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Original Research Article

PHARMACOLOGICAL EVALUATION OF CRUDE SAPONIN FRACTION FROM FENUGREEK SEED EXTRACT ON ETHYLENE GLYCOL INDUCED NEPHROLITHIASIS IN RATS.

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Abstract: Introduction: Fenugreek is a folklore medicine prescribed for treatment of renal stones in Sudan. There has been many studies evaluating the effect of fenugreek seeds and leaves in renal stones. These studies have used the aqueous, methanolic extracts of seed and/or whole seed to establish the effect in nephrolithiasis. In the current study we evaluated the effect of saponins from the methanolic extract of the fenugreek seeds on nephrolithiasis in Wistar Rats.
Method: 24 Wistar rats were divided into 4 goups: Normal, Low dose TSf, high Dose Tsf and Ethylene glycol. The duration of treatment was 28 days. Following parameters like serum BUN, serum Creatinine, dry oxalate weight in kidney and histopathological examination were evaluated.
Statistical analysis: the result presented as mean ± SEM, and the comparisons between the experimental groups were performed using Student's *t*-test.
Results & Conclusion: Crude saponin fraction has shown a significant decrease in serum BUN and Serum creatinine as compared to Ethylene glycol treated rats. The crude saponins fraction of Fenugreek seeds significantly reduced the formation and deposition of (CaOx) stone as compared to Ethylene Glycol treated rats.

Key words: Wistar Rats, Fenu Greek seed, Crude Saponin fraction, nephrolithhiasis, Blood Urea Nitrogen, Serum Creatinine, Ethylene glycol.

Introduction:

Renal stone disease (urolithiasis, nephrolithiasis) covers many conditions causing kidney, ureteric or bladder stones. These include metabolic and inherited disorders, anatomical defects of the upper or lower urinary tract, and chronic urinary infection. Renal stone disease is common; in the UK, about 8% of men and around 4% of women form at least one stone by their 60s.Urolithiasis is more common in affluent, industrialized countries than in poorer countries with agrarian economies. In the USA, the male lifetime prevalence rises to 15% and in the oil-rich states of the Arabian Gulf to

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over 20%. Stone formation tends to recur without preventative measures after a first stone, 40% of sufferers return within 3 years with at least one more stone, by 10 years this figure has increased to 75% of patients, and by 25 years virtually everyone has formed at least one more stone. Some patients may form only a couple of stones in their lifetime, while others may get them annually, or even more frequently *(Ajayi et al., 2007).*

There are many studies indicating that incidence of urolithiasis has been increasing continually in the past decades *(Tanagho et al., 2000).* High incidence of urinary calculi has been reported in countries in the Afro-Asian stone belt. Countries in tropical and subtropical areas have also reported a high incidence of urolithiasis *(Rizvi et al., 2002).*

Causes of stone formation

The salts and acids that normally crystallize in kidney stones do so because of their relative insolubility in urine. The most insoluble is calcium oxalate (CaOx); once a CaOx stone trapped in the urinary tract, it is almost impossible for it to re-dissolve. Its solubility is independent of urinary pH, unlike the solubility of other common stone constituents such as cystine and uric acid (soluble in alkali) or calcium phosphate (CaP) and magnesium ammonium phosphate (soluble in acid) *(Vijaya et al., 2013, Ajayi et al., 2007).* Calcium oxalate (CaOx), representing up to 80% of analyzed stones *(Daudon et al., 1993).*

Currently open renal surgery for nephrolithiasis is unusual and used rarely since the introduction of extracorporeal shockwave lithotripsy (ESWL), which has revolutionized urological practice and almost become the standard procedure for eliminating kidney stone. However, in addition to the traumatic effects of shockwaves, persistent residual stone fragments, and the possibility of infection, it seems that ESWL may cause acute renal injury, a decrease in renal function and an increase in stone recurrence *(Kishimoto et al., 1986; Begun et al., 1991).* Therefore, it is worthwhile to look for an alternative to these means by using medicinal plants or Phytotherapy.

