed by the same letter differ significantly (P<0.05).

**Table 6.** Serum oxidative stress and antioxidant parameters (mean±SE) of rats treated with ethylene glycol and *Origanum vulgare* extracts.

Group	Control	Ethylene glycol	Ethanol	Aqueous	N-Hexane
MDA (µmol/ml)	9.15 ± 0.72 <sup>c</sup>	15.14 ± 0.41 <sup>a</sup>	10.12 ± 0.59 <sup>c</sup>	12.11 ± 0.31 <sup>b</sup>	14.24 ± 0.51 <sup>a</sup>
GSH (µmol/ml)	2.12 ± 0.04 <sup>a</sup>	1.37 ± 0.05 <sup>c</sup>	2.29 ± 0.07 <sup>a</sup>	2.15 ± 0.07 <sup>a</sup>	1.85 ± 0.06 <sup>b</sup>
SOD (U/ml)	6.1 ± 0.41 <sup>a</sup>	5.13 ± 0.27 <sup>b</sup>	6.77 ± 0.52 <sup>a</sup>	5.99 ± 0.37 <sup>a</sup>	6.14 ± 0.1 <sup>a</sup>
TAC (U/ml)	4.6 ± 0.04 <sup>d</sup>	4.16 ± 0.05 <sup>e</sup>	6.05 ± 0.02 <sup>a</sup>	5.1 ± 0.05 <sup>c</sup>	5.48 ± 0.04 <sup>b</sup>

Means in the same row not followed by the same letter differ significantly (P<0.05).

**Table 7.** Serum kidney markers (mean±SE) of rats treated with ethylene glycol and *Origanum vulgare* extracts.

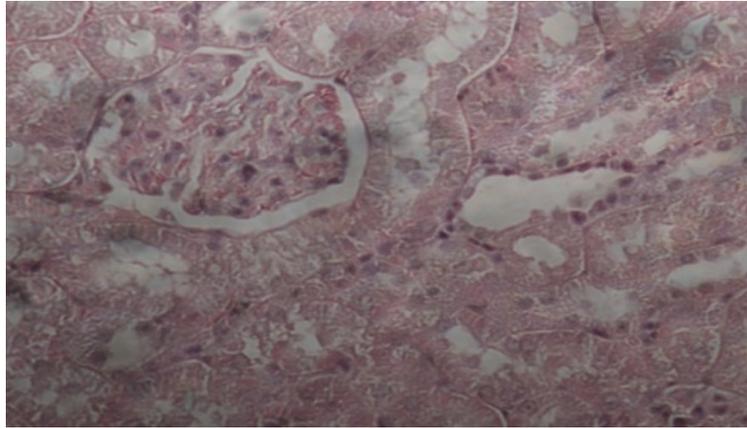
Group	Control	Ethylene glycol	Ethanol	Aqueous	N-Hexane
Urea mg/dl	32 ± 1.3 <sup>c</sup>	57.17 ± 1.9 <sup>a</sup>	29.5 ± 3.1 <sup>c</sup>	33.7 ± 1.98 <sup>c</sup>	40.8 ± 2.9 <sup>b</sup>
Creatinine mg/dl	0.57 ± 0.12 <sup>c</sup>	1.38 ± 0.10 <sup>a</sup>	0.51 ± 0.12 <sup>c</sup>	1.1 ± 0.04 <sup>b</sup>	1.13 ± 0.02 <sup>b</sup>
Uric acid mg/dl	0.88 ± 0.11 <sup>c</sup>	1.73 ± 0.07 <sup>a</sup>	0.95 ± 0.12 <sup>c</sup>	1.32 ± 0.14 <sup>b</sup>	1.7 ± 0.21 <sup>a</sup>
Calcium mg/dl	9.02 ± 0.1 <sup>a</sup>	6.45 ± 0.33 <sup>b</sup>	8.92 ± 0.39 <sup>a</sup>	8.6 ± 0.21 <sup>a</sup>	7.1 ± 0.25 <sup>b</sup>
Phosphorus mg/dl	4.18 ± 0.2 <sup>b</sup>	6.15 ± 1.1 <sup>a</sup>	3.9 ± 0.18 <sup>b</sup>	4.42 ± 0.12 <sup>b</sup>	4.38 ± 0.43 <sup>b</sup>
Albumin g/dl	3.83 ± 0.12 <sup>a</sup>	2.63 ± 0.07 <sup>c</sup>	3.02 ± 0.15 <sup>b</sup>	2.8 ± 0.18 <sup>b</sup>	3.12 ± 0.14 <sup>b</sup>
T. Protein g/dl	7.30 ± 0.13 <sup>a</sup>	6.23 ± 0.22 <sup>c</sup>	6.67 ± 0.13 <sup>b</sup>	6.82 ± 0.17 <sup>b</sup>	7.01 ± 0.19 <sup>b</sup>

Means in the same row not followed by the same letter differ significantly (P<0.05).

