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Research Article

Radish Juice Promote Kidney Stone Deposition in Ethylene Glycol-induced Urolithiasis in Rats

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ABSTRACT

Urolithiasis is a well-known problem that stones could form in various parts of the urinary system and it is the most common disease of the urinary tract. The current study was planned to investigate the effect of radish juice on ethylene glycol (EG)-induced urolithiasis. Twenty-one rats randomly divided into three groups. The first group was the control group was received normal standard diet and drinking water and the second group represented the model group received 0.75% EG in drinking water *ad libitum*. The third group received radish juice (2 ml/kg of body weight) by gavage plus EG (0.75%) in drinking water *ad libitum*. The experiment was conducted for 28 days. The light microscope examination revealed a disturbed histological architecture of the kidney tissues, including dilated renal tubules, aggregation of infiltrated leukocytes inflammatory cells, and crystal deposition in the model group. The EG plus radish juice treated rats showed higher crystal density with improved renal tubule structure and alleviated inflammation. Both treated groups showed various biochemical alterations compared to control group, but the most interest biochemical result was the significant decrease of malondialdehyde, a lipid peroxidation marker, and in the radish plus EG group compared to the EG group. Scanning electron microscopy showed clear structural detail about calcium oxalate crystals in which radish-treated group showed higher crystal deposition and calcified tissue compared to EG group. The present study concluded that radish juice promotes stone deposition but exerted an antioxidant effect.

Keywords: Ethylene glycol, kidneystone, radis, urolithiasis

INTRODUCTION

Urolithiasis is a well-known problem that stones could form in various parts of the urinary system.[1] Mankind has been afflicted by urinary stones for centuries dating back to 4000 B.C. and it is the most common disease of the urinary tract.[2] Urolithiasis affects about 12% of the world population at some stage in their lifetime.[3] Urolithiasis is a complex process that occurs due to imbalance between promoters and inhibitors.[4] The chemicals that can promote stone formation are calcium, sodium, oxalate, urate, and cystine, while magnesium, citrate, and pyrophosphate can inhibit the urolithiasis process.[5] Calcium containing stones are the most common renal stones with a percentage of 80% of cases.[6] The process of stone formation begins with supersaturation that is the driving force for crystallization in solutions like urine.[7] As a result of supersaturation, solutes precipitate in urine leads to nucleation and then crystal growth. Crystallization happens when chemical concentrations exceed their point of saturation.[8] Then after, small hard mass of crystals sticks to each other to form a larger stone that is called crystal aggregation.[7] Plants such as *Oxalis corniculata,*[9] *Phyllanthus niruri,*[10] *Bergenia ligulate,*[11] and *Nigella sativa*[12] have been used as a remedy for renal calculi. Radish (Raphanus sativus) is a member of the family Cruciferae. This family consists of annual, biennial, or perennial herbs with pungent oils in the sap. There are about 380C Genera and about 3000 species.[13] Varieties of radish are now broadly distributed around the world, but there are almost no archeological records available to determine its early history and domestication.[14] Radish has been shown medicinal benefits such as gastroprotective effects[15] as a remedy for diabetes treatment[16] and anti-microbial activity.[17] The present study has been designed to evaluation of protective effect of radish juice on renal stone.

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MATERIALS AND METHODS

Experimental Animals and Radish Juice Preparation

For conducting this experiment, 21 mature male rats were bred under standard animal house conditions. For radish juice preparation, radish obtained from local market in Erbil city, systematic identity confirmed in College of Science/ Salahaddin University, washed, cut to small pieces, and homogenized then filtered by gauze. 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 (EG)-induced urolithiasis model. The rats of this group were given standard rat chow and EG (0.75%) in drinking water *ad libitum* for 28 days. Group 3, radish, the rats received standard rat chaw, radish juice (2 ml/kg of body weight) by gavage, and 0.75% EG in drinking water *ad libitum* for 28 *days*.

The animals were anesthetized by intraperitoneal injection of combination ketamine hydrochloride 80 mg/kg (Trittau, Germany) and xylazine 12 mg/kg (Interchem, Holland). After sacrification, kidneys removed then fixed in desired fixative according to the type of microscopical preparation.

Kidney Weight Recording

Both kidneys have removed, weighted, and expressed as gram per 100 *g* of body weight.

Urine Flow

Animals remained in urine collector and urine was collected for 24 *h* then urine flow measured as ml/h/kg.

Fluid Intake

Fluid intake recorded every 4 *day*s during the experiment.

Blood Collection

At the end of the treatment period, blood samples were collected from all anesthetized rats of four groups through cardiac puncture in which the collected blood samples were immediately placed into gel tube for serum collection, later were centrifuged (Hettich D-78532/Germany) at 3000 *rpm* for 15 *min*. The sera were stored at −80°C (Sanyo – Ultra-low Temperature, Japan) until assayed.

