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Effect of Fenugreek (*Trigonella foenum-graecum*) on Ethylene Glycol Induced Kidney Stone in Rats

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Abstract

Fenugreek is one of several herbal medicines whose seeds and leaves are used either as food or as an ingredient in folk medicine. In the existing literature, there is evidence regarding the effects of fenugreek on ethylene glycol induced kidney stone formation of rats. Regarding the other drugs, such as Cystone, the seeds of *Trigonella foenum-graecum* (fenugreek) are reported to have been used as anti-urolithiatic in traditional medicine. Thus, the present study was undertaken to investigate the effect of fenugreek on the prevention of kidney stone formation. Twenty male albino rats were divided into 4 groups: Normal, Ethylene Glycol (EG), Cystone and Fenugreek. The duration of the experiment was 28 days. Ethylene glycol group led to increases in kidney weight, malondialdehyde (MDA) and platelet count, while Cystone and Fenugreek combat the effect of EG. Haematological examination showed that the hemoglobin and red blood cell count in rats treated EG were significantly lower than those in the controls while Fenugreek and Cystone decreased the EG effect. Our studies demonstrate the anti-urolithiatic and anti-oxidative potential effects of *T. foenum-graecum*, which could exert beneficial effects against the kidney stone formation and the associated free radicals complications in kidney tissues. Further clinical trials are needed for evaluating its benefits and the possible side effects.

Keywords: Fenugreek, kidney stone, Ethylene glycol, Cystone, rats.

1. Introduction

Trigonella foenum-graecum (Linn.) (Tfg) is an annual dicotyledon herb belonging to the family Fabaceae commonly known as fenugreek; it is 30 to 60 cm tall, cultivated throughout Asian countries (Shishtoppo *et al*., 2009). 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). Its seeds are considered to be of commercial interest as a source of a steroid diosgenin, which is important in the pharmaceutical industry. In Iranian traditional medicine, the seeds are used as tonic and blood sugar lowering (Nasroallah and Kolsum, 2013). Fenugreek was used to ease childbirth and to increase milk flow. It is still taken by Egyptian women for menstrual pain and as hilba tea to ease stomach problems of tourists (Rashmi *et al*., 2011). Renal stone is one of the most painful urologic disorders. Urolithiasis is the medical term used to describe stones occurring in the urinary tract. Calcium containing stones, especially calcium oxalate monohydrate (whewellite), calcium oxalate dihydrate (weddellite) and basic calcium phosphate (Javed *et al*., 2011). Kidney stone formation is a complex process that results from a succession of several physico-chemical events including supersaturation, nucleation, growth, aggregation and retention within renal tubules (Vijaya, *et al*., 2013).

Rashmi and his worker showed that the fenugreek seeds reduces the amounts of calcium oxalate in the kidneys, which often contributes to the production of kidney stones (Rashmi *et al*., 2011). Laroubi and his worker showed that the amount of calcification in the kidneys and the total calcium amount of the renal tissue in rats treated with Tfg were significantly reduced compared with untreated group (Laroubi *et al*., 2009). Consequently, Tfg may be a useful agent in the treatment of patients with calcicurolithiasis (Laroubi *et al*., 2007).

The present study was designed to investigate the anti-urolithiatic activity of fenugreek and its effect on some physiological parameters.

2. Materials and Methods

2.1 Plant Materials

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

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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 normal group (N) which was supplied with normal water and diet.

Depending on Cystone leaflet it was comprised of the following substances: shilapuspha (Didymocarpus pedicellata) 130mg, Pasanabheda (Saxifaga lgulata Syn. Bergenia ligulata/ciliata) 98 mg, Manjishtha (Rubia cordifolia) 32 mg, Nagarmusta (Cyperus scariosus) 32 mg, Apamarga (Achyranthes aspera) 32 mg, Gohija (Onosma bracteatum) 32 mg, Sahadevi (Vernonia cinerea) 32 mg, Shilajeet (Purified) 26 mg, and Hajrul yahood bhasma 32 mg. Group (E): drinking water was supplemented with EG (1%) for 28 days.

Group (C) was given 2.5 tablets of Cystone (750 mg\ kg body weight) in 100 ml of water and 2.5 tablets in 100 g of standard diet.

Group (F) was given 10 gm of fenugreek in 100 ml of water and 10 gm in 100 gm of standard diet.