Based on, the fact that fenugreek seeds decoction used as a traditional medicines for the treatment of the renal colic associated with renal stone in north Africa , In previous studies, the anti nephrolithiasic effect reported for fenugreek seeds powder 500 mg/kg against glycolic acid-induced calcium oxalate renal stone *(Ahsan et al., 1989)* ,and the aqueous fraction, against ethylene glycol-induced calcium oxalate renal stone *(Mudhir et al., 2014, Rashmi et al., 2011, Laroubi et al., 2009, Laroubi et al., 2007).*

Material and Methods:

Plant material: *Trigonella foenum-graecum* L. seeds were procured from Al-Oshara market, Khartoum city, Sudan. The seeds were identified and authenticated by Mr. Wail E. Abdalla, plant taxonomist, Medicinal and Aromatic Plant Research Institute (MAPRI), Khartoum. Sudan. A specimen was deposited at the Medicinal and Aromatic Plant Research Institute (MAPRI) Herbarium, Khartoum-Sudan with voucher specimen №: Y91/07.

Preparation of extract: The seeds were dried and coarsely powdered. Then 50 g powder was extracted in a Soxhlet apparatus using methanol. The methanol extract then concentrated under reduced pressure to 10 ml, then 50 ml of ethyl acetate was added in order to precipitate crude saponins fraction (2 g) (TSf) which was used in the study.

Animal and Treatments: 24 Wistar rats weighing 180-260 g, maintained for 3 days under experimental conditions, were divided equally into four groups of six animal each. All animal had free access to drinking water (*ad libitum*) and regular chow every day, and were kept under controlled 12 h light/dark cycle at 22±2°C. Hyperoxalurea and CaOx deposition in the kidney was induced by ethylene glycol (EG) in the drinking water to a final concentration of 0.75% for the 28 days of the study *(Atmani et al., 2004).*

For three groups, the drinking water was supplemented with EG (0.75%): two groups were given 0.5 ml of 25 mg/kg (low dose, group LD) and 50 mg/kg (high dose, group HD) of TSf, the third group (control toxic, group CT) received 0.5 ml of normal saline/ animal. The last group (control normal, group CN) served as a control normal (not supplemented with EG).

All the rats were fed on the standard laboratory diet and weighed weekly.

At the end of the experiment, each rat was anaesthesized by ethyl ether inhalation. Blood was collected for the analysis of serum blood urea nitrogen (BUN) (by alkaline picrate method-kit) and creatinine (end-point DAM method-kit).

Both kidneys were removed. The left kidneys were dried at 100° for 24 hour and weighed. They were homogenized individually and analyzed for oxalate contents. The right animal's kidneys (3 of each group) were fixed in 10% formalin solution (7.4-7.6 PH) to prepare L.S section stained with haematoxylin and eosin for histological examination under normal light and polarized light microscopy.

Analysis of kidney oxalate content

A sample of 100 mg of the dried kidney homogenate was dissolved in 1 N HCl (10 ml) in a 25 ml volumetric flask and heated in a water bath at 70°C for 1h *(Chow et al., 1974).* The solution was later centrifuged (2000 rpm) for 10 min and supernatant separated. Oxalate was precipitated by adding 0.5 ml of 1M $CaCl_2$ solution and left over night at 4°C. Oxalate content was determined by titration with 0.02N $KMnO_4$ while solution was kept at 90°C.

Statistical analysis: the result presented as mean ± SEM, and the comparisons between the experimental groups were performed using Student's *t*-test.

Results

Serum analysis

The control toxic group (group CT) showed significantly higher serum creatinine and blood urea nitrogen (BUN) level ($P< 0.05$), compared to control normal group (group CN) (Table 4.1). Kidney tissues oxalate content was very significantly higher in control toxic group (group CT) compared to control normal group (group CN) ($P< 0.001$) (Table 4.2).

