HYDROALCOHOLIC EXTRACT OF *APIUM GRAVEOLENS* AMELIORATES ETHYLENE GLYCOL-INDUCED HYPEROXALURIC OXIDATIVE STRESS AND RENAL INJURY IN WISTAR RAT

PREETHA PETER. P*, SIVA KRISHNAN. S[1]

*&1 Department of Pharmacy, Annamalai University, FEAT, Annamalai nagar, Chidambaram

*Corresponding author: PREETHA PETER. P

Abstract

Background: *Apium graveolens* Linn. (Apiaceae) is widely used in traditional Indian medicines against renal disorders including oxalate urolithiasis and is known for antioxidant activity.

Aim: The present study was designed to investigate the ameliorating effect of hydroalcoholic extract of *A.graveolens* in hyperoxaluric oxidative stress and renal cell injury.

Experimental procedure: Wistar albino rats were given 0.75% v/v ethylene glycol in drinking water to induce chronic hyperoxaluria and simultaneously *A.graveolens* was given to nephrolithiatic treated rats at the dose of 200 and 400 mg/kg b.w. orally for 28 days. Urinary volume, oxalate, serum creatinine, blood urea nitrogen (BUN), malondialdehyde (MDA) and antioxidant enzyme (SOD, CAT, GST, GPx) were evaluated.

Results and discussion: Hydro alcoholic extract of *Apium graveolens* was found to possess a high total phenolic content and exhibited significant free radicals scavenging activity. Oxalate excretion significantly increased in hyperoxaluric animals as compared to control which was protected in Hydro alcoholic extract of *Apium graveolens* treated animals. *A.graveolens* treatment significantly reduced level of MDA and improved the activity of antioxidant enzymes followed by reduction in BUN and serum creatinine. Histological analysis reveals that *A.graveolens* treatment inhibited renal cell damage.

Conclusion: The present study reveals that antioxidant activity of *A.graveolens* significantly protects against oxidative stress and renal cell injury.

Keywords: Ethylene glycol, Oxidative stress, antioxidant, lipid peroxidation

Introduction

Kidney stone disease is a multifactorial condition caused by the interaction of epidemiological, biochemical, and genetic risk factors.It affects both men and women, but the risk is higher in men,and it is becoming more prevalent among young women.(1) According to studies, calcium oxalate and calcium phosphate make up 80% of renal stones, while struvite, which contains magnesium ammonium phosphate, makes up 10%. Uric acid 9% and cystine or ammonium acid urate are the causes of drug-related renal stones 1%.(2,3)

Hyperoxaluria is one of the major risk factors of human idiopathic calcium oxalate (CaOx) urolithiasis. Oxalate is a natural byproduct of metabolism and harmlessly excreted through urine in normal individuals. However, increased urinary excretion of oxalate (hyperoxaluria) can be highly toxic because of its propensity to crystallize at physiologic pH and form CaOx (4). Exposure to oxalate generates toxic responses in renal epithelial cells, including altered membrane surface properties, mitochondrial dysfunction, formation of reactive oxygen species (ROS) and decreased cell viability.(5).

Overproduction of ROS and reduction in cellular antioxidant capacities, leads to the development of oxidative stress. Oxidative stress followed by renal cell and loss of membrane integrity, which subsequently facilitates the retention and growth of CaOx stones in renal tubules (6). Recent studies suggest that treatment with antioxidants reduced CaOx crystal induced renal injuries. Pretreatment with vitamin
E along with mannitol abolished the deposition of CaOx crystals in the kidneys of rats injected with sodium oxalate (7). Therefore, treatment with natural antioxidants seems to be the possible therapeutic strategy for ameliorating hyperoxaluria-induced oxidative stress and renal cell injury in urolithiasis.

Celery, botanically known as *Apium graveolens* is belongs to the family of apiaceae, an annual or bionomical herbaceous plant that is native of Mediterranean regions like Asia, Africa and Europe. *Apium graveolens* is an important plant with great Ayurvedic medicinal properties. The medicinal properties of celery include antioxidant, anti-inflammatory, antispasmodic, antibacterial, antifungal, anticancer, diuretic and sedative activities. (8)

The constituents of the celery include glycosides, steroids, and different types of phenolic including furanocoumarins, flavones, and trace elements (sodium, potassium, calcium and iron).The roots contain falcarinol, falcarindiol, panaxidol, and polyacetylene 8-O-methylfalcarindiol. The stem contains pectic polysaccharide (apiuman) containing d-galacturonic acid, l-rhamnose, l-arabinose, and d-galactose. Caffeic acid, chlorogenic acid, apiol, apigenin, rutaretin, ocimene, bergapten, and isopimpinellin are reported to be found in celery seed. which possibly contribute to its various uses in tradition medicine. (9-15)

These studies incorporated multidisciplinary interests that antioxidant activity of plant may have a role in protecting the kidney from hyperoxaluric oxidative stress in urolithiasis. Therefore, in the present study, an effort has been made to establish the scientific validity for the ameliorating effect of hydroalcoholic extract of *Apium graveolens* in ethylene glycol (EG)-induced hyperoxaluric oxidative stress and renal cell injury in rat kidney.

Experimental

Plant Material

The collection of the plant materials of *Apium graveolens* were done in the month of December-2024 at Nilgiris hills,ooty,Tamilnadu. The identification and authentication of the plant was carried out by Dr. K.N.Sunil kumar, Research Officer, Department of Pharmacognosy, Siddha central Research Institute, Arumbakkam, Chennai, Tamil Nadu. Voucher specimen (No.SCRI/AG/245) was prepared and preserved in the Department of Pharmacognosy, Siddha central Research Institute,Arumbakkam,Chennai for future reference.

Preparation of extracts of *Apium graveolens* by hot continuous percolation method

About 500 gm of dried powder was properly packed in Whatmann filter paper (grade no.1) and kept in thimble and the soxhlet apparatus was set up. The extraction of powder was done with different solvents with solvents of increasing polarities like petroleum ether (60-80º C), chloroform, and hydro alcohol. Here temperature maintenance is based on the solvents used

for extraction. The solvents were removed under reduced pressure using rotary evaporator and stored in desiccators. (16)

Drugs and chemicals

EG was obtained from Qualigens Fine Chemicals, Mumbai, India, and Cystone was procured from Himalaya Health Care, Bangalore, India. All other chemicals and reagents used in this study were of analytical grade. Apparatus such as the metabolic cage (Dolphin, Mumbai), the biochemistry analyzer (Benspera clinical Chemistry Analyzer C-61) and centrifuge (Remi) were used in the study.