At the end of the experiment, the blood (hematological parameter and serum for electrolyte determination) and urine samples were collected for determining the presence of any CaOx in kidney.

2.3 Statistical Analysis

The results were presented as means ± S.E.M and the comparison between the experimental groups were made using Newman-keuls test and ANOVA. *: $P < 0.05$ was considered as indicative of degree of significance by using GrphaPad prism 6.0.

3. Results and Discussion

From the results of the present study, it was observed that ethylene glycol caused an increase in kidney weight and kidney/body weight ratio when compared with normal; our results are comparable with results of Schladt *et al*. (1998) who concluded that a significant increase in kidney weights in rats treated with ethylene glycol which induced necrosis, fibrosis and crystal deposition in renal tubules (Figure 1).

Cystone decreases kidney weight and kidney/body weight ratio when compared with EG group (Figure 2). This might mean that Cystone prevents the accumulation, deposition and super saturation of calciurologenic chemicals in the kidneys. Likewise, oxalic acid and calcium hydroxyproline were reduced in urine. This action inhibits the formation of kidney stones (Rafiq *et al*., 2012). The fenugreek highly significant decrease of kidney weight is clear when compared with EG group; this indicates that fenugreek may be protective against kidney stones.

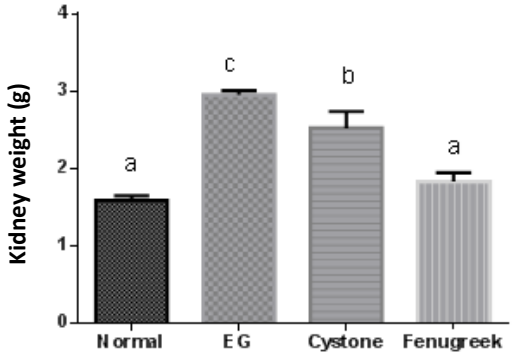

Figure 1. The effect of Fenugreek seeds on kidney weight of rats. Each column and vertical bar represents mean ± SEM of 5 animals. Normal, EG, Cyctone and Fenugreek.

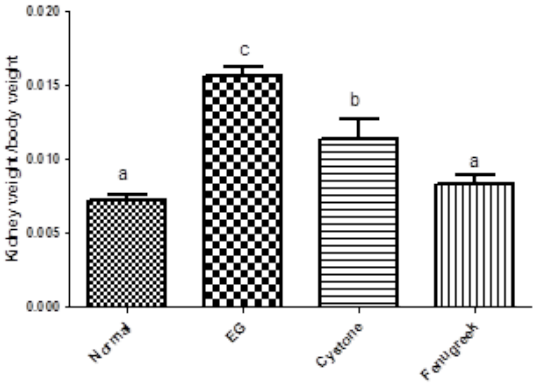

Figure 2. The effect of Fenugreek seeds on kidney/body weight ratio of rats. Each column and vertical bar represents mean ± SEM of 5 animals. Normal, EG, Cyctone and Fenugreek

Body weight gain was significantly reduced in rats of the ethylene glycol group during the days of the experiment (Figure 3); these results are in agreement with the result of Gaunt *et al*. (1974). While treated groups of Cystone and fenugreek, when compared with the untreated group, decreased body weight because fenugreek decreases body fats and is effective on obesity but the differences between them is not statistically significant (Nasroallah and Kolsum, 2013) (Figure 3).

Supplementation of fenugreek in group 4 significantly countered EG induced renal hypertrophy when compared to the control group 1 since its seeds contain alkaloids, including trigonelline, gentianine and carpine compounds, unique amino acid, 4-hydroxy isoleucine (Ajaya *et al*., 2009).

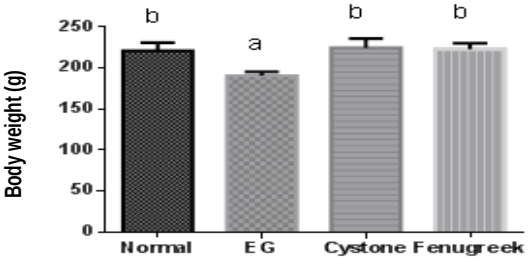

Figure 3. The effect of Fenugreek seeds on body weight of rats. Each column and vertical bar represents mean ± SEM of 5 animals. Normal, EG, Cyctone and Fenugreek.