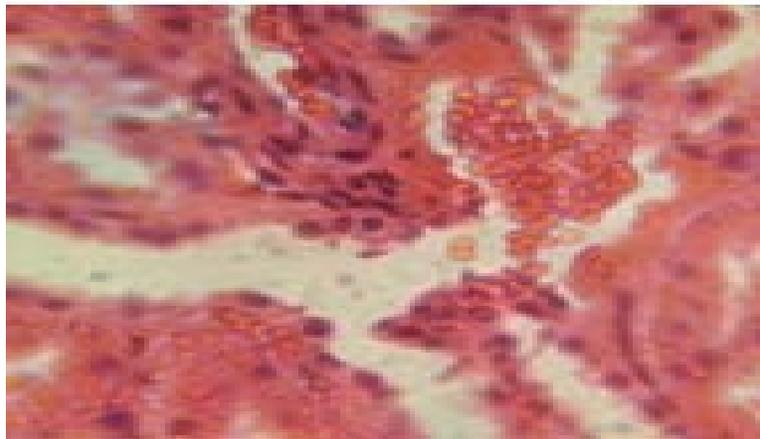
aggregation, retention and stone development (Escobar et al., 2008; Byer and Khan, 2003; Vanachayangkul et al., 2011).

The antiurolithic effect of *O. vulgare* was evaluated on the ethylene glycol-induced model for urolithiasis (Divakar et al., 2010; Tsai et al., 2008). The changes in

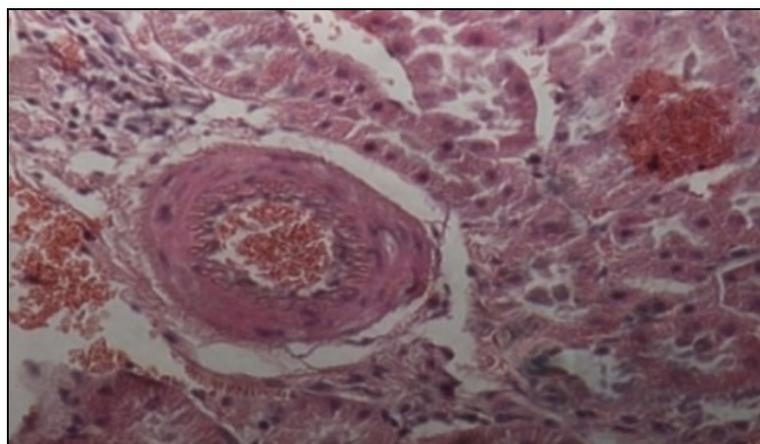
urine electrolytes and calcium oxalate super-saturation were used to a greater extent due to their greater sensitivity to ethylene glycol toxicity (Bashir and Gilani, 2011; Lia and McMartin, 2009). In our study, administration of EG and AC resulted in the increase in crystalluria due to hyperoxaluria, which might be due to



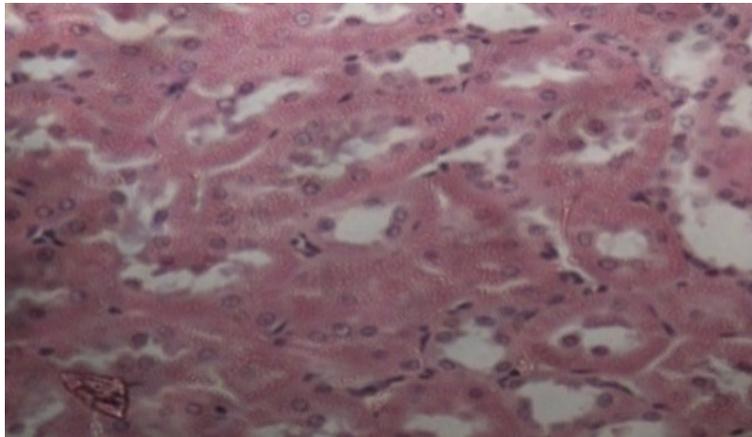
**Figure 1.** A photomicrograph of right kidney of controlled adult male albino rat showing the normal histology of renal parenchyma  $\times 400$