The group treated with 50 mg/kg of TSf (group HD) showed significantly lower creatinine and BUN serum level ($P< 0.05$) compared to control toxic group (group CT) (Table 4.1). Both group HD and group LD (treated with 25 mg/kg of TSf), showed very significantly lower kidney tissues oxalate content ($P < 0.001$) compared to control toxic group (Table 4.2).

Table 4.1: Effect of crude saponins fraction of *Trigonella foenum-graecum* seeds on the serum parameter

Group (n)	BUN mg/dl	Creatinine mg/dl
Group CN (6)	40.38±2.3	0.101±0.004
Group CT (6)	57.49±4.5‡	0.159±0.02‡
Group LD (6)	52.53±4.5	0.135±0.01
Group HD (6)	41.66±1.6†	0.105±0.01†

Values are mean± SEM.

(n): number of rats.

† $P < 0.05$: significantly different compared with Group CT (control toxic).

‡ $P < 0.05$: significantly different compared with Group CN (control normal).

Table 4.2: Dry kidney tissues oxalate content

Group (n)	Oxalate content (mg/100 mg of dry kidney weight)
Group CN (6)	0.99±0.01
Group CT (6)	26.06±1.07‡
Group LD (6)	6.11±0.16†‡
Group HD (6)	2.06±0.02†

Values are mean± SEM.

(n): Number of rats.

† $P < 0.001$: significantly different compared with Group CT (control toxic).

‡ $P < 0.001$: significantly different compared with Group CN (control normal).

The histological examination of the kidney sections revealed that in the control normal group (group CN) there was no deposition of calcium oxalate (CaOx) stone (Fig 4.1c). In the control toxic group (CT), there was several tubular deposits of (CaOx) crystals in large aggregates (Fig 4.2c), with high indication of renal tubule damage and interstitial inflammation in the cortex (Fig 4.2b) (Table 4.3). In contrast, the crude saponins fraction of *Trigonella foenum-graecum* significantly reduced the formation and deposition of (CaOx) stone in the high dose group (group HD) which showed an isolated tubular deposit of CaOx crystals (Fig 4.4c), and slightly significant in low dose group (LD) 25 mg/kg which showed few tubular deposits of CaOx crystals (Fig 4.3c).

Table 4.3: Histological changes in kidney

Group	Cortex			Medulla	
	Interstitial inflammation	Glomular damage	Tubular damage	Interstitial inflammation	Tubular damage
Group CN	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
Group CT	++	0	++	+	++
	++	0	++	-	++
	+	0	+	+	+
Group LD	+	0	+	0	+
	+	0	+	0	+/-
	+	0	+	0	+
Group HD	+	0	0	+	0
	+	0	0	+	0
	+	0	0	+	0

(0)Not detected. (-): slight. (+): moderate. (++): high.

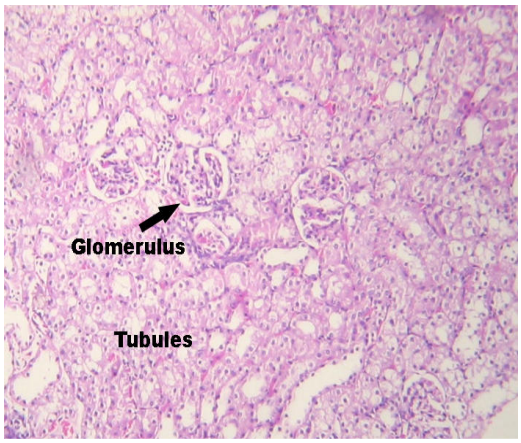

Figure 4.1a: Group CN - Low power photomicrograph of section of kidney from normal control group showing a normal histological appearance of renal cortex. (HE x 100)

