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Anti-Urolithiatic and Anti-Oxidant Effects of Fenugreek on Ethylene Glycol-Induced Kidney Calculi in Rats

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Abstract

Renal calculi formation is one of the most common urological disorders. Urinary stone disease is a common disease which affects 10-12% of the population in industrialized countries. The objective of the present study is to investigate the antiurolithiatic and antioxidant activity of fenugreek on ethylene glycol-induced urolithiasis in rats. Twenty male rats weighing 203-263 were used for this study. Group A animals received distilled water for 28 days. Group B to group D animals received 1% v/v ethylene glycol in distilled water for 28 days. Groups C and D received cystone and fenugreek, respectively, from day 15 to day 28. On day 28, blood was collected for serum malondialdehyde (MDA) and calcium level monitoring. Kidneys of rats from all the groups were removed, and histopathologically examined using Paraffin method. Samples of kidney were viewed under a scanning electron microscope. Histologically, the kidney of fenugreek treated group appeared mostly to be calculi-free, even better than the cystone treated group. Similarly, the calcium oxalate deposits, the serum (MDA), the renal tissue calcium content were all significantly lower than those in the other groups. The results of the present study suggest that fenugreek has a strong antiurolithiatic and antioxidant activity.

Keywords: Antilithiatic, Fenugreek, Nephrolithiasis, Urolithiasis, Renal Calculi, Ethylene Glycol, Cystone.

1. Introduction

Kidney stone disease is a common disorder that has been on the rise in Western societies for the last five decades. Kidney stone formation is a complex process and it results as a cascade of events, including crystal nucleation, growth and aggregation, crystal retention within the renal tubules (Coe and Parks, 1983). Usually kidney stones are yellow or brown color with a smooth or gagged structure. Some common type of kidney stones are calcium oxalate, calcium phosphate, struvite, uric acid and cysteine, among of which calcium stones are the most common form of kidney stones in both humans and rats (Sunitha *et al.*, 2012). Urolithiasis, also called calculi or uroliths, is a condition which involves the process of stone formation in the kidney. Renal stones are a universal cause of blood in the urine and pain in the abdomen, with a reported incidence about 12% in the general population (Araújo Viel *et al*., 1999; Kumar *et al.*, 2005).

Fenugreek is one of several herbal medicines whose seeds and leaves are used either as food or as an ingredient in folk medicine (Bellakhdar, 1997). Fenugreek leaves and seeds are consumed in different countries around the world for various purposes, such as medicinal uses, e.g., as an anti-diabetic, for lowering blood sugar and cholesterol levels (Lafta, 2010), as an anti-cancer and anti-microbial; it is also used in food and drinks in many Eastern and Western countries. Fenugreek can be a very useful legume crop for the incorporation into a short-term rotation and for hay and silage for the livestock feed, for the fixation of nitrogen in soil and its fertility (Sadeghzadeh-Ahari, 2009). The fenugreek seeds have been proven to have both hypoglycaemic and anticholesterolemic properties (Sharma, 1986 a; b). It can attenuate oxidative stress indirectly by reducing the lipid peroxidation (Shekha *et al.*, 2014). Calcium oxalate urolithiasis model has commonly been used to investigate the influence of urolithiasis on experimental model in rats. This model is induced by ethylene glycol (EG), a precursor to oxalate formation (Bayir *et al.*, 2011). EG poisoning can lead to acute renal failure which is characterized by a proximal tubular necrosis and an accumulation of calcium oxalate monohydrate crystals in the urine and kidney tissue. The prices mechanism is probably due to the calcium oxalate monohydrate's adherence to tubular cells primary hyperoxaluria and kidney stone formation (McMartin, 2009). Many studies indicated that the Cystone® has a potent anti-lithiatic (prevents the formation of kidney stones) and lithotriptic (dissolves kidney stones) properties by decreasing urinary supersaturation or micropulverizes and diuretic that flushes out small kidney stones (Karamakar and Patki, 2010; Kumaran and Patki, 2011). On the other hand, it was also reported that malondialdehyde production

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increased in the presence of oxalate and stone forming since oxalate in the kidney induces cell death mediated by a cellular necrosis because it induces changes in the membrane integrity, cellular enzyme release and membrane lipid peroxidation (Park *et al.,* 2008). In fact, several of the published studies reported that serum calcium did not change in ethylene glycol induced kidney stone in rats (Laroubi *et al*., 2007; Pareta *et al.,* 2011), while other studies reported an increased in serum calcium (Jafar *et al.,* 2011); sometimes decreased in serum calcium (Soundararajan *et al.,* 2006). Some of the therapeutic uses of fenugreek include its use as an anti-urolithiasis (Laroubi *et al.,* 2007). Fenugreek seeds have been used by traditional medicine for problems of kidney (Snehlata and Payal, 2012). The mechanism underlying the anti-urolithiasis effect is still unknown, but apparently it is related to increased diuresis, antioxidant activity and lowered urinary concentrations of stone forming constituents (Laroubi *et al*., 2007).