Animals

Twenty-four inbred male Wistar albino rats (180–200 g body weight) were used in this study. Animals were procured from the Institutional animal House
(Reg no. 661/PO/Re/S/02/CPCSEA) of K.M.College of Pharmacy,Madurai.Tamil Nadu. All animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 24–27°C and humidity 60–65% with 12:12 light:dark cycles). Food was provided in the form of dry pellets and water *ad libitum*. The animals were allowed to get acclimatized to the laboratory conditions for 7 days before the commencement of the experiment. All experiments involving animals comply with the ethical standards of animal handling and were approved by Institutional Animal Ethical Committee. **Proposal No:(IAEC/P.PREETHA PETER/ AU/Ph.D/1761130002/KMCP/14/2023-24)**

Induction of oxidative stress by oxalate

EG-induced hyperoxaluria model was used to induce oxidative stress and renal cell injury in Wistar albino rats. Animals were divided into four groups comprising six animals in each. Group 1 was used as normal control and given water only and groups 2–4 were given 0.75% v/v EG for 28 days in drinking water to induce low chronic grade hyperoxaluria. Simultaneously, the following treatment was given once daily:
Group 1 was given normal saline 10 ml/kg body weight p.o served as normal control group; Group 2 was given normal saline 10 ml/kg body weight p.o. served as untreated hyperoxaluric rats group; groups 3 and 4 were given hydroalcoholic extract of *Apium graveolens* at a dose of 200 and 400 mg/kg body weight p.o., respectively, served as treated hyperoxaluric rats group.. During the study of 28 days, various biochemical parameter of urine and serum was estimated. At the end of the experimental period, animals were sacrificed and dissected to isolate kidneys for estimation of antioxidant markers and histopathological analysis.

Assessment of renal function and oxalate excretion

Animals were kept in metabolic cages individually for the collection of 24 h urine on 28th day and volume were measured immediately after collection. Urinary oxalate level was estimated by the colorimetric method using commercial oxalate kit (Sigma). Blood was obtained by cardiac puncture under ether anesthesia at the end of experiment. Blood urea nitrogen (BUN), serum and urine creatinine were estimated using commercial kits.

Assessment of parameters for oxidative stress and antioxidant status in renal tissue

The isolated right kidneys were used for the preparation of kidney homogenate. Renal cortex was separated and subsequently homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The renal cortical homogenates were centrifuged at 1500×*g* for 10 min at 4°C (17). The resulting supernatant were used for the determination of malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione *S*-transferase (GST), reduced glutathione (GSH) and glutathione peroxidase (GPx).

MDA assay
According to the method of Esterbauer and Cheeseman (1990),(18) MDA was estimated in terms of thiobarbituric acid reactive species (TBARS). Homogenized renal tissue (1 ml) in 2 mL of normal saline was mixed with 1 mL trichloro acetic acid (20%), 2 mL thiobarbituric acid (0.67%) and heated for 1 h at 100°C. After cooling, the precipitate was removed by centrifugation. The absorbance of the sample was measured at 535 nm using a blank containing all the reagents except the sample. As 99% TBARS are MDA, TBARS concentrations of the samples were calculated using the extinction coefficient of MDA (1.56 × 105 M−1cm−1) (El-Demerdash et al., 2009).(19)

CAT assay
The CAT activity was measured using the method of Chance and Maehly (1955)(20) by following the decomposition of hydrogen peroxide. The reaction mixture consisted of 2 mL of 100 mM phosphate buffer (pH 7.0), 0.90 mL of hydrogen peroxide (30 mM) and 0.1 mL of supernatant in a final volume of 3 mL. Absorbance was recorded at 240 nm at every 10 s interval for 1 min. One unit of CAT is defined as the amount of enzyme required to decompose 1 μM H2O2/min, at 25°C.

SOD assay
An indirect method of inhibiting auto-oxidation of epinephrine to its adrenochrome was used to assay SOD activities. Kidney homogenate (0.05 ml) was added to 2.0 mL of carbonate buffer and 0.5 mL of 0.01 mM EDTA solution. The reaction was initiated by addition of 0.5 mL of epinephrine (3 × 10−4 M) at pH 10.2 and the change in optical density every minute was measured at 480 nm against reagent blank for 5 min. A graph of absorbance against time was plotted for each sample and the rate of auto-oxidation calculated. One unit of SOD activity is defined as the concentration of the enzyme (mg protein/mL) in the plasma that caused 50% reduction in the autooxidation of epinephrine..(21,22)

GST assay
GST activity was estimated by the method of Habig et al.(1974)(23) from the rate of increase in conjugate formation between GSH and 1-chloro-2,4-dinitrobenzene (CDNB) by measuring the increase in absorbance at 340 nm. In a 3.00 mL reaction mix, the final concentrations were 97 mM potassium phosphate, 0.97 mM EDTA, 2.5 mM GSH, 1.0 mM CDNB, 3.2% (v/v) ethanol and 0.0075–0.015 unit GST. In the test, kidney homogenate added instead of enzyme. Immediately, absorbance was taken at 340 nm at 30 s interval for 5 min.

GPx assay
GPx activity was measured by using the method of Paglia and Valentine (1967).(24) Reaction mixture contained 2.5 mL of 0.1 M/l Tris-HCl buffer (pH 7.2), 75 μL of 0.04 M/l GSH,

100 µL of 0.1 M/l nicotinamide adenine dinucleotide phosphate (NADPH) and 100 µL of GSH (0.24 units). Homogenate (20 µL) was added to the reaction mixture. Reaction was initiated by adding 100 µL of 0.75 mM hydrogen peroxide. The decrease in absorbance was measured at 340 nm for 3 min at every 30 s interval. The activity was expressed as unit/mg protein using molar extinction coefficient of 6.22 × 103 (mM/L)−1cm−1.

Reduced glutathione

GSH was measured by using the method described by Dringen and Hamprecht (1996)(25) with slight modifications. Tissue homogenate 50 µL was diluted with 50 µL of 100 mM phosphate buffer containing 1 mM EDTA. To this mixture, 100 µL of reaction buffer (295 µM 5,5′- dithiobis (2-nitrobenzoic acid) (DTNB) made in 10 mL of phosphate buffer) was added and change in absorbance was measured at 412 nm for 5 min at every 30 s interval.Reduced pure GSH was used to obtain a standard curve. Reduced GSH was expressed as µM GSH/mg tissue.

Protein estimation

Protein estimation was done by using standard protocol of Lowry et al. (1951)(26). Bovine serum albumin was used as standard, and the color developed was read at 660 nm.

Histopathological studies

The left kidney excised from animal was immediately fixed in 10% buffered formalin (pH 7.0). The tissues were dehydrated with ascending grade of alcohol and embedded with paraffin wax (M.P. 55°C). Paraffin kidney sections (6 µm thick) were cut, mounted on slides with Mayer’s albumin solution, deparaffinized, rehydrated with descending grade of alcohol and finally stained with hemotoxylin and eosin(27). The kidney sections were examined under light microscope to evaluate pathological changes and photomicrographs were taken.

