Histological and Ultrastructural Study on the Effect of Celery Juice on Ethylene Glycol Induced Urolithiasis in Male Albino Rats

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1. INTRODUCTION

Renal stones (Renal Calculi) is a universal disease afflicting human beings for several centuries. The life Time risk of developing Urolithiasis is about 10–15% in the western world, but can be as high as 20–25% in the Middle East. Renal stones are caused by a variety of conditions, including metabolic and inherited disorders, and anatomical defects with or without chronic urinary tract infection (Johri et al., 2010).

Renal stone or urinary calculus can be classified by its place in the kidney (Nephrolithiasis), ureter (Ureterolithiasis), and bladder (Cystolithiasis) (Potts, 2012) or classified according to their chemical composition to calcium containing stone, uric acid stone, cysteine stone and struvite (Frassetto and Kohlstadt, 2011). The majority of human renal stones are comprised as combination of calcium oxalate monohydrate (COM) and Calcium oxalate dehydrate(COD) (Xie et al., 2015).
Renal tubular epithelial cell injury, apoptosis, and inflammation are involved in kidney stone formation (Lu et al., 2012). Oxalate-induced tissue damage may play an initiating role in the pathophysiology of calcium oxalate nephrolithiasis (Schepers et al., 2005). Severe glomerular damage, RBCs deposition, which leads to hematuria, numerous and large size CaOX crystal deposition in the renal tubule and dilation of the proximal tubules with interstitial inflammation were observed in the renal tissue (Patel et al., 2012). Renal epithelial cells had more tubular dilatation and damage shown by large spaces in the tissue. Renal stone deposition damages the renal tissue and deteriorates the renal function. Tissue injury and inflammation in animal induced stones are due to exposure to phosphate and Calcium phosphate crystals, leading to the generation of reactive oxygen species, development of oxidative stress, lipid peroxidation and depletion of antioxidant enzymes. Renal epithelial injury further promotes crystal retention, as epithelial injury exposes a variety of crystal adhesion molecules on epithelial surfaces and promotes stone formation (Thangaratinam et al., 2013).

Celery (Apium graveolens) is a herbaceous herb growing to a height of 60 to 90 cm. It has a shallow tape root system, the stem is branched succulent and ridged (Rastogi and Mehrotra, 1990). The native habitual of celery is the lowland of Italy from where it spread to Sweden, Egypt, Algeria and Ethiopia in Asia to India. Celery firstly cultivated food plant in France in 1623 (Fazal and Singla, 2012). Celery leaf contains limonene and myrecene essential oils with antibacterial activity (Sipailiene et al., 2003). Beside antimicrobial activity, gupta et al. (2015) mentioned antiproliferative effects of limonene in breast cancer cells. (Gupta et al., 2015)

Celery belongs to the plant family of Apioideae with several medicinal usage like: hypotensive effect (Moghadam et al., 2013), neutralizing agent (Asif et al., 2011) and antimicrobial activity (Marongiu et al., 2013).

The aims of the present study was:

-To study the protective effect of celery against Ethylene glycol induced renal stone formation in rats from histological and ultrastructural points of view.

2. MATERIALS AND METHODS

The present study was conducted on 21 mature male albino rats (Rattus norvegicus). All rats were healthy, weighing 250 – 300 gm and 8 weeks old at the time when the experiment started. The animals were bred and housed in plastic cages (56 x 39 x 19 cm) bedded with wooden chips in groups of seven rats per cage in standard animal house condition.

Celery was grown then aerial part of plant was harvested and washed by tap water. Aerial part cut by homogenizer and macerated in pistil and mortar then filtered by gauze. Systematic identity of the plant confirmed in College of Science/ Salahaddin University and, the convenient dose for the plant was determined by preliminary test (1 ml/kg).

The rats were divided randomly into three groups, each group consisted of seven rats per cage: Group 1, Control, the rats of this group were given standard rat chow and tap water for 28 days. Group 2, ethylene glycol induced urolithiasis model. The rats of this group were given standard rat chow and ethylene glycol (0.75%) in drinking water ad libitum for 28 days. Group 3, Celery (1 ml/kg), the rats received standard rat chow, (1 ml/kg) celery by gavage and 0.75% ethylene glycol in drinking water ad libitum for 28 days.
The animals were anesthetized by intraperitoneal injection of combination Ketamine hydrochloride 80mg/Kg (Trittau, Germany) and Xylazin 12mg/Kg (Interchem, Holland). After sacrificing, kidneys removed then fixed in desired fixative according to the type of microscopical preparation.