Serum Urea Determination

Urine and serum urea was determined by urea kit (BIOLABO, France). Enzymatic and colorimetric method of urea determination is based on hydrolysis of urea to ammonium and carbon dioxide by urease. Ammonium reacts with chloride and salicylate and makes a blue-green complex that is proportional to urea concentration and measured at 600 *nm*.

Estimation of Creatinine and Glomerular Filtration Rate

Serum-urine creatinine was determined spectrophotometrically using BIOLABO kit (France).

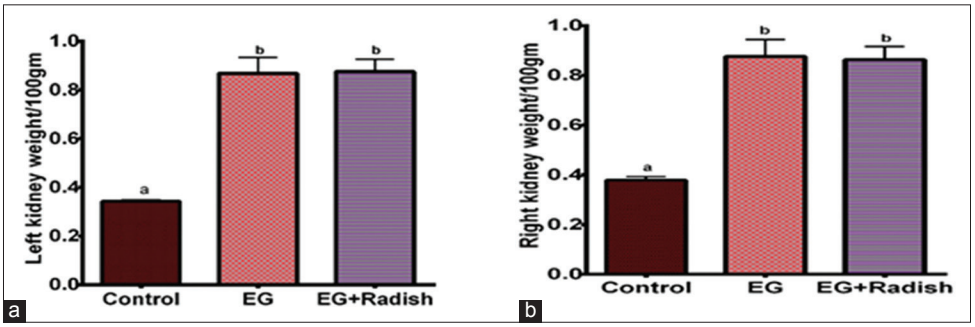

Figure 1: Left (a) and right (b) kidney weight per 100 g of body weight. Different letters on bars mean significant change and the same letters means no significant change

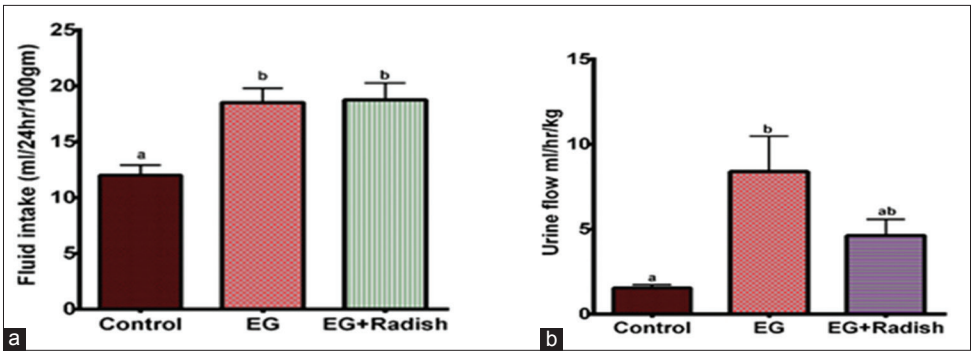

Figure 2: Fluid intake (a) and urine flow (b) of control and ethylene glycol treated groups. Different letters on bars mean significant change and the same letters means no significant change

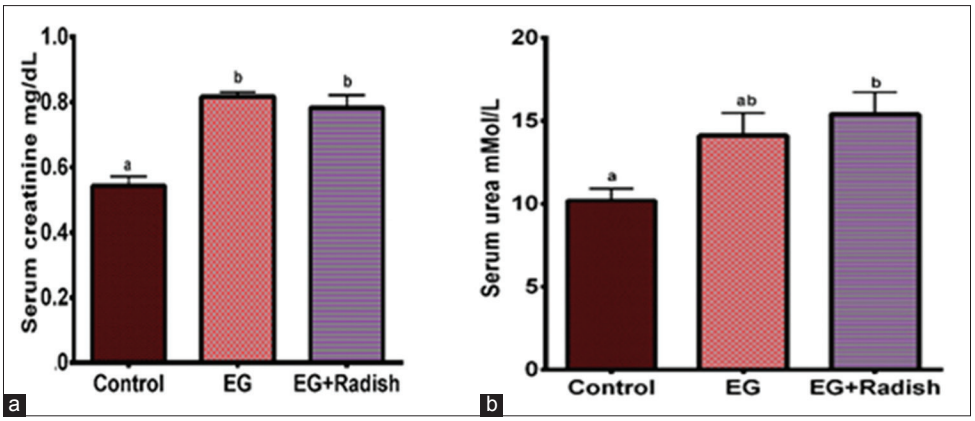

Figure 3: Serum creatinine (a) and serum urea (b) of control and ethylene glycol treated groups. Different letters on bars mean significant change and the same letters means no significant change