Statistical calculations

All the data were expressed as mean ± standard error of mean (SEM). All statistical comparisons between the groups were made by means of one way analysis of variance (ANOVA) with Newmann keuls multiple range tests using Graphpad Prism 3.0 software.

Results

Table 1. Effect of HAEAG treatment on oxalate excretion and renal function in rats.

Groups		Urine volume (mL/24 h)	Oxalate (mg/mL)	Blood urea nitrogen (mg/dl)	Serum creatinine (mg/dl)
G1	Normal control 10ml/kg normal saline	6.18 ± 0.40	0.448 ± 0.064	36.25 ± 1.95	0.589 ± 0.033
G2	Hyperoxaluric acid control rats 0.75% v/v Ethylene Glycol	13.10 ± 0.82*a	1.834 ± 0.68*a	51.68 ± 2.35*a	0.898 ± 0.047*a
G3	Treatment control HAEAG 200mg/kg	16.55 ± 0.96*b	0.628 ± 0.32*b	44.30 ± 2.20*b	0.645 ± 0.041*b
G4	Treatment control HAEAG 400mg/kg	25.05 ± 1.32*b	0.530 ± 0.051*b	40.90 ± 2.05*b	0.608 ± 0.038*b

Values are represented as Mean±SEM.
*a Values are significantly different from Normal control at P<0.01
*b Values are significantly different from Lithiasis control at P<0.01

Table 2. Effect of HAEAG treatment on renal enzymes and other antioxidant markers in rat kidney homogenate analysis.

Groups		Malondialdehyde (MDA) (μM/mg protein)	Catalase (μM H_2O_2 decomposed /min)	Super oxide dismutase (U/mg protein)	Glutathione *s*-transferase (μM/min/mg protein)	Glutathione peroxidase (U/mg protein)	Total protein content (mg/dl)
G1	Normal control 10ml/kg normal saline	6.55 ± 0.24	70.4 ± 1.48	1.765 ± 0.12	0.748 ± 0.08	0.778 ± 0.14	244.10 ± 5.40
G2	Hyperoxaluric acid control rats 0.75% v/v Ethylene Glycol	10.10 ± 0.88*a	41.0 ± 0.75*a	0.842 ± 0.07*a	0.457 ± 0.04*a	0.410 ± 0.07*a	324.40 ± 6.35*a
G3	Treatment control HAEAG 200mg/kg	7.20 ± 0.48*b	50.4 ± 0.84*b	1.472 ± 0.10*b	0.672 ± 0.06*b	0.680 ± 0.10*b	295.20 ± 5.90*b
G4	Treatment control HAEAG 400mg/kg	6.85 ± 0.40*b	46.3 ± 0.78*b	1.518 ± 0.11*b	0.704 ± 0.07*b	0.714 ± 0.12*b	258.15 ± 5.75*b

Values are represented as Mean±SEM.
*a Values are significantly different from Normal control at P<0.01
*b Values are significantly different from Lithiasis control at P<0.01

Evaluation of renal functioning

The urine output was increased significantly ($p < 0.001$) in untreated as well as hydroalcoholic extract of *Apium graveolens* (HAEAG) treated hyperoxaluric rats. The urine output in control group was 6.18 ± 0.40 mL/24 h/rat,which was increased to about 52.82% in hyperoxaluric rats (Group II) and 62.65 and 75.32% in HAEAG treated rats, which still remains significantly ($p < 0.01$) higher in HAEAG treated rats in dose-dependent manner as compared to untreated hyperoxaluric group. Hyperoxaluric treatment (EG) induced hyperoxaluria in rats and urinary excretion of oxalate in untreated hyperoxaluric rats (Group II) increased tremendously ($p < 0.001$), as compared to control rats (Group I). In HAEAG treated rats (group III and IV) again the oxalate excretion is high as compared to control rats but there was a significant ($p < 0.001$) reduction as compared to untreated hyperoxaluric rats (Table 1).,

Hyperoxaluric treatment caused impairment of renal functions in untreated hyperoxaluric rats as evident from the markers of glomerular and tubular damage, viz, raised BUN ($p < 0.001$) and serum creatinine ($p < 0.01$) which were dose-dependently prevented in the animals receiving a simultaneous treatment with HAEAG.

Effect on oxidative stress parameters in kidney tissue

Ethylene glycol administration significantly ($p < 0.001$) increased the MDA level and Total protein content and decreased GSH,GPX content and activities of CAT and SOD in lithiatic control group when compared to normal control group [Table 2]. The treatment with HAEAG (200 and 400 mg/kg) significantly ($p < 0.001$, in all cases) reduced the levels of MDA and Total protein content and increased GSH content, GPX. CAT and SOD activities when compared to lithiatic group.