Kidneys were removed from the anesthetized animals, immediately fixed in Bouin’s solution for 24 hours, followed by dehydration using a series of ethanol in ascending concentrations (50%, 70%, 95% and 100%), then immersed in xylene for clearing process, infiltrated with paraffin wax and embedded in paraffin wax. Five micrometer thick paraffin sections were obtained using rotary microtome (Bright, MIC) and stained by hematoxylin and eosin (H&E) (Bancroft et al., 1977). The specimens were examined and photographed under light microscope (digital binocular compound microscope 40x-2000x, built-in 3MP USB camera).

Scanning electron microscopy was done in University of Niece, France. Kidneys were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer pH 7.2-7.4. After washing dehydrated in ethanol (50%, 70%, 85%, 100% and 100%), the samples were put in desiccator for air drying, after mounting they were coated by coater machine (JEOL fine coat, JFC-1100E ION SPUTTER, JEOL TOKYO JAPAN) with gold and then examined by SEM (JEOL scanning microscope JSM-5310 LV, JEOL TOKYO JAPAN).

Samples of kidneys (<1mm3) were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer pH 7.2 - 7.4 for 24 hours, washed by cacodylate buffer 0.1M, postfixed in 1% Osmium tetroxide for one hour, dehydrated through a graded series of ethanol (50%, 70%, 95% and 100%) and then cleared in acetone for 15 minutes (twice). Then infiltrated with acetone plus resin mixture (TED PELLA, INC, USA) in ratio of (1:1) for one hour, then with acetone plus resin mixture (1:3) over night and finally embedded in resin medium. Polymerization accomplished in oven at 60°C for 48 hours. Thin sections (90 nm) of Plastic blocks were taken on Ultramicrotome (RMC PT-XL Boeckler Instruments, Inc., Tucson, Arizona) and placed on 200 mesh copper grids and stained by uranyl acetate and lead citrate. Sections were viewed and photographed at a variety of magnifications on transmission electron microscope (Hitachi H-7500) of Washington University in St. Louis.

3. RESULTS AND DISCUSSION

After 28 days of oral treating of EG, several histological and ultrastructure alteration were seen in the kidney of rats. Kidney sections through the control group has shown healthy and normal histological kidney structure with normal appearance of tubules and glomeruli (Fig. 1). Paraffin sections through the kidney of EG treated group showed dilated kidney tubules with accumulated crystals inside some of renal tubules. The tubular crystal accumulation has led to compression on adjacent tubules or inserting into epithelial lining cells (Fig. 2) . This may be one of the reasons beyond the death of cells in the renal crystal deposited cells. Mitotic figures in epithelial cells of renal tubules were also observed. The study by Tanimoto et al. (1993) were especially noteworthy because the authors also identified proximal tubular cells that appeared to be proliferating as part of the response to apoptotic injury. An accumulation of inflammatory leucocytes in the kidney of EG treated group was appeared and this also might be attributed to oxalate (Makasana et al., 2014).

paraffin sections through kidney rats treated with celery having approximately normal kidney structure and few number of
dilated tubules filled with crystals (Fig. 3). Corticomedullary region of kidney has shown some CaOx crystals deposition. The protective role of celery against renal stone formation may be due to alkalization effect of celery on urine (Asif et al., 2011). Acidic urine comprised as a strong promoter for renal crystal deposition. Celery contains high amount of magnesium and potassium (Fazal and Singla, 2012). Magnesium makes complex with oxalate and decreases the absorption of oxalate (Liebman and Costa, 2000) and also was found to increase urinary citrate excretion (Reungjui et al., 2002). On the other hand potassium increases the urinary citrate excretion that acts as renal stone inhibitor (Johri et al., 2010).

Images that were captured by scanning electron microscope showed the three dimensional image of crystals more clearly in comparison to other techniques.

The kidneys in the control group (Fig. 4) were appeared with normal and healthy structure having no crystals, while clear large crystals (Fig. 5) were revealed in the kidney tissue of EG treated rats in addition to several morphological alterations due to renal stone formations such as tubule shape disturbance and blood vessels congested with red blood cells.

SEM of rat kidneys belong to EG+ celery juice treated group shown approximately normal structural appearance (Fig 6). These SEM images added more information about the size of damage that such renal crystal may cause through showing the three dimensions of them and how they can produce pressure on cells, tubules and blood vessels. The present investigation, as was seen in the SEM images revealed the surface of the stones. Such views couldn’t be obtained by the other techniques (Marickar et al., 2009).