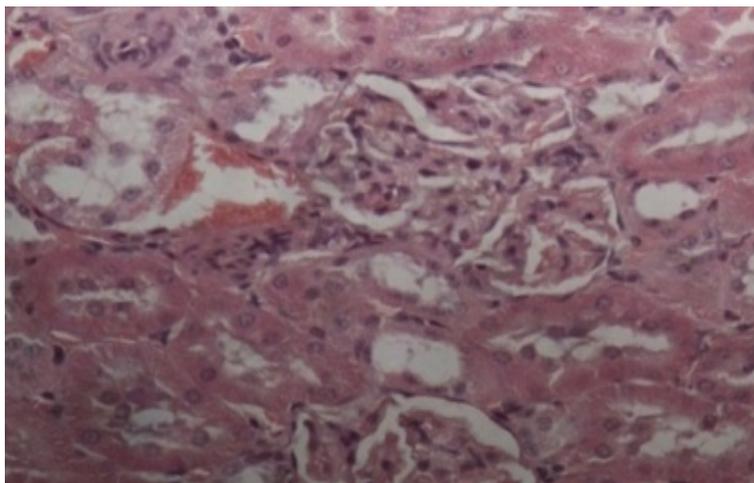
**Figure 2.** A photomicrograph of right kidney of untreated urolithitic adult male albino rat showing shrinkage glomerulus with crystals in duct,  $\times 400$ .



**Figure 3.** A photomicrograph of right kidney of untreated urolithitic adult male albino rat showing thickened fibrosed blood vessels and inflammatory infiltrates,  $\times 400$ .



**Figure 4.** A photomicrograph of right kidney of treated urolithitic adult male albino rat group showing normal renal tubules,  $\times 400$ .

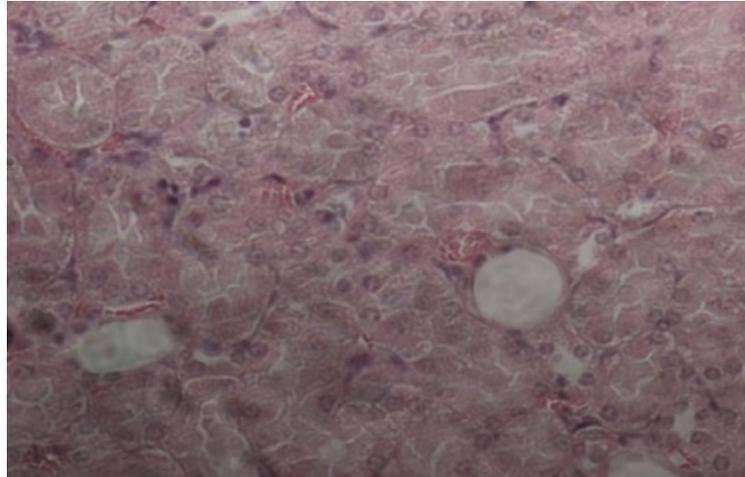


**Figure 5.** A photomicrograph of kidney of treated urolithitic adult male albino rat group 4 showing normal glomerulus and injured renal tubules,  $\times 400$ .