Effect on histopathology of kidney

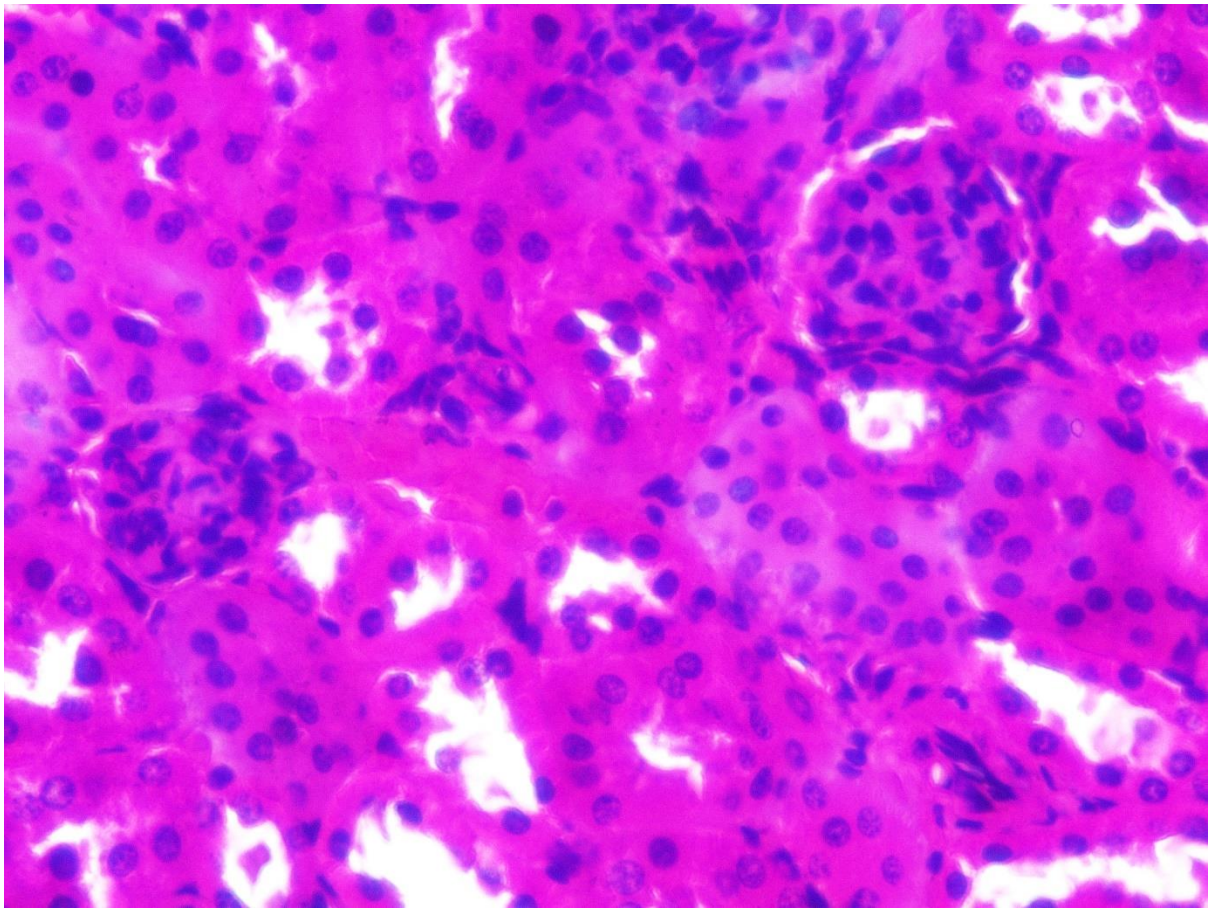

FIG.NO:A NORMAL CONTROL

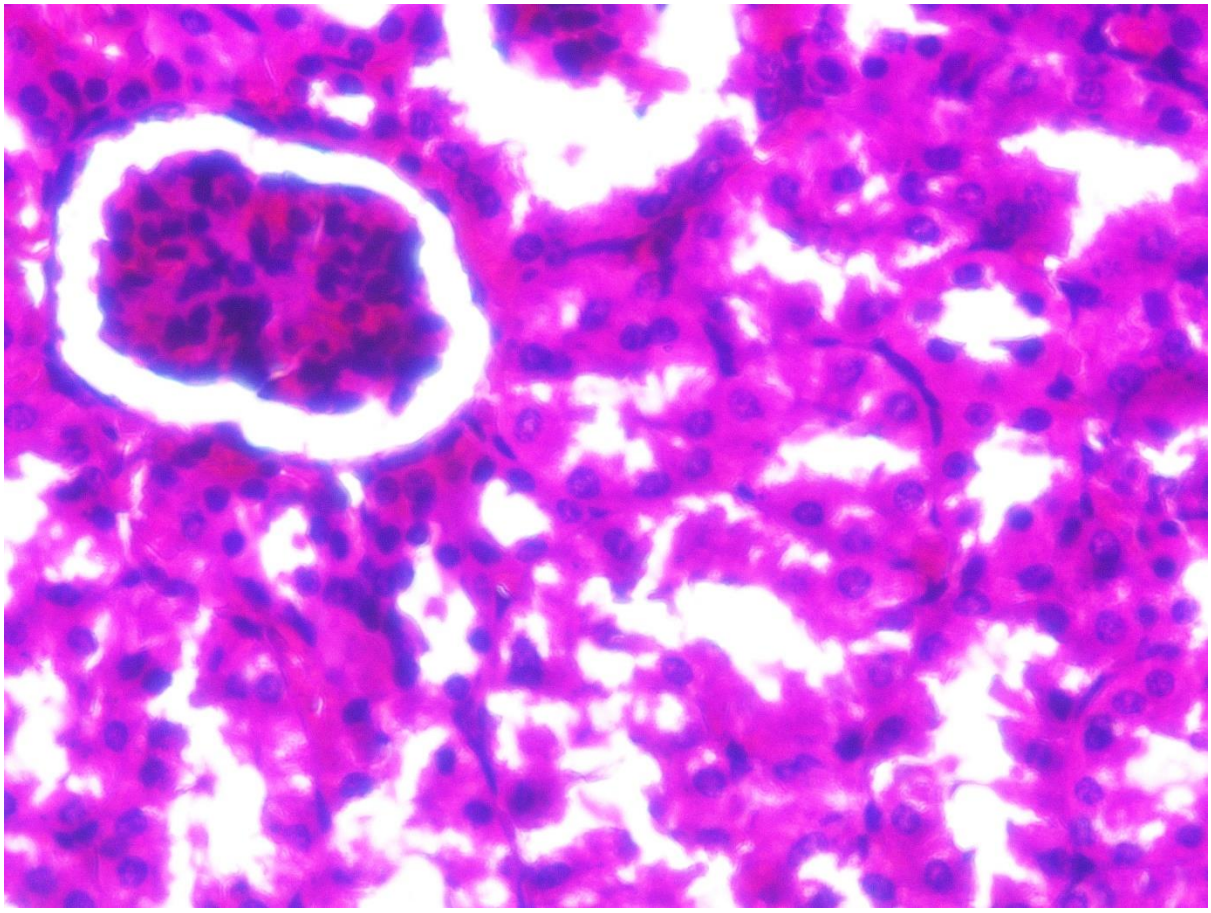

FIG.NO:B TOXIC CONTROL

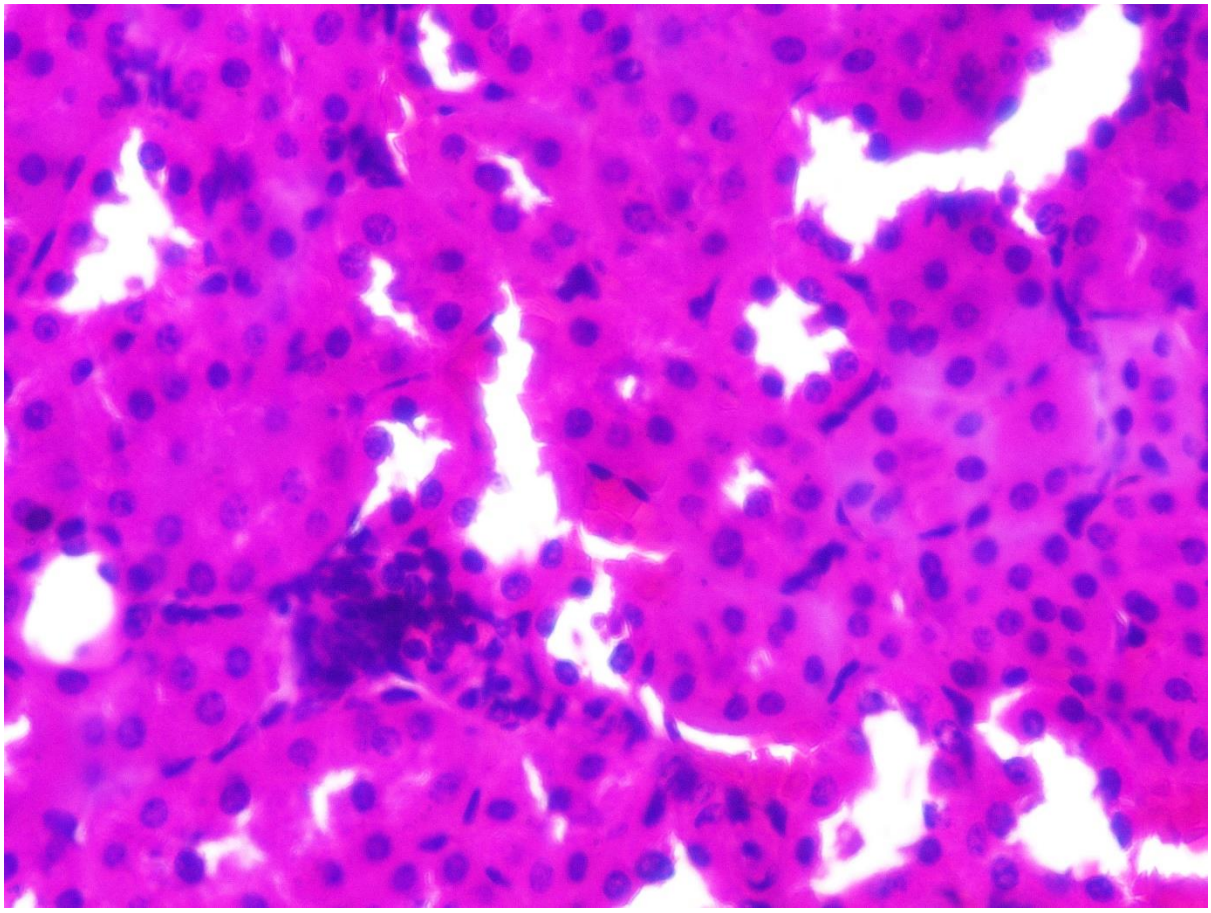

FIG.NO:C TREATMENT CONTROL HAEAG 200MG/KG

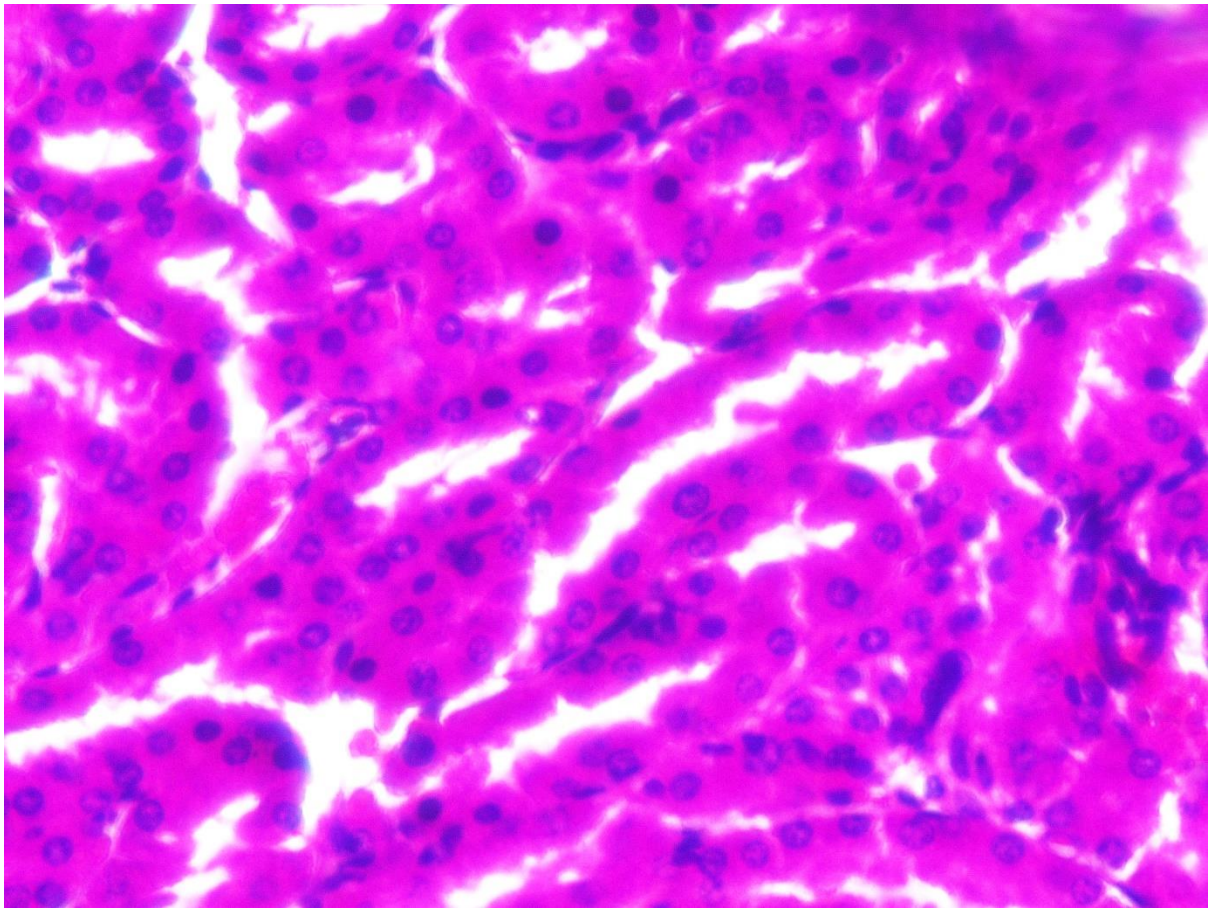

FIG.NO:D TREATMENT CONTROL HAEAG 400MG/KG

There were no histopathological changes in renal tubules, glomeruli and blood vessels in normal group of rats [Figure A]. The EG induced lithiatic group showed the presence of CaOx crystals in lumen of tubules accompanied by inflammation and cast formation which causes dilation of tubules and blood vessels. The lithiatic group also revealed the presence of crystals in interstitial spaces with moderate to marked glomerular congestion and tubular degeneration [Figure B]. Very few or no crystal deposition and changes in the architecture of kidneys were found in HAEAG [Figure C], (200 mg/kg) and [Figure D] HAEAG (400 mg/kg) groups when compared to lithiatic control group.