The present study is designed to investigate the anti-urolithiatic activity of fenugreek and its effect on kidney abnormalities induced by ethylene glycol in rats.

2. Materials and Methods

2.1. Plant Material

Trigonella foenum graecum L. seeds were purchased from a local market, Erbil city, Kurdistan, Iraq. A voucher specimen was deposited at the Herbarium of Department of Biology, College of Science.

2.2. Animal and Treatment

Male Wistar albino rats (203 to 263 g) were obtained from the Animal House, College of Science, University of Salahaddin, Kurdistan region of Iraq. Twenty Wistar rats, maintained for ten days under experimental conditions, were divided equally into four groups, each of five animals.

All animals had a free access to drinking water ad libitum and regular food, and they were kept under controlled conditions.

Hyperoxalurea and CaOx deposition in the kidney was induced by adding Ethylene Glycol (EG) to the drinking water to a final concentration of 1% for all groups except for the control group (A) which was supplied with normal water and diet. Group (B) received drinking water supplemented with EG (1%) for 28 days. Group (C) was given 2.5 tablets of Cystone in 100 ml of water and 2.5 tablets in 100 g of standard diet+ 1% EG, while group (D) was given 10 gm of fenugreek in 100 ml of water and 10 gm in 100 gm of standard diet+ 1% EG. At the end of the experiment, the serum (MDA) and calcium were determined.

2.3. Determination of Serum Malondialdehyde (MDA)

The level of serum (MDA) was determined spectrophotometrically with a thiobarbituric acid (TBA) solution. In brief, to a 150µl serum sample following were added: 1ml of trichloroacetic acid (TCA) 17.5 % with 1ml of 0.66% TBA were mixed well by vortex, incubated in boiling water for 15 minutes, then allowed to cool. One ml of 70 % TCA was added and the mixture was left to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15minutes, then the supernatant was taken out for scanning spectrophotometrically. The concentration of (MDA) was calculated as follows:

MDA= (AxD)/(LxE)

(MDA) (µmol/L) = Absorbance at 532 nm X D/ L x Eo,

where L: light bath (1cm)

Eo: Extinction coefficient 1.56 x 105 M-1.Cm-1

D: Dilution factor = 1 ml Vol. used in ref. / 0.15 =6.7(Burtis and Ashwood, 2005)

2.4. Paraffin Method

Kidney pieces were removed and fixed in Bouin's fluid, dehydrated, cleared, embedded in paraffin and cut into 4-5µm thick section, then stained by hematoxylin and eosin (Kierrnan, 1981).

2.5. Scanning Electron Microscopy

Kidneys were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer pH 7.2-7.4 for 24 hours. After being washed by cacodylate buffer 0.1M, they were postfixed in 1% Osmium tetroxide for two hours, and dehydrated in ethanol (50%, 70%, 85%, 100% and 100%). Then the samples were put in desiccator for air drying, after mounting they coated with gold by coating system (E5200 AUTO SPUTTER COATER) and then examined by SEM in Malaysia (ZEISS, super A, 55VP) (Rasul and Aziz, 2012).

3. Results and Discussion

. The histological structure of the ethylene glycol treated rat kidney showed a lot of alterations compared with the normal structure of control kidney (Figures 1-3). As seen in Figure 2, highly inflammated regions were seen in the kidney of the EG treated rat kidney in which large numbers of leukocytes forming large foci occupied the cortical region. Furthermore, a kidney tubule lumen widening was obvious. The high number of kidney tubules filled with stone crystals is shown in Figure 2b. The crystals caused a further widening of the tubules. The crystals appeared colorless. Further occurrence of crystals within the tubulesis is shown in Figures 3a-d. In Figure 3c, the dilatation of kidney tubules and the deposition of crystals caused a compression on the glomerulus. The scanning electron images showed the crystals more clearly (Figures 3e and f).