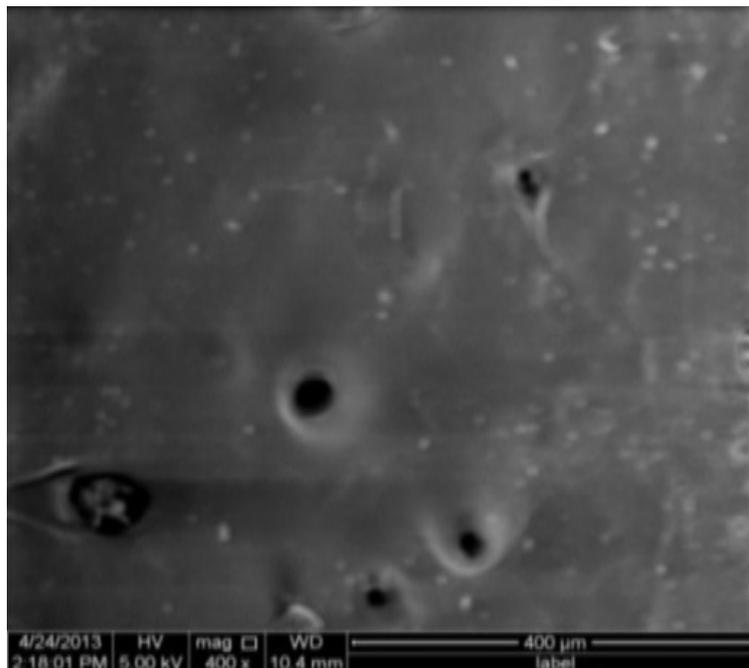
the renal impairment (Atmani et al., 2003; Bashir and Gilani, 2011). There was a significant increase in serum creatinine, blood urea nitrogen and total protein loss in the lithogenic group as compared to normal control group, which has been restored by the *O. vulgaris* treatment. Consistent with some previous reports, crystal deposition by hyperoxaluria caused an increase in oxalate and decrease in the  $\text{Ca}^{2+}$  excretion in the lithogenic group (Atmani et al., 2003; Thamilselvan et al., 2003; Santhosh and Selvam, 2003; Park et al., 2008) which was prevented by *O. vulgaris*. There was hypertrophy and extensive calcium oxalate crystal deposition in the kidneys of lithogenic group. The renal tubules were markedly dilated, which might be due to the

obstruction in the distal renal tubular flow of large crystals (Bashir and Gilani, 2011).

Several *in vivo* studies have demonstrated that hyperoxaluria, a major risk factor for calcium oxalate nephrolithiasis (Atmani et al., 2003), results in production of superoxide and hydroxyl free radicals causing oxidative stress, cell membrane rupture and cell death (Wiessner et al., 2001) and leads to calcium oxalate crystal adherence and retention in renal tubules (Revelto et al., 1997). It can be speculated that the inhibitory effect of *O. vulgaris* on calcium oxalate crystal deposition in renal tubules could have also been caused by its antioxidant activity (Tsai et al., 2008; Srihari et al., 2008). In this study, *O. vulgaris* enhanced the



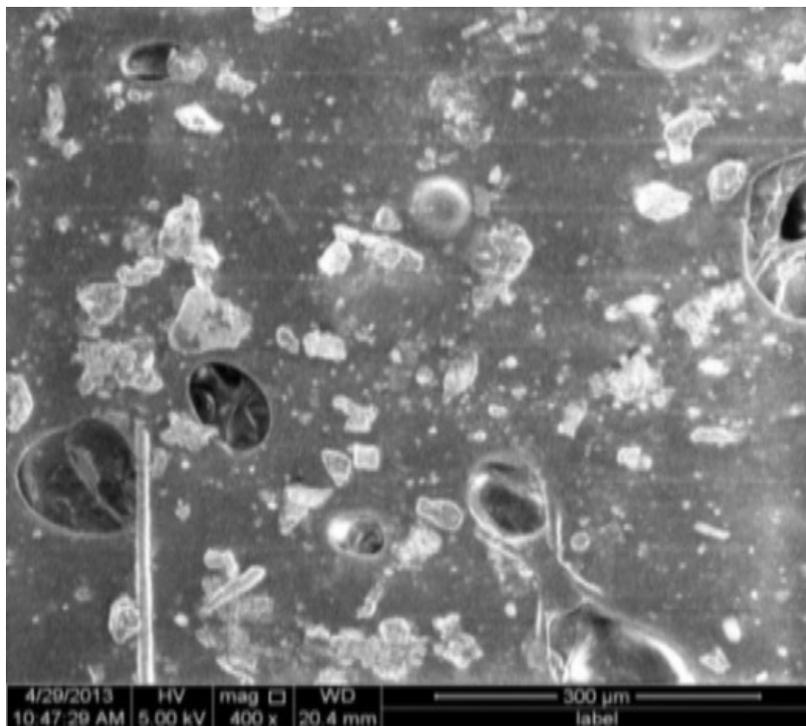
**Figure 6.** A photomicrograph of right kidney of treated urolithitic adult male albino rat group 5 showing normal renal tubules, × 400.