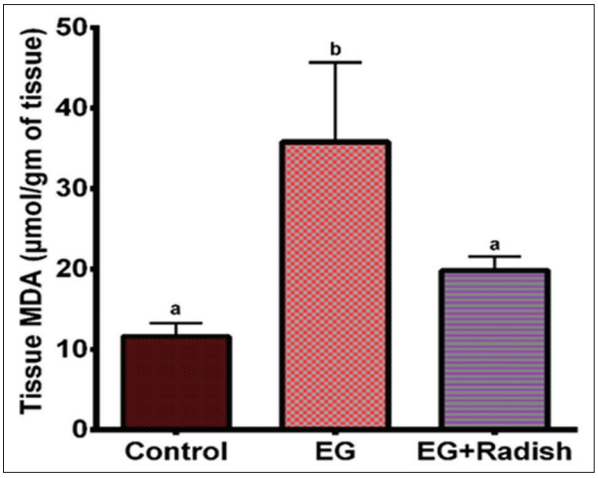

Figure 4: Level of tissue malondialdehyde in control, model, and ethylene glycol +radish treated groups. Different letters on bars mean significant change and the same letters means no significant change

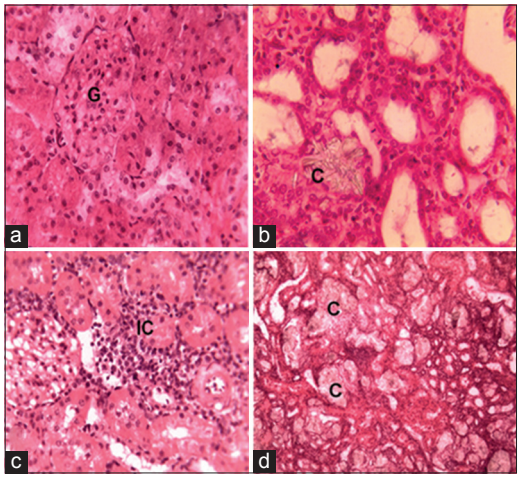

Figure 5: Kidney sections of control group (a) show normal appearance(G): Glomerulus.×400. Hematoxylin and eosin (H&E). (B) Kidney sections of model group (b) show a lot of crystal deposition(C) ×400. H&E. Kidney section of radish treated group (c and d) shown inflammatory cells and crystal deposition (C) ×400. H&E

Creatinine reacts with picrate to form a colored complex in alkaline solution. Absorbency of samples was read at 500 *nm*.

Determination of Kidney Tissue Malondialdehyde (MDA)

The right kidney of each rat was washed in ice-cold normal saline solution then homogenized in 20 mM phosphate buffer (pH = 7.4; tissue/buffer ratio, 1/10 *w/v*) by handheld glass homogenizer (Chowdhury *et al*., 2013). Homogenates were centrifuged at 4000 *g* at 4°C for 10 *min* (Beckman J2-21). The supernatants were collected and stored at −8°C until assayed. MDA level was estimated according to method of Kartha and Krishnamurthy (Kartha and Krishnamurthy, 1978). 1 *ml* of tissue homogenate was added to 20% trichloroacetic acid. After centrifugation for 10 *min*, 2 *ml* of supernatant was taken in a test tube and 2 *ml* of 0.7% thiobarbituric acid was added to each tube and kept it in the boiling water bath for 20 *min*. The development of pink color was measured at 535 *nm* and MDA concentration was calculated by the following equation:

$$\text{MDA nmol/g of tissue} = \text{Absorbance at 535 nm} \times D / (L \times Eo)$$

L: Lightpath (1 cm)

E o: Extinction coefficient 1.56×105 M−1.cm−1

D: Dilution factor.

Light Microscopy

Kidneys were removed from the anesthetized animals, immediately fixed in Bouin's solution for 24 *h* 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. 5 μm thick paraffin sections were obtained using rotary microtome (Bright, MIC) and stained by hematoxylin and eosin. The specimens were examined and photographed under light microscope (digital binocular compound microscope ×40–×2000, built-in 3MP USB camera).

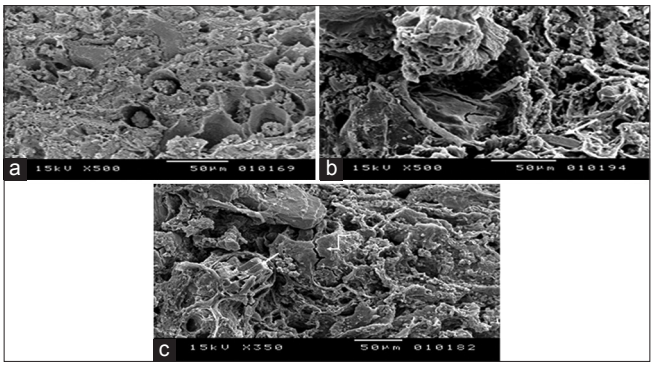

Figure 6: Scanning electron microscopy of kidney (a) control group showing normal appearance (b) ethylene glycol (EG)-treated group showing crystal deposition (white arrow) and calcified tissue (blue arrow). (c) EG + radish treated group showing more crystal deposition (white arrow) and calcified tissue (elbow arrow)

Electron Microscopy

Scanning electron microscopy was done at the 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 with gold and then examined by scanning electron microscope (SEM).