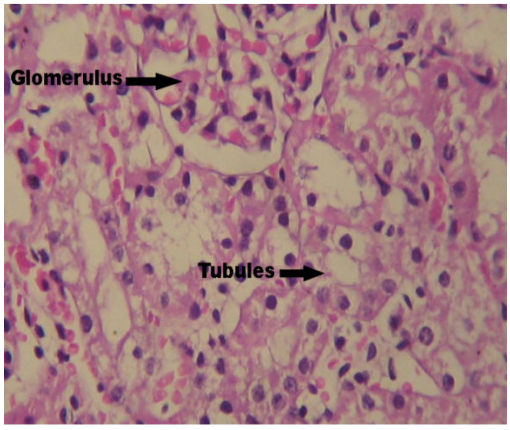

Figure 4.1b: Group CN - High power photomicrograph of section of kidney from normal control group showing a normal glomerulus and tubular epithelial cells. (HE x 400)

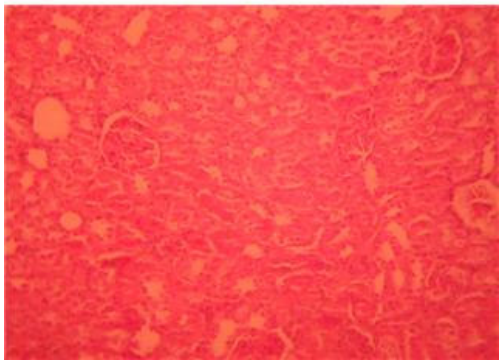

Figure 4.1b: Group CN - Photograph taken under polarized light. There was no presence of crystal deposition. (x 100)

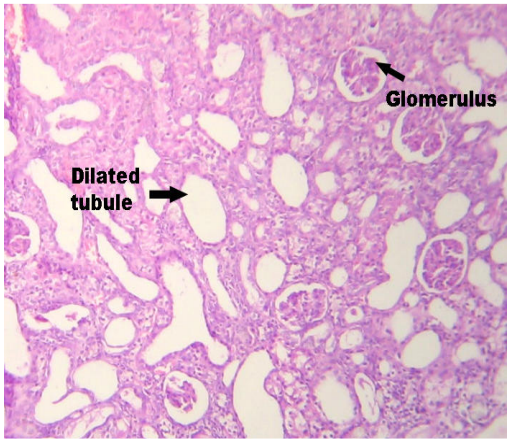

Figure 4.2a: Group CT - Low power photomicrograph of section of kidney from toxic control group showing pronounced tubular damage leading to dilated tubules in the renal cortex. Glomeruli appear normal. (HE x 100)

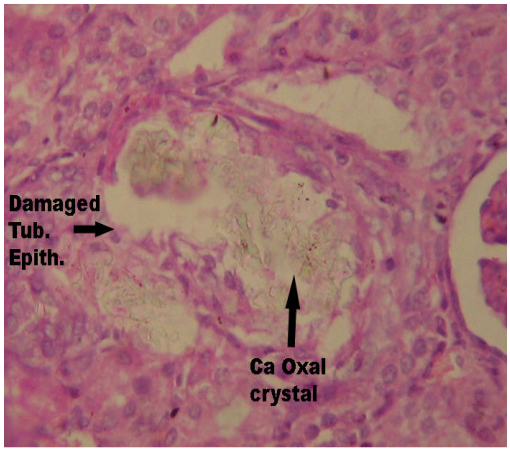

Figure 4.2b: Group CT - High power photomicrograph of section of kidney from toxic control group showing damaged tubular epithelium around a large deposit of CaOx crystals. (HE x 400)

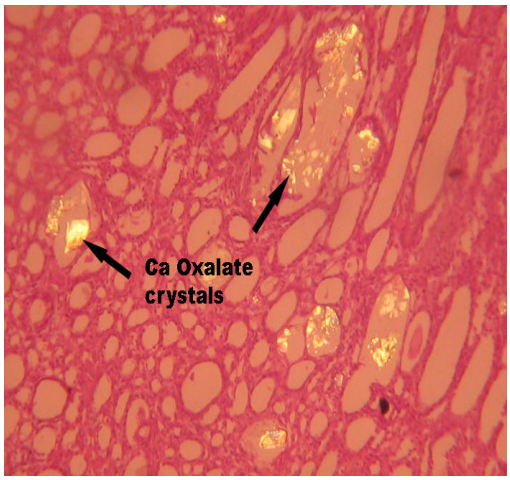

Figure 4.2c: Group CT -Photograph taken under polarized light showing several tubular deposits of CaOx crystals. (x 100)