Discussion

Epidemiological studies have shown that the majority of stones are commonly composed of calcium oxalate (CaOx). A number of animal models using rats have been used to induce calcium oxalate urolithiasis. Out of these models, ethylene glycol induced hyperoxaluria rat model causes rapid formation calcium oxalate crystals in renal tubules of experimental animals and hence commonly employed for rapid screening of anti-urolithiatic drugs. It has been postulated that oxalate-induced damage to renal cells may contribute to a number of renal pathologies, including the deposition of CaOx stone in kidney (Hackett et al., 1995; Scheid et al., 1995).(28,29) High concentration of oxalate is toxic for renal epithelial cells, producing injury, alteration in membrane integrity and death of renal cells through the generation of ROS and increased oxidative stress (Kurien & Selvam, 1989; Miller et al.,

2000)(30,31). Compounds with antioxidant potential have been found to be fairly useful in combating these disorders. Therefore, a huge body of research is focused on exploring safe and effective antioxidant compounds. Plant extracts or plant-based antioxidants are not only efficient, but also relatively safer than the synthetic ones.

Celery (*Apium graveolens*) is a medicinal plant in traditional medicine with numerous health benefits. Celery involves in the prevention of cardiovascular disease,(32) lowering blood glucose in diabetic mice,(33) lowering blood pressure and strengthening the heart.(34) Experimental studies report antifungal.(35) and anti-inflammatory effects of celery.(36) Celery has an anticoagulant activity.(37) Its root leads to an increase of calcium and decrease of potassium in the heart tissue.(38) Essential oil of celery has antibacterial effects. This plant has cooperation in the molecular mechanisms and cellular targets that have a significant effect on the treatment of human cancers.(39) Celery root and leaves have the property of eliminating OH and DPPH radicals. It also reduces the severity of liposomal peroxidation that represents renewal and conservation activities of it.(40) Celery has a protective effect on the gastric mucosa and it is anti- gastric ulcer, (41) also a diuretic plant (42) with antioxidant properties.
Celery leaves and stem contain phenols. Flavonoid is the main component of apigenin celery leaves whose amount is 202 milli-grams per kilo-gram. There are also luteolin and chrysoeriol 7-glucosides with the amounts of 48 mg per kg and 27 mg kg respectively in celery leaves. Celery leaves also contain furanocomarin,psoralen, bergapten, xanthotoxin and isopimpinellin.(43) These phenolic constituents are responsible for antioxidant activity of *Apium graveolens.*

The most harmful consequence of hyperoxaluria is deposition of the CaOx crystal in kidney. Primary or secondary hyperoxaluria is the major risk factor for the urolithiasis (Sellaturay & Fry, 2008).(44) In the present study, low chronic grade hyperoxaluria in animals was induced by adding the 0.75% EG in drinking water to mimic the idiopathic hyperoxaluric condition in human (Atmani et al., 2009).(45) Oxlate excretion significantly increased in hyperoxaluric animals as compared to control and protected in HAEAG-treated animals. EG increase oxalate production by way of increase substrate availability that induce the activity of oxalate synthesizing enzyme. Glycolic acid oxidase (GAO) and lactate dehydrogenase (LDH) catalyses the oxidation and reduction of glyoxalate results in formation of glycolate and oxalate (Soundararajan et al., 2006).(46) HAEAG inhibits the oxalate synthesizing enzyme and prevent the increased urinary excretion of oxalate. HAEAG-treated animals showed increased urine volume with respect to control and hyperoxaluric animals. Increase of palatability due to sweetness of EG increased the water intake in untreated rats followed by increased urine volume, but it still remains significantly higher in HAEAG-treated group. This is due to well-established diuretic activity of Apium graveolens.(42) Increased urine volume decreases the saturation of the oxalate and prevents the precipitation of the CaOx at physiological pH. Diuresis also flushes out the renal system and helps in mechanical expulsion of the stone.

CaOx crystal agglomerate tends to retain in kidney by trapping in renal tubules and develop into renal stones, which damage the renal tissue and deteriorate the renal function. Impairment of renal functions of untreated rats is evident from the markers of glomerular and tubular damage: raised BUN and serum creatinine (Karadi et al., 2006)(47). Renal dysfunction diminishes the ability to filter urea and creatinine clearance, so the BUN and creatinine level rises in blood (Cao et al 2003)(48) The normalization of BUN and creatinine

in HAEAG-treated animals as compared to hyperoxaluric animals shows that it protects the deterioration of the renal function by minimizing tubular damage and crystal deposition.

Further, we assessed the effect of oxalate exposure on the rat kidneys by estimating oxidative stress markers. Hyperoxaluric treatment caused extensive CaOx crystal deposition in kidneys of untreated rats accompanied by oxidative damage as reflected from increased levels of markers of oxidative injury such as MDA and protein carbonyl content and decreased activities of antioxidant enzymes along with GSH level,GPX and CAT and SOD activity.

MDA is a major end product of lipid peroxidation in membrane fatty acids representing oxidative tissue damage caused by ROS resulting in structural alteration of membrane with release of cell and organelle contents, loss of essential fatty acids with formation of cytosolic aldehyde and peroxide products (Kato et al., 2007).(49)

In the present investigation, the level of MDA was found to be significantly elevated with oxalate exposure in hyperoxaluric rats. Exposure to oxalate generates toxic responses in renal epithelial cells, including altered membrane surface properties, changes in gene expression (NF-κB), disruption of mitochondrial function and formation of ROS (Jonassen et al., 2005).(50) Mitochondria are a major site of ROS formation and oxalate-induced activation of NADPH oxidase is another source of ROS in renal cells.

Free radical scavenging enzymes such as CAT, SOD and GPx are the cellular defense enzymes against oxidative injury, decomposing superoxide and peroxide before their interaction to form the more reactive hydroxyl radical. Under oxidative stress conditions, ROS are reduced by conjugation with GSH directly or by means of GSH-related enzymes, which decrease GSH levels (Li et al., 2010).(51) GST also plays a key role in cellular detoxification by catalyzing the reaction of glutathione with toxicants to form an *S*-substituted glutathione (El-Demerdash et al., 2009).(52)

Several *in vivo* and *in vitro* studies have demonstrated that exposure to high level of oxalate results in greater production of superoxide and peroxide free radicals, leading to redox imbalance and have been manifested as antioxidant depletion, peroxidation of lipid and oxidation of protein (Hackett et al., 1995; Thamilselvan & Selvam et al., 1997).(53,54)

Recent studies have provided evidence that CaOx kidney stone patients excrete significantly higher amounts of GST and MDA in their urine, indicating ROS in kidneys of CaOx stone patients (Huang et al., 2003; Puntel et al., 2007).(55,56) The accumulation of these products was concomitant with the decrease in the antioxidant enzymes SOD, CAT, and GPx as well as GSH and protein thiol. All the above parameters were reported to decrease in hyperoxaluria induced urolithiasis (Rodrigo & Bosco, 2006)(57). Recent studies evidenced that vitamin E therapy prevents CaOx deposition in the rat kidney and reduced renal cell injury by restoring these enzymes (Thamilselvan & Menon, 2005).(58)

Similarly, antioxidant constituent (flavanoids) of *Apium graveolens* effectively scavenge the superoxide and peroxide radicals and protect the renal cell from oxidative stress induced injuries, which is evident from restoration of SOD, CAT, GPx, GST and GSH level in HAEAG-treated animal as compared to hyperoxaluric animals.