Statistical Analysis

Data entry was done by Microsoft excel 2010 and analyzed statistically by one-way analysis of variance using GraphPad Prism *version* 6.01 and SPSS *version* 20 and data expressed as mean ± standard error. Newman–Keuls tests were chosen as *post hoc* tests.

RESULTS AND DISCUSSION

As shown in Figure 1, both the left and right kidneys weight in model and radish-treated group increased significantly when compared with control group. Although many cells may degenerate in response to EG toxicity or to the formation and presence of crystals in the kidney, the increase in kidney weight in EG and EG-treated plant juice was expected at least due to the presence of renal crystals.[18] Fluid intake [Figure 2a] in EG and radish-treated groups elevated in compare of control group. Increasing fluid intake in EG-induced group maybe return to sweet taste of EG[19] or nephron damage that could not reabsorb glomerular filtrate again.

As illustrated in Figure 2b, urine flow in model group increased significantly when compared to control group; however, there is no statistical difference between model and radish-treated group. With respect of serum creatinine, Figure 3a shows significant raising in EG and radish-treated groups when compared to control group, while there is no statistical difference between both EG-treated groups. The elevation of serum creatinine may be due to renal tubules obstructions.[20] Figure 3b revealed that there was no statistical difference of serum urea between control and model group and between model and radish treated group but serum urea elevated in radish group when compared to control group. Increasing of serum urea is related to the level of nephron injury[21] and since more crystals were deposited in the kidney of EG plus radish group leading to more kidney damage, this may explain the higher serum urea noticed in this group in comparison to the control group. Furthermore, urea is by product of protein metabolism and this rising in serum urea may return to the fact that radish itself contain total protein of 6.5%.[22] As shown in Figure 4, the level of MDA in model group raised when compared with control group while there was no significant difference between control and radish treated group. MDA is by product of lipid peroxidation and is used as marker for this process and oxidative stress induced by EG through elevating MDA level confirmed previous findings.[23,24] Under hyperoxaluria, oxalate reacts with polyunsaturated fatty acid in cell membrane[25] and make damage. Calcium oxalate which induced oxidative stress can help crystal attachment to epithelial cells of nephrons (cell-crystal attachment).[26] Radish juice succeeded in lowering the MDA level compared with EG group and it may be due to the antioxidant contents of radish since it has been found to contain phenolic compounds and ascorbic acid.[27]

Paraffin sections of kidney belong to control group (Figure 5a) show normal and healthy appearance. Kidney sections of model group have shown crystal deposition and dilated tubules [Figure 5b] that is match with finding of the previous study.[28] Inflammatory cells (IC) accumulation also observed that is maybe due to ability of calcium oxalate to triggering immune system.[29] Oxalate, the major stone-forming constituent, has been reported to induce lipid peroxidation and causes tissue damage by reacting with polyunsaturated fatty acids in cell membrane.[23] On the other hand, some investigations say that lipid peroxidation is not the underlying cause of renal injury in hyperoxaluric rats.[30] Paraffin section through the kidney of EG + radish juice treated rats has shown more quantity of crystal deposited in lumen of kidney tubules [Figure 5d] and a lot of inflammatory leucocytes were observed in kidney tissues [Figure 5c]. Radish has been found to contain four major oxalic, malic, malonic, and erythroic acid.[31] Oxalic acid itself is one of the promoters of renal stone formation. Despite promoter activity of oxalic acid, total acidity of these four acids may decrease urine pH and makes a favor environment for calcium oxalate crystals.

Scanning electron microscopy showed the three-dimensional image of crystals more clearly in comparison to other techniques. The kidneys in the control group were appeared with normal and healthy structure having no crystals [Figure 6a]. Clear large

crystals were revealed in the kidney tissue of EG-treated rats [Figure 6b]. SEM of rat kidneys belong to EG + radish juice treated group showed crystals deposition in wide distribution and in the most area, the tissue appeared calcified [Figure 6c].

CONCLUSION

Radish treated group has shown more crystal deposition, damage, and IC in compare to model; however, radish juice decreased MDA level significantly. Radish juice could not improve the state of experimental rats in kidney weight, renal function tests, and fluid intake.

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