With respect to the rats exposed to EG plus cystone, still dilatation of kidney tubules and the occurrence of tubules containing crystals were detected but with a lower density compared with EG treated group. Infiltration of inflammatory leukocytes also appeared in this group (Figure 4).

Treating the rats with fenugreek in addition to EG showed a disappearance of the crystals, and the normal kidney tubules structure was approximately similar to the control group (Figure 5).

As seen in Figure 6, a significant elevation in MDA (µmol/L) levels resulted after 28 days of EG administration (36.67±1.188) compared to control group (4.804±0.3053). Fenugreek and cystone significantly diminished (P<0.0001) the levels of serum (MDA)

(8.180±1.125, 8.484 ±1.023, respectively). Malon-dialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes an overproduction of the MDA. Malondialdehyde level is commonly known as a marker of oxidative stress (Gawel *et al.*, 2004). It seems that fenugreek plays an antioxidant role against oxidative stress that is induced by the ethylene glycol, since the MDA level was elevated in this group. However, serum calcium did not show a significant change (Figure7).

Several experiments suggested the primary contribution of the increased production of ROS in ethylene glycol group (Green *et al.*, 2005), cystone treatment may lead to an increase in the citrate concentration which might have reduced the crystallization of calcium oxalate (Ruckmani *et al.*, 1998).

The present investigation showed a quite disappearance of stone crystals in the EG treated rats after being exposed to fenugreek extract compared to the EG treated group in such a way that exceeds the anti-urolithial effect of cystone. In addition to the disappearance of kidney stone in the fenugreek plus EG treated group, no inflammation was noticed and much less cell degeneration was detected; this may be due to the antioxidant effect of this plant (Shekha *et al.*, 2014), since the oxidative stress is an important cause of cell death, especially the necrotic mode of cell death (Choi *et al.*, 2009; Hanus *et al*., 2013) which is accompanied by infiltration of inflammatory leukocytes(Zitvogel *et al.*, 2010).

The extract of some plant leaves treatment suppresses the increase of the intracellular calcium. The exact reason of this effect is not clear; however it might be due to the increased bioavailability of NO (nitric oxide) which, in turn, activates cGMP (3,5 cyclic guanosine monophosphate) that controls the increase of the intracellular calcium levels (Makasana *et al.*, 2014). Nitric oxide donors have the capacity to control the intracellular rise in the calcium levels. Thus, the plant extract could effectively control the levels of both the salts by the mechanism, such as inhibiting the oxalate or increasing the bioavailability of NO to sequester calcium through the cGMP pathway (Pragasam *et al.*, 2005).

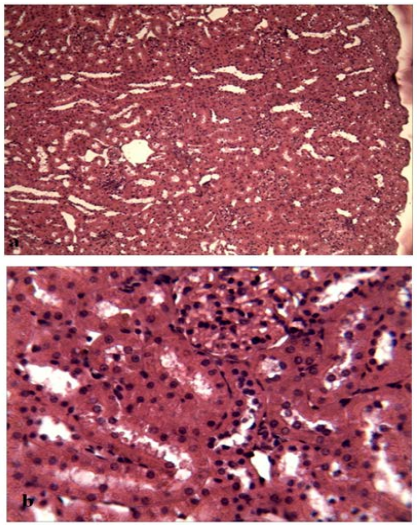

Figure 1. Section through rat kidney in control group, a) lower power, 100X, b) high power, 400X.Both images show the normal structure of the kidney.

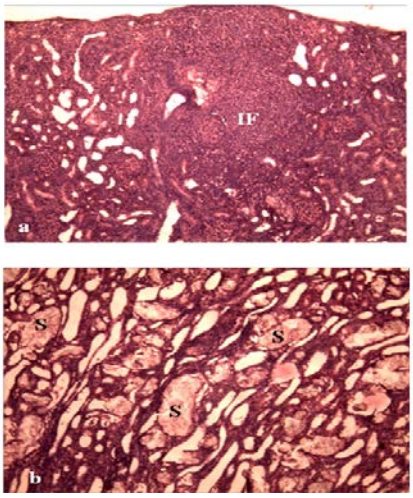

Figure 2 . Sections through the kidney of rat exposed to ethylene glycol showinga) accumulation of high number of inflammatory blood cell infiltration (IF), 100X, b) the high number of the kidney tubules filled with stone crystals (S), 100X. Both images show kidney tubule dilation.