Animals treated with EG for 28 days revealed a significant increase in the MDA level with a mean value as compared with control group which was 36.67 ± 1.188 nmol/ml (Figure 4). On the other hand, animals treated with Cystone showed a decrease in MDA level in sera compared with EG group; after 28 days, the mean value was 8.484 ± 1.023 while the mean value for fenugreek was 8.18 ± 1.125 which showed high significant differences ($P < 0.001$) when compared with EG group. It counteracts its effect to the normal level 7.804 ± 0.3053.

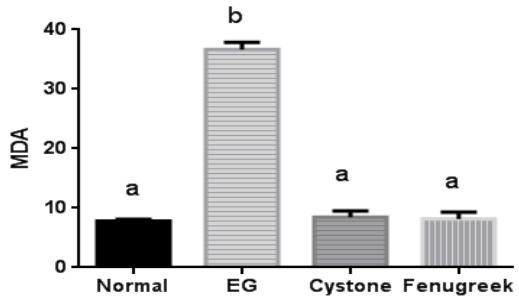

Figure 4. The effect of Fenugreek seeds on MDA of rats. Each column and vertical bar represents mean ± SEM of 5 animals. Normal, EG, Cyctone and Fenugreek

Devi *et al*. (2012) showed that fenugreek led to decrement in MDA level in fenugreek treated rats, which could be due to the consumption of the extracellular antioxidant, ceruloplasmin, by the oxidants, to combat oxidative stress.

The ethylene glycol administration resulted in non-significant increases in the level of serum values of sodium, potassium, chloride and calcium (Figures 5, 6 and 7) compared with normal rats; these results are in agreement with the results of Sunitha *et al*. (2012). The present results are comparable with the study of Al-Atwi (2010), who investigated the serum values of sodium, potassium, chloride and calcium and did not reveal any significant change in Cystone and fenugreek treated rats when compared with the control values.

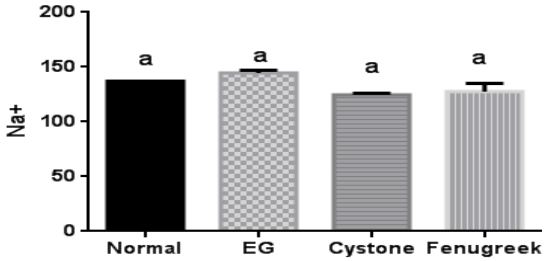

Figure 5. The effect of Fenugreek seeds on Na+ of Rats. Each column and vertical bar represents mean ± SEM of 5 animals. Normal, EG, Cyctone and Fenugreek

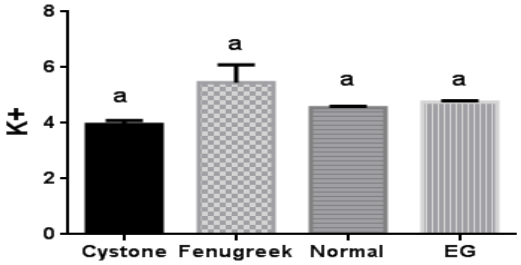

Figure 6. The effect of Fenugreek seeds on K+ of rats. Each column and vertical bar represents mean ± SEM of 5 animals. Normal, EG, Cyctone and Fenugreek.

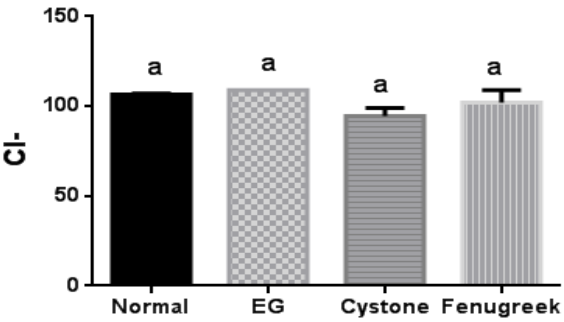

Figure 7. The effect of Fenugreek seeds onCl-+ of Rats. Each column and vertical bar represents mean ±SEM of 5 animals. Normal, EG, Cyctone and Fenugreek

The results of the present study showed a significant decrement in RBC count and hemoglobin concentration (Figures 8, 9), which leads to anaemia in the EG group and our results are comparable with the study of Shih *et al*. (2000) who proposed that anemia is caused by a bone marrow toxin rather than haemolysis or peripheral toxicity.