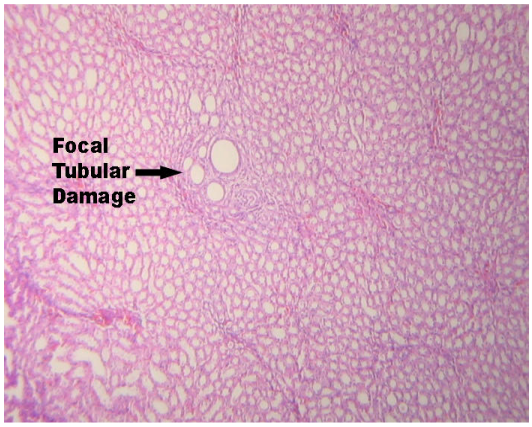

Figure 4.3a: Group LD - low power photomicrograph of section of kidney from Plant Extract Low Dose group showing a small focus of tubular damage in the medulla leading to dilated tubules (HE x 100)

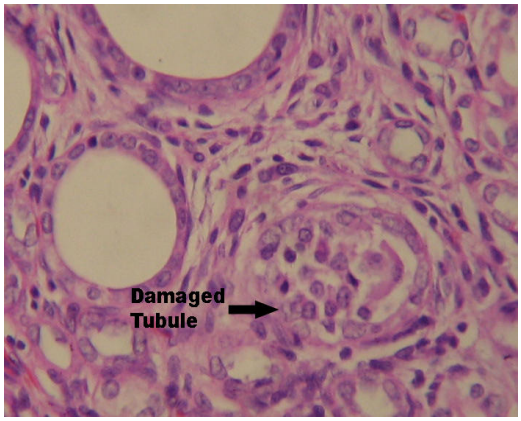

Figure 4.3b: Group LD - high power photomicrograph of section of kidney from Plant Extract Low Dose group showing the dilated tubule in the medulla with flattened tubular epithelial cells. (HE x 400)

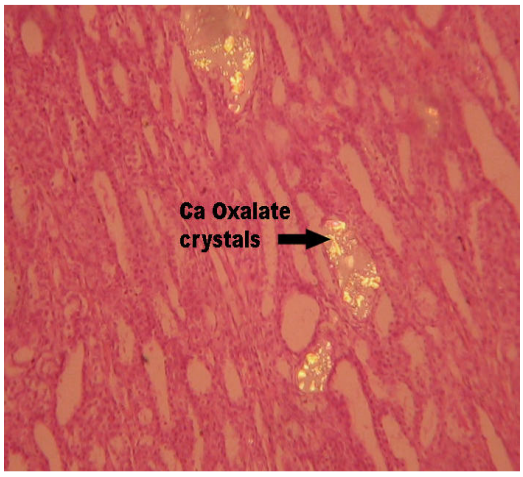

Figure 4.3c: Group LD - Photograph taken under polarized light showing fewer tubular deposits of CaOx crystals. (x 100)

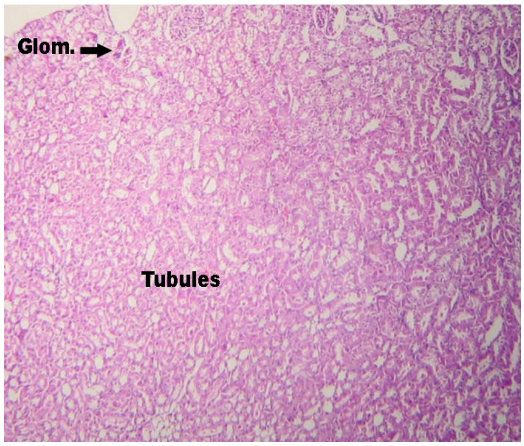

Figure 4.4a: Group HD - Low power photomicrograph of section of kidney from plant extract high dose group showing a normal histological appearance in the renal cortex. (HE x 100)