Renal histopathology also supports the above results as evident from CaOx crystal deposition, shrinkage of glomeruli and tubular damage in kidneys of untreated rats. Tissue injury, loss of membrane integrity and inflammation in kidney of these animals are due to hyperoxaluria-induced lipid peroxidation and depletion of antioxidant enzymes (Santhosh Kumar & Selvam, 2003; Itoh et al., 2005).(59,60).However, treatment with HAEAG (Figure C and D) inhibited crystal deposition and ameliorates renal injury through free radical scavenging, inhibition of lipid peroxidation and restoration of antioxidant enzyme.

Conclusion

Apium graveolens extracts exhibited preventive effect on ethylene glycol-induced formation of renal calculi. The antilithiatic effect of extracts may be through dissolution of preformed stones and/or prevention of the formation of CaOx crystals and antioxidant activity. Thus, various phytoconstituents of *Apium graveolens* synergistically combats with hyperoxaluria-induced oxidative stress and renal cell injury, possibly mediated through antioxidant activity of plant. This study validates the ethnomedicinal use of *Apium graveolens* leaves in treatment of urinary stones.

References

1. Selvam P, Kalaiselvi P, Govindaraj A, Murugan VB, Sathishkumar AS. Effect of A. lanata leaf extract and vediuppu chunnam on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria. Pharmacol Res. 2001;43:89e93.

2. Coe FL, Evan A, Worcester E. Kidney stone disease. J Clin Invest. 2005;115: 2598e2608.

3. Divakar K, Pawar A, Chandrasekhar S, Dighe S, Divakar G. Protective effect of the hydro-alcoholic extract of Rubia cordifolia roots against ethylene glycol induced urolithiasis in rats. Food Chem Toxicol. 2010;48:1013e1018.

4. Khan SR. (2005). Hyperoxaluria-induced oxidative stress and antioxidants for renal protection. *Urol Res*, 33, 349–357.

5.Jonassen JA, Kohjimoto Y, Scheid CR, Schmidt M. (2005). Oxalate toxicity in renal cells. *Urol Res*, 33, 329–339.

6. Selvam R. (2002). Calcium oxalate stone disease: Role of lipid peroxidation and antioxidants. *Urol Res*, 30, 35–47.

7. Thamilselvan S, Selvam R. (1997). Effect of vitamin E and mannitol on renal calcium oxalate retention in experimental nephrolithiasis. *Indian J Biochem Biophys*, 34, 319–323.

8. Ruchi Roper, Dharam Pal Pathak, Vikash Gupta, Uma, Garima Kapoor , Rubina Bhutani , Ravi Kant. *Apium Graveoleons* - A Health Boon. Int. J. Pharm. Sci. Rev. Res., 46(1), September - October 2017; Article No. 18, Pages: 95-99

9. Al-Asmari AK, Al-Elaiwi AM, Athar MT, et al. A review of hepatoprotective plants used in Saudi traditional medicine. Evid Based Complementary Alter n Med 2014, 2014, 1-23.

10.Tyagi S, Dhruv M, Ishita M, Gupta AK, Usman MRM, Nimbiwal B, *et al* . Medical benefits of *Apium graveolens* (celery herb). J Drug Discov Ther 2013;1:36-8

11.Hussain MT, Ahmed G, Jahan N, Adiba M. Unani description of Tukhme Karafs (seeds of *Apium graveolens* Linn) and its scientific reports. Int Res J Biol Sci 2013;2:88-93.

12.Zidorn C, Jöhrer K, Ganzera M, Schubert B, Sigmund EM, Mader J, *et al* . Polyacetylenes from the *Apiaceae* vegetables carrot, celery, fennel, parsley, and parsnip and their cytotoxic activities. J Agric Food Chem 2005;53:2518-23

13.Ovodova RG, Golovchenko VV, Popov SV, Popova GY, Paderin NM, Shashkov AS, *et al* . Chemical composition and anti-inflammatory activity of pectic polysaccharide isolated from celery stalks. Food Chem 2009;114:610-5.

14.Nagella P, Ahmad A, Kim SJ, Chung IM. Chemical composition, antioxidant activity and larvicidal effects of essential oil from leaves of *Apium graveolens*. Immunopharmacol Immunotoxicol 2012;34:205-9.

15.Sastri B. The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products. Raw Materials. The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products. Raw Materials; 1956. p. 4.

16. WHO, Research Guidelines for evaluating the safety and efficacy of herbal medicines, World Health Organizations, Geneva, 1998; 30-33.

17. Bashir S, Gilani AH. (2009). Antiurolithic effect of *Bergenia ligulate* rhizome: An explanation of the underlying mechanisms. *J Ethnopharmacol*, 122, 106–116.

18. Esterbauer H, Cheeseman KH. (1990). Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. *Meth Enzymol*, 186, 407–421.

19. El-Demerdash FM, Yousef MI, Radwan FM. (2009). Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs. *Food Chem Toxicol*, 47, 249–254.

20. Chance B, Maehly C. (1955). Assay of catalase and peroxidase. *Methods Enzymol*, 11, 764–775.

21. Misra HP, Fridovich I. (1972). The generation of superoxide radical during the autoxidation of hemoglobin. *J Biol Chem*, 247, 6960–6962.

22. Saggu H, Cooksey J, Dexter D, Wells FR, Lees A, Jenner P, Marsden CD.(1989). A selective increase in particulate superoxide dismutase activity in parkinsonian substantia nigra. *J Neurochem*, 53, 692–697

23. Habig WH, Pabst MJ, Jakoby WB. (1974). Glutathione *S*-transferases.The first enzymatic step in mercapturic acid formation. *J Biol Chem*, 249, 7130–7139.

24. Paglia DE, Valentine WN. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *j Lab Clin Med*, 70, 158–169.

25. Dringen R, Hamprecht B. (1996). Glutathione content as an indicator for the presence of metabolic pathways of amino acids in astroglial cultures. *J Neurochem*, 67, 1375–1382.

26.Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with the Folin phenol reagent. *J Biol Chem*, 193, 265–275.

27. Atmani F, Sadki C, Aziz M, Mimouni M, Hacht B. (2009). *Cynodon dactylon* extract as a preventive and curative agent in experimentally induced nephrolithiasis. *Urol Res*, 37, 75–82.

28. Hackett RL, Shevock PN, Khan SR. (1995). Alterations in MDCK and LLC-PK1 cells exposed to oxalate and calcium oxalate monohydrate crystals. *Scanning Microsc*, 9, 587–596.

29. Scheid CR, Koul HK, Kennington L, Hill WA, Luber-Narod J, Jonassen J, Honeyman T, Menon M. (1995). Oxalate-induced damage to renal tubular cells. *Scanning Microsc*, 9, 1097–105; discussion 1105.