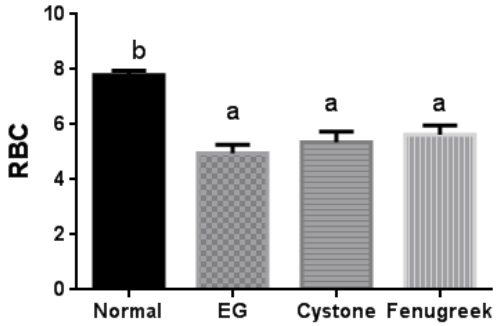

Figure 8. The effect of Fenugreek seeds on RBC of Rats. Each column and vertical bar represents mean ± SEM of 5 animals. Normal , EG, Cyctone and Fenugreek

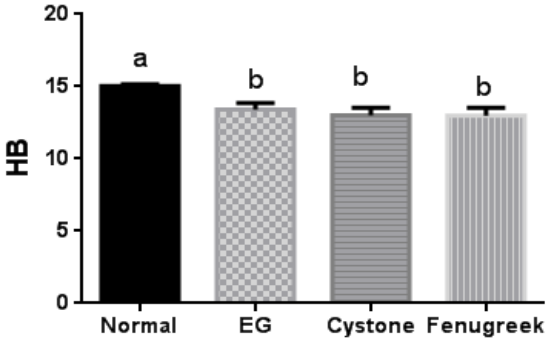

Figure 9. The effect of Fenugreek seeds on Hb of Rats. Each column and vertical bar represents mean ± SEM of 5 animals. Normal, EG, Cyctone and Fenugreek

These findings are consistent with previous studies which showed that the high exposure to EG will induce haematological defects. Haematological disorders induced by exposure to EGME have also been confirmed in animal studies. The haematological toxicity in animals included decreased concentrations of haemoglobin, packed cell volume, white and red blood cell counts, bone marrow cellularity, and erythropoiesis and enhanced haemolysis (NIOSH, 1992).

The reduction in RBC count and hemoglobin was observed in the 15 days treated animal with EG and was also found to be significantly restored in fenugreek and

Cystone treated rats (Sindhu *et al*., 2012) (Figures 8 and 9). Platelet count, obtained in the present study, demonstrated that non-significant change in EG, Cystone and fenugreek when compared with normal rats (Figure 10).

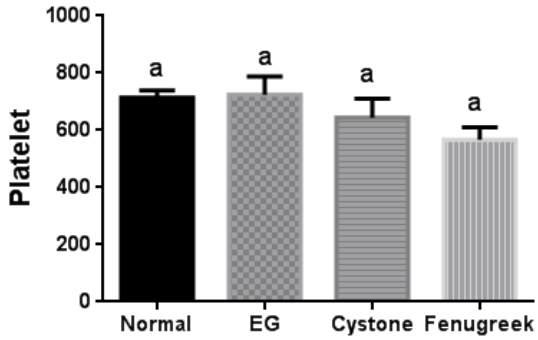

Figure 10. The effect of Fenugreek seeds on platelet of rats. Each column and vertical bar represents mean ± SEM of 5 animals. Normal, EG, Cyctone and Fenugreek

The microscopic examination of urine revealed the presence of CaOx in EG rats, but in Cystone and fenugreek groups, no CaOx appeared, it was similar more or less to that normal group as shown in Figure 11.

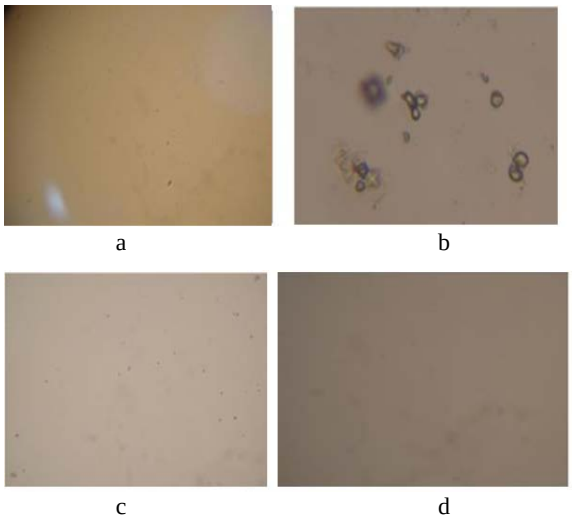

Figure 11. (a) Presence of CaOx in urine smear in Normal rats; (b) Urine smear in EG rats; (c) Urine smear in Cystone rats, (d) Urine smear in Fenugreek rats

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