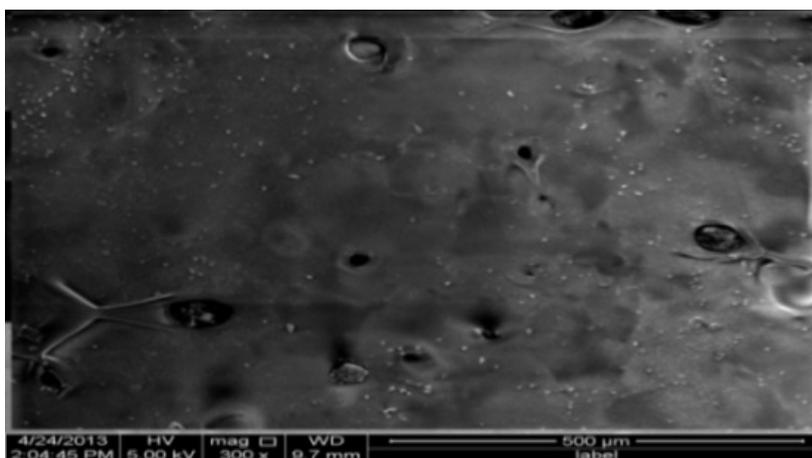
**Figure 7.** A scanning electron micrograph of kidney of control rats showing normal urothelium. Ductal openings are identifiable. Epithelium appears normal (arrow), × 500.

spontaneous recovery in the treated group, which was clearly shown by the gain in the body weight, significant decrease in urinary oxalate, renal crystal deposition and improvement in renal functions compared to the lithogenic group. Phytochemical screening revealed the presence of saponins, alkaloids, coumarins, sterol, terpenes, flavonoids and tannins. Different activities

observed in the crude extract might be due to the presence of these phytochemicals. For example, flavonoids are known to possess antispasmodic and  $\text{Ca}^{++}$  channel blocking as well as antioxidant and diuretic activities (Pietta, 2000; Gürocak and Küpeli, 2006; Ramamoorthy et al., 2010; Chishti et al., 2013). Saponins are known to possess anti-crystallization property by



**Figure 8.** A scanning electron micrograph of kidney of untreated rats showing disrupted urothelium. Ductal openings are crystalline obstructed. Covering epithelium sloughed at ductal opening, × 500.

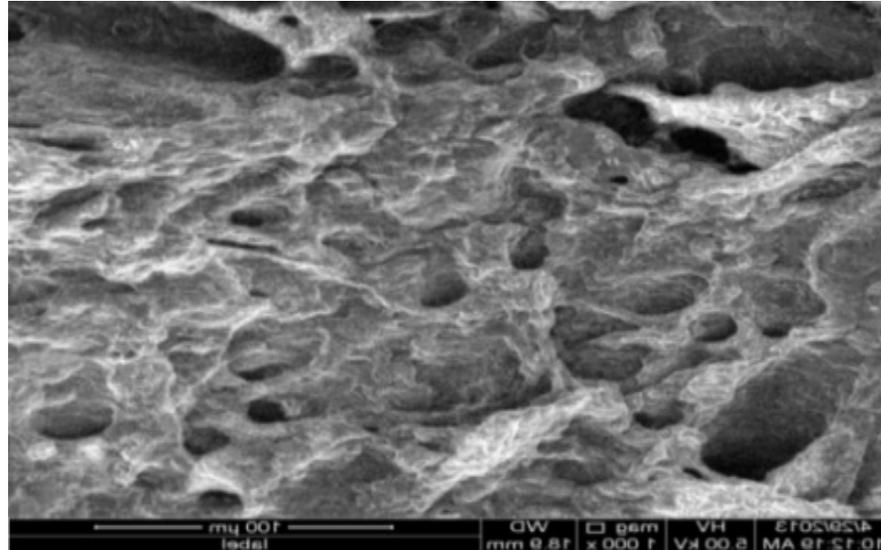


**Figure 9.** A scanning electron micrograph of kidney of treated rats showing urothelium has been reconstituted. Ductal openings are again identifiable and appeared opened. The epithelium appears normal (arrow), × 500.