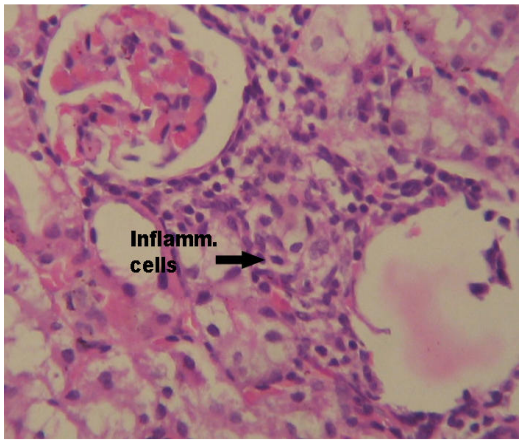

Figure 4.4b: Group HD - High power photomicrograph of section of kidney from plant extract high dose group showing a focus of interstitial inflammation. (HE x 400)

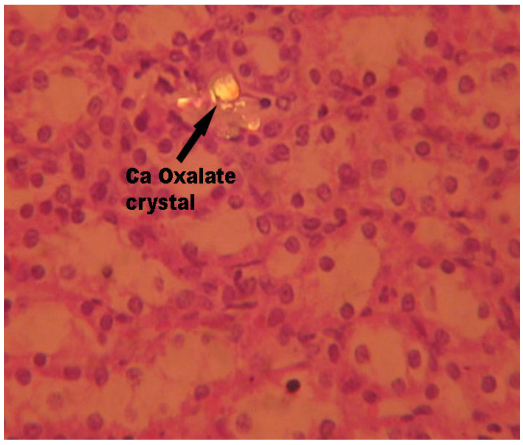

Figure 4.4c: Group HD - Photograph taken under polarized light showing an isolated tubular deposit of CaOx crystals. (x 400)

Discussion

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).* Urinary super saturation with respect to stone- forming constituents is generally considered one of the causative factors in calcugenesis. In response to 28 days period of EG (0.75%) administration, young rats formed renal calculi composed mainly of calcium oxalate *(Atmani et al., 2004).* The biochemical mechanisms for this process are related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxalurea. In nephrolithiasis, the glomerular flirtation rate (GFR) decrease

due to the obstruction of the out flow of urine by stone in the urinary system. Due to this, the waste products, such as urea and creatinine accumulate in blood *(Ghodkhar, 1994).* In addition, oxalate has been reported to induce lipid peroxidation and to cause renal damage by reacting with polyunsaturated fatty acid in the cell membrane *(Ernester and Nordebrand, 1967).*

In the control toxic group (CT), significant renal tissues damage was seen as indicated by elevated serum levels of creatinine, urea (Table 4.1) and the histological investigation (Table 4.3). Crude saponins in both the doses have shown that there is no interstitial inflammation in the medulla and cortex region of kidney. There has been less formation of Calcium oxalate in the kidney *(Mudhir et al., 2014, Rashmi et al., 2011, Laroubi et al., 2009).* However, the prophylactic effect of the crude saponins fraction of Sudanese fenugreek seeds (TSf) which is markedly occurred in group HD (50 mg/kg/day) and indicated by the significantly low oxalate kidneys content ($P < 0.001$) seems to be either through inhibition of oxalate synthesis, or by enhancing pathway of calcium oxalate stone dissolving factor such as citric acid. It was reported that the female sex hormone inhibited or decreased the formation of calcium containing renal stone suggesting that the mechanism was induced citric acid formation that was known to inhibit formation of calcium renal calculi *(Igughi et al., 1999),* which may be one of mode of action of the fenugreek crude saponins fraction used in or study. To determine the exact possible mechanism of action could be the aim for the coming research.

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