Transmission Electronmicrographs of the kidney of control group (Fig.7) have shown normal ultrastructure of glomeruli with healthy podocytes, blood capillaries and mesangial cells.

Transmission electron microscopy of rat kidneys treated by EG showed different results. Shrinkage and degeneration of podocytes, dilatation of capillaries (Fig. 8) and thickening of capillaries basement membrane of glomeruli were seen (Fig. 9). Such damages which included most of the cells of renal tubules and glomeruli is induced by the oxidative stress, which is produced during the attachment of crystals to renal tubular epithelial cells (Khan, 2004).

Proximal convoluted tube cells of this group were seen contained large numbers of vacuoles filled with amorphous materials and lysosome like electron dense vacuoles as well as swelled mitochondria (Fig. 10). In renal tubular cell injury, mitochondrial damage has been recognized as a crucial cause for tubular cell death which involves disruption of respiration complexes and loss of mitochondrial membrane potential (Kaushal et al., 2004), (Servais et al., 2008). Cell apoptosis is precipitated by mitochondrial outer membrane permeabilization and consequent release of apoptogenic factors such as cytochrome c (Brooks et al., 2009).

Electronmicrographs of rat kidney treated with EG+celery juice have shown approximately normal structure of glomeruli, normal mesangial cells and podocytes (Fig. 11. Cells of proximal convoluted tubules were in normal state with normal microvilli but some swelled mitochondria and electron dense vacuoles can be also seen (Fig. 12).

Such attenuation role of celery juice to the ultrastructural changes and even the histological alteration that induced by ethylene
glycol can be explained by the oxidant – antioxidant mechanism, since celery is found to have an antioxidant content (Yao et al., 2010). As ROS (reactive oxygen substance) plays an important role in renal calcium crystallization, celery may have this therapeutic effect.

**Figure 1.** Paraffin section through the kidney of control rats showing normal histological structure A) Cortical region containing glomeruli (arrows) surrounded by kidney tubules, 100X, H&E. B) higher magnification showing the glomerulus (G), healthy kidney tubule lining cells (blue arrow) and macula densa (black arrow), 400X, H&E.
Figure 2. A & B) Paraffin sections through the kidney of ethylene glycol treated rats showing dilation of kidney tubules (*) some are filled with crystals(C) and inflammatory cells are seen (IC) 100X, H & E.
Figure 3. Paraffin sections of EG+ celery juice treated rat kidney showing approximately healthy kidney structure, few number of tubules filled with crystals (arrow) and dilated tubules (*) are still seen. 40X H & E.

Figure 4. Scanning EM image showing kidney rat of control group with normal histological structure of glomeruli (G).

Figure 5. Scanning EM image of EG induced rat kidney shown crystals(C) and congested blood vessels and red blood cells (G).
Figure 6. Scanning EM image of EG kidney rat treated with celery juice showing approximately normal histological structure. Glomerulus (Arrows).

Figure 7. Electronmicrograph of control group rat kidney showing normal ultrastructure of the podocyte (PC) with their foot processes and pedicles, capillaries lined by endothelial cells (EN), Mesangial cells (MC) and the red blood corpuscles (RBC) in the lumen of capillaries (CL).
Figure 8. Electronmicrograph of EG treated rat kidney showing shrinkage of podocyte (PC) and dilatation of capillaries (CL). Phagocytosis in the lumen of capillary is seen (arrow). Endothelial cell (EN), mesangial cells (MC), glomerular basement membrane (elbow arrow).

Figure 9. Electronmicrograph of EG treated rat kidney showing part of glomerulus with degenerated podocytes (elbow arrow), endothelial remnant of blood capillary (arrow) and thickening of capillary basement membrane is seen (BM). Podocyte (PC), capillary lumen (CL).
**Figure (10)** Electronmicrograph of proximal convoluted tubule of EG treated rat kidney showing variable sized vacuoles containing amorphous materials (arrow), destructed microvilli (MV) and swelled mitochondria (M). Glomerular basement membrane (GBM).

**Figure 11.** Electronmicrograph of rat kidney treated with EG+ celery juice showing approximately normal mesangial cell (MC) and podocyte (PC) in glomerulus. Normal endothelial cell also seen (EN).
4. CONCLUSIONS

Celery has lowered renal crystal deposition and approximately protected the organ against EG toxicity. Celery has given good results in ameliorating the ultrastructure of the EG treated rat kidney, especially the degeneration of the cells and mitochondrial changes.

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