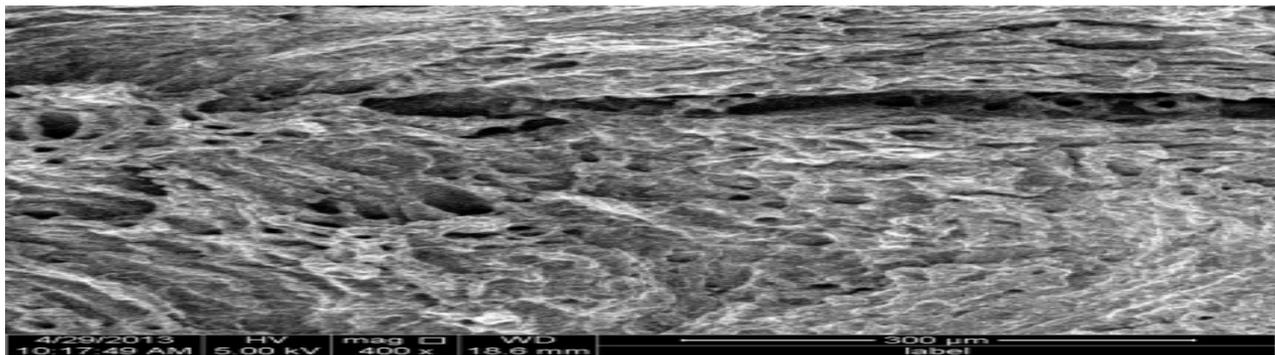
disaggregating the suspension of mucoproteins, promoters of crystallization (Ocana-Fuentes et al., 2010).

The *O. vulgaris* showed antiurolithic activity *in vivo* models in addition to its antioxidant, renal epithelial cell protective, antispasmodic and diuretic activities reported

in this study, all of which could be beneficial in urolithiasis (Butterweck and Khan, 2009). The plant was also reported to possess anti-inflammatory (Ocana-Fuentes et al., 2010) and antimicrobial (Sarac and Ugur, 2008) activities, which could also be supplementing its



**Figure 10.** A scanning electron micrograph of kidney of treated rats showing urothelium has been reconstituted. Ductal openings are again identifiable. The epithelium appears to be hanging through a ductal opening. At some places urothelium appears to be migrating laterally (arrow),  $\times 500$ .



**Figure 11.** A scanning electron micrograph of kidney of treated rats showing damaged urothelium. Covering epithelium sloughed exposing the underlying layer,  $\times 500$ .

beneficial effect, as the infection and inflammation are likely to be associated with urolithiasis process. A few studies on the effectiveness of herbal remedies in urolithiasis exists, such as *Hernaira hirsute*, *phylanthusniruri* and *Hibiscus sabdariiffa*, which showed promising results in the management of urolithiasis (Aguila et al., 2004; Butterweck and Khan, 2009; Chishti et al., 2013).

This study was designed to investigate if *O. vulgaris* supplementation can prevent the development of ethylene glycol overdose intoxication and its associated life-threatening sequels. Interestingly, the data showed that concomitant administration of *O. vulgaris* successfully prevented the acute lethal effect of ethylene glycol

toxicity and protected the rat kidney and bone marrow from the destructive effects of ethylene glycol overdose intoxication. Also, El-Nekeety et al. (2011) and Chishti et al. (2013) in a similar study, reported that reactive oxygen species (ROS) play a major role in the progression of renal disease. Substances that can attenuate the production of ROS, such antioxidant activities as *O. vulgaris*, can potentially slow or stop the progression of renal disease (Kulisical, 2004). Decreasing ROS will lead to better resolution of acute glomerulonephritis (Park et al., 2008; Srihari et al., 2008). Various reports have suggested that damage to the vascular endothelial cells associated with ethylene glycol may promote accumulation of macromolecules particularly lipoproteins

in the intima (Revuelto et al., 1997; Cullen-McEwen et al., 2003). The damage of vascular endothelial cells may be due to exposure to oxygen radical stress associated with aging and hypertension, which cause vascular pathological changes (Cullen-McEwen et al., 2003; Aguila et al., 2004).