30. Kurien TB, Selvam R. (1989). Induction of lipid peroxidation in calcium oxalate stone formation. *Indian J Exp Biol*, 27, 450–453.

31. Miller C, Kennington L, Cooney R, Kohjimoto Y, Cao LC, Honeyman T, Pullman J, Jonassen J, Scheid C. (2000). Oxalate toxicity in renal epithelial cells: Characteristics of apoptosis and necrosis. *Toxicol Appl Pharmacol*, 162, 132–141.

32. Sowbhagya HB, Srinivas P, Krishnamurthy N. Effect of enzymes on extraction of volatiles from celery seeds. Food chemistry. 2010; 120(1): 230-234.

33. Gelodar G, Nazify Habib Abadi S. Effect of celery, apple tart and carrots on some biochemical parameters in diabetic rats. J Kerman Univ Med Sci. 1997; 3(40): 114-119.

34. Lans CA. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. J Ethnobiol Ethnomed. 2006; 2: 45.

35. Momin RA, Nair MG. Mosquitocidal, nematicidal, and antifungal compounds from Apium graveolens L. seeds. J Agric Food Chem. 2001; 49(1):142-5.

36. Mencherini T, Cau A, Bianco G, Della Loggia R, Aquino RP, Autore G. An extract of *Apium graveolens* var.dulce leaves: structure of the major constituent, apiin, and its anti-inflammatory properties. J Pharm Pharmacol. 2007; 59(6): 891-7.

37. Sowbhagya HB, Srinivas P, Krishnamurthy N. Effect of enzymes on extraction of volatiles from celery seeds. Food Chem. 2010; 120(1): 230- 234.

38. Bernard B, Stiehl B. Effect of atmospheric modification on the incidence of blackheart and the cation content of celery. Ecotoxicol Environ Saf. 1986; 28(1-2): 19-28.

39. Atta A. Anti-nociceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts. J Ethnopharmacol. 1998; 60(2): 117-24.

40. Zidorn C, Johrer K, Ganzera M, Schubert B, Sigmund EM, Mader J, et al. Polyacetylenes from the apiaceae vegetables carrot, celery, fennel, parsley,and parsnip and their cytotoxic activities. J Agric Food chem. 2005; 53(7): 2518-23.

41. Belanger JT. Perillyl alcohol: applications in oncology. Altern Med Rev. 1998; 3(6):448-57.

42. Al-Howiriny T, Alsheikh A, Alqasoumi S, Al-Yahya M, ElTahir K , Rafatullah S.
Gastric antiulcer, antisecretory and cytoprotective properties of celery in rats. Pharm Biol. 2010; 48(7):786-93.

43. Taher M, Ghannadi A, Karmiyan R. Effects of volatile oil extracts of *Anethum graveolens* L. and *Apium graveolens* L. seeds on activity of liver enzymes in rat . J Qazvin Univ Med Sci. 2007; 11 (2):8-12.

44. Sellaturay S, Fry C. (2008). The metabolic basis for urolithiasis. *Surgery*, 26, 136–140.

45. Atmani F, Sadki C, Aziz M, Mimouni M, Hacht B. (2009). *Cynodon dactylon* extract as a preventive and curative agent in experimentally induced nephrolithiasis. *Urol Res*, 37, 75–82.

46. Soundararajan P, Mahesh R, Ramesh T, Begum VH. (2006). Effect of *Aerva lanata* on calcium oxalate urolithiasis in rats. *Indian J Exp Biol*, 44, 981–986.

47. Karadi RV, Gadge NB, Alagawadi KR, Savadi RV. (2006). Effect of *Moringa oleifera* Lam. root-wood on ethylene glycol induced urolithiasis in rats. *J Ethnopharmacol*, 105, 306–311.

48. Cao ZG, Liu JH, Radman AM, Wu JZ, Ying CP, Zhou SW. (2003). An experimental study of effect of different extracts of *Alisma orientalis* on urinary calcium oxalate stones formation in rats. *Zhongguo Zhong Yao Za Zhi*, 28, 1072–1075.

49. Kato J, Ruram AA, Singh SS, Devi SB, Devi TI, Singh WG. (2007). Lipid peroxidation and antioxidant vitamins in urolithiasis. *Indian J Clin Biochem*, 22, 128–130.

50. Jonassen JA, Kohjimoto Y, Scheid CR, Schmidt M. (2005). Oxalate toxicity in renal cells. *Urol Res*, 33, 329–339.

51. Li ZH, Velisek J, Zlabek V, Grabic R, Machova J, Kolarova J, Randak T. (2010). Hepatic antioxidant status and hematological parameters in rainbow trout, *Oncorhynchus mykiss*, after chronic exposure to carbamazepine. *Chem Biol Interact*, 183, 98–104.

52. El-Demerdash FM, Yousef MI, Radwan FM. (2009). Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs. *Food Chem Toxicol*, 47, 249–254.

53. Hackett RL, Shevock PN, Khan SR. (1995). Alterations in MDCK and LLC-PK1 cells exposed to oxalate and calcium oxalate monohydrate crystals. *Scanning Microsc*, 9, 587–596.

54. Thamilselvan S, Selvam R. (1997). Effect of vitamin E and mannitol on renal calcium oxalate retention in experimental nephrolithiasis. *Indian J Biochem Biophys*, 34, 319–323.

55. Huang HS, Ma MC, Chen CF, Chen J. (2003). Lipid peroxidation and its correlations with urinary levels of oxalate, citric acid, and osteopontin in patients with renal calcium oxalate stones. *Urology*, 62, 1123–1128.

56. Puntel RL, Roos DH, Paixão MW, Braga AL, Zeni G, Nogueira CW, Rocha JB. (2007). Oxalate modulates thiobarbituric acid reactive species (TBARS) production in supernatants of homogenates from rat brain, liver and kidney: Effect of diphenyl diselenide and diphenyl ditelluride. *Chem Biol Interact*, 165, 87–98.

57. Rodrigo R, Bosco C. (2006). Oxidative stress and protective effects of polyphenols: Comparative studies in human and rodent kidney. A review. *Comp Biochem Physiol C Toxicol Pharmacol*, 142, 317–327.

58. Thamilselvan S, Menon M. (2005). Vitamin E therapy prevents hyperoxaluria-induced calcium oxalate crystal deposition in the kidney by improving renal tissue antioxidant status. *BJU Int*, 96, 117–126.

59. Santhosh Kumar M, Selvam R. (2003). Supplementation of vitamin E and selenium prevents hyperoxaluria in experimental urolithic rats. *J Nutr Biochem*, 14, 306–313.

60. Itoh Y, Yasui T, Okada A, Tozawa K, Hayashi Y, Kohri K. (2005). Examination of the anti-oxidative effect in renal tubular cells and apoptosis by oxidative stress. *Urol Res*, 33, 261–266.