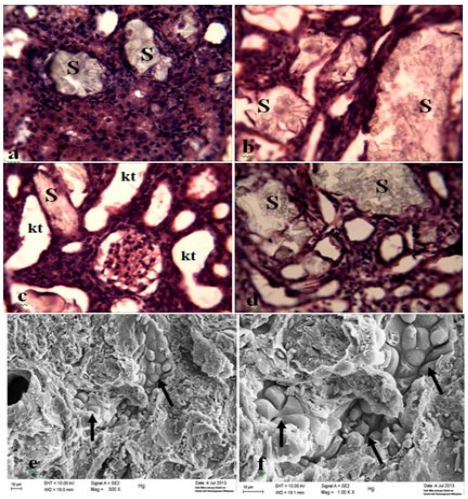

Figure 3. Ethylene glycol treated rat kidney showing the appearance of the crystals within the ratkidney tubules (S), inflammatory leucocytes (IF) and tubule dilation (kt), a-d) paraffin section, 400X, e, and f) scanning electron microscopy showing the crystal in the tubules (arrows).

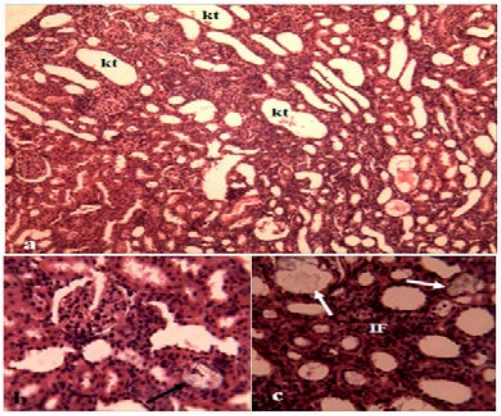

Figure 4 . Sections through the kidney of rat exposed to cystone plus ethylene glycol, still few crystals deposition (arrows) are seen in addition to inflammatory cells(IF) and the kidney tubule dilatation(kt) , a) 100X and c)400X.

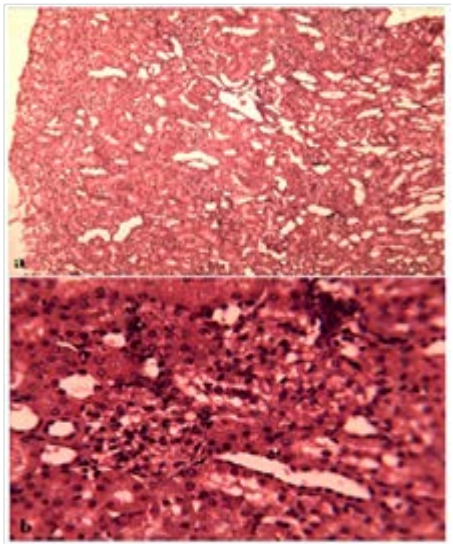

Figure 5 . Sections through ethylene glycol plus fenugreek treated rat kidney showing normal histological structure, a) 100X and c)400X.

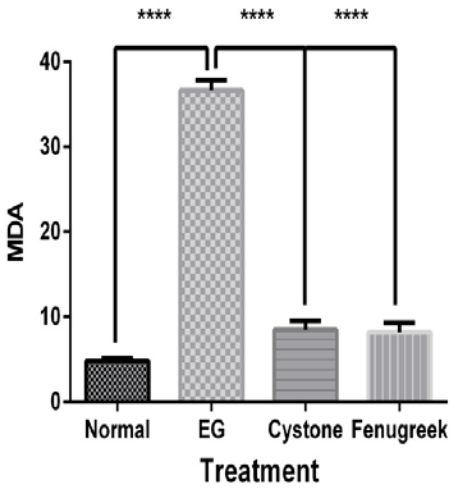

Figure 6. The effect of Fenugreek seeds on (MDA) of rats. Each column and vertical bar represents mean ± SEM of 5 animals.

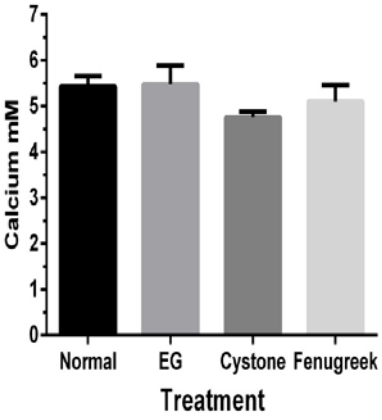

Figure 7. The effect of Fenugreek seeds on serum calcium of rats. Each column and vertical bar represents mean ± SEM of 5 animals.

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