In the current study, elevated ROS, lipid peroxidation (MDA), were the damage of vascular endothelial cells which may be due to exposure to oxygen radical stress associated with hypertension, which cause vascular pathological changes (Cullen-McEwen et al., 2003; Aguila et al., 2004). Ethylene glycol induces ROS and may accelerate the development of glomerulosclerosis and pathological changes in hypertensive nephropathy (Brent, 2001). The present study supported the idea of the beneficial effect of *O. vulgaris* intake supplementation to toxic rats. The ethylene glycol treated rats in combination with *O. vulgaris* showed significant improvement in antioxidant system (GSH, CAT and TAC) compared with ethylene glycol treated alone. Kulisic et al. (2004) and Srihari et al. (2008) approved the antioxidant activities of *O. vulgaris* as well as inhibition of lipid peroxidation and ROS. In the present work, the major effect of *O. vulgaris* intake was observed to be renoprotective and reduce both blood pressure (BP) and glomerular hypertrophy and decreasing loss of glomeruli. The control of blood pressure by *O. vulgaris* in treating hypertensive group markedly delayed and slightly improved the histological changes to delay progression of renal disease in metabolic toxicity (Eddouks et al., 2002; Esch et al., 2002; Cullen-McEwen et al., 2003; Xue et al., 2005). In this study, a significant increase in heart rate and blood pressure was found in ethylene glycol treated group, in contrast to non-significant changes in treating a group with *O. vulgaris* extract. The significant drop of systolic BP in treating rats with *O. vulgaris* oil as shown in this study agreed and fully explained by Aguila et al. (2004) who reported that *O. vulgaris* oil reduced the BP by reduction of vascular reactivity to nor-adrenaline blunting the rennin-angiotensin-aldosterone system by decreasing the adrenal synthesis of aldosterone. The major effect of *O. vulgaris* intake was observed to reduce glomerular hypertrophy and decreasing loss of the glomeruli, which coincided with who reported deleterious effects on kidney nephrons and functions as well as blood pressure in aged heterozygous mice (Cullen-McEwen et al., 2003). Another study reported that the common renal injury of hypertension is a complex dynamic process involving several players, such as an inflammatory agent, cytokines, vasoactive agents and enzymes participating in an extracellular matrix assembly, anchoring or degradation (Eddouks et al., 2002; Esch et al., 2002). The progression starts in glomeruli with loss of separation of tuft and Bowman's capsule by forming cell bridge passing on damage from lost and/or damaged nephrons to so far healthy kidney

(Xue et al., 2005). Similar to our ultrastructure result, Smith et al. (1990), Fan et al. (1999) and Hovda et al. (2009), reported a change in Bowman's capsule and glomerular tufts including shrinkage of glomeruli, glomerulosclerosis, widening of space beneath the Bowman's capsule, decreased number of glomeruli and thickened glomerular basement membrane with the prominent vesicular cytoplasm.

In this study, serum creatinine, urea and uric acid has shown a significant increase in ethylene glycol treated group as well as hypoproteinemia, hypoalbuminemia and hypocalcemia, which was due most likely to a drop of number of functioning nephrons. Our results agreed with Fan et al. (1999) and Cullen-McEwen et al. (2003) who reported that different aspects should be considered concerning the loss of the glomeruli and estimation of the glomeruli number and glomerular volume. Accumulating evidence indicates that vascular remodeling contributes to end-organ damage, apoptosis and ultra-structural destruction is increased in the kidney of untreated rats, resulting in vascular remodeling and renal dysfunction (Cullen-McEwen et al., 2003). In support, a significant elevation was observed in the serum creatinine level in untreated rats, but not in *O. vulgaris* treated animals. The renal markers, reduced to the normal value in *O. vulgaris* treated rats; the renoprotective could be the inhibition oxidative stress damage with antioxidant activities of the *O. vulgaris* (Kulisic et al., 2004; Srihari et al., 2008). The histopathology in the present work approved renoprotective effect of *O. vulgaris* in rats treated with ethylene glycol.

The present study demonstrated a survival rate of 100% in treating rats and severe decrease in body weight, in contrast to 66.6% survival rate in untreated rats. Also, the administration of ethylene glycol led to extreme weight loss and ultimately death of the rats (Fan et al., 1999; Li and McMartin, 2009). The protective effect in the present work was attributed to the protective effect of oxidative stress urolithiasis and hypertensive.

In conclusion, the present study approved that the *O. vulgaris* extracts have promising antioxidant activity, antiurolithic action, antihypertensive and renoprotective effect against nephrotoxic agents.

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## Conflict of interest

The authors have none to declare.

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