

## Original Article

# The preventive and therapeutic effect of potassium citrate combined estradiol on lithangiuria

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**Abstract:** Urinary calculus is a common disease. Potassium citrate is usually used to prevent lithangiuria. Urinary calculus formation is affected by sex hormone. This study investigated the preventive and therapeutic effect of potassium citrate combined estradiol on lithangiuria. SD rats were randomly divided into three groups, control group, model group established by administration of 3% melamine (Mel), and potassium citrate combined estradiol group. The weight, 24 h urine volume, water intake, Mel level, Scr, BUN, UA1b, ratio of kidney and weight, and calculus formation rate were measured. TNF- $\alpha$  and interleukin (IL)-1 $\beta$  levels were detected by real-time PCR and ELISA. Rats from model group displayed reduced body weight, increased 24 h urine volume, water intake, Mel level, Scr, BUN, UA1b, ratio of kidney to body weight, but decreased urine potassium and urine citric acid compared with those parameters in control group ( $P < 0.05$ ). TNF- $\alpha$  and IL-1 $\beta$  levels were significantly elevated in model group compared those in control group ( $P < 0.05$ ). The rats in combination treatment group exhibited reduced calculus formation rate, 24 h urine volume and water intake, increased body weight, urine potassium, and urine citric acid, but declined levels of Mel, Scr, BUN, UA1b, TNF- $\alpha$  and IL-1 $\beta$ , and decreased ratio of kidney to weight as well as urinary calcium compared with those parameters in model group ( $P < 0.05$ ). Potassium citrate combined estradiol treatment suppresses urinary calculus formation induced by Mel, inhibits TNF- $\alpha$  and IL-1 $\beta$  secretion, reduces urine calcium, as well as improves urine potassium and urine citric acid, leading to improved renal function.

**Keywords:** Urinary calculus, melamine, potassium citrate, estradiol, renal function

## Introduction

Urinary calculus is a worldwide common disease in urinary surgery [1]. Its incidence keeps rising that occurs at each age stage and mainly in males [2, 3]. China is one of the three high-prevalence areas of calculus. Following increased incidence and recurrence rate, urinary calculus seriously threatens global health and social economy [4, 5]. Multiple factors affect urinary calculi, such as environment, gender, genetic factors, metabolic abnormalities, abnormal immune state, dietary, and lifestyle [6, 7]. Melamine (Mel) is a kind of triazine nitrogen heterocyclic compound that can polymerize with other substances through hydrogen bond under vacuum or acid solution [8]. Mel can be discharged in urine directly without metabolism in the body. In addition, Mel can damage kidney and form urinary calculus [9, 10]. Potassium citrate is a common drug to prevent urinary calculus. Except hypokalemia, it can be used to treat urinary calculus which is

induced by low citric acid and elevated urinary calcium, and prevent new calculus formation [11, 12]. Urinary calculus exhibits significant difference in gender, suggesting that sex hormone may play a role in the formation of urinary calculus [13, 14]. Thus, estrogen drug can affect urinary calculus formation to a certain extent [15, 16]. However, the effect of application of potassium citrate and estradiol on the prevention and treatment of urinary calculus remains poorly understood. This study aimed to investigate the impact of potassium citrate combined estradiol on urinary calculus and related mechanism on rat urinary calculus model established by administration of Mel.

## Materials and methods

### Experimental animals

A total of 30 healthy SD rats aged 3 months and weighted  $250 \pm 30$  g were purchased from experimental animal center, Hubei University of

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**Table 1.** Primer sequences

Gene	Forward 5'-3'	Reverse 5'-3'
GAPDH	ACCAGGTATCTTGTTG	TAACCATGTCAGCGTGGT
TNF- $\alpha$	CAGCTCTAAGTCCGTGGTCC	TCACCCACTCAATTCAGAACC
IL-1 $\beta$	CAGGTACTACCGTATGG	ATGTCTTTCACTCACA

Medicine and raised in SPF grade experimental animal center. The temperature of living conditions was  $21 \pm 1^\circ\text{C}$ , with a relative humidity of 50-70%, and 12 h day/night cycle.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Renmin Hospital, Hubei University of Medicine.

### Main reagents and instruments

Mel (chemical purity  $\geq 98\%$ ) was purchased from Fujian chemical company. Potassium citrate and estradiol were obtained from Huari pharmaceutical company (Hunan, China). Serum creatine detection kit was from Roche. TNF- $\alpha$  and IL-1 $\beta$  ELISA kits were purchased from R&D (USA). Rat urine protein detection reagent was from Beijing Furui. RNA extraction kit and reverse transcription kit were purchased from Axygen (USA). Labsystem Version 1.3.1 microplate reader was obtained from Bio-rad (USA). CX5CE automatic biochemistry analyzer was purchased from Beckman (Germany). AB-I7900 HT Real-time PCR was bought from ABI (USA). Surgical instrument was bought from Suzhou medical apparatus factory. EASY-nLC™ 1200 system high performance liquid chromatography was from Thermo Fisher (USA). Operation microscope was obtained from Zhenjiang optical instruments company. LZJ-4D operating microscope was got from Optical Instrument Company (Zhenjiang, China). Other reagents were purchased from Sangon (China).

### Methods

**Experimental animal grouping and urinary calculus modeling:** The rats were randomly and equally divided into three groups with  $n = 10$  in each group. The rats in model group were feed by fodder with 3% Mel and normal drinking for 8 weeks [17]. The rats in combined group were treated with 0.2 mg/kg potassium citrate through gavage once a day and 0.1 mM estradiol caudal via vein injection once a week during modeling. The experiment was in accordance with animal ethics.

**Sample collection:** After treatment, blood was extracted from the aorta to the vacuum biochemistry tube using the negative pressure acquisition method. After 30 min, blood was centrifuged at 3600 rpm at  $4^\circ\text{C}$  for 10 min.

Next, the supernatant was stored at  $-20^\circ\text{C}$ . The left renal tissue was obtained and stored at  $-80^\circ\text{C}$ .

The urine was collected from the metabolism cage and stored at  $-20^\circ\text{C}$ .

**Renal function detection:** BUN, Scr, urinary calcium, urinary potassium, and urinary citric acid were tested by automatic biochemistry analyzer. UAlb was determined by radioimmunoassay. Ratio of kidney to body weight was also calculated.

**Urine Mel concentration detection:** Urine Mel level was measured by high performance liquid chromatography. 5 ml trichloroacetic acid was added into 0.15 ml sample. Next, the sample was added with 5 ml ddH<sub>2</sub>O and filtrated for detection. Phenomenex C8 was adopted. Heptane sulfonic acid sodium citrate buffer + acetonitrile (v/v, 90:10) was selected as mobile phase and the flow velocity was 1.12 ml/min. At last, a total of 10  $\mu\text{l}$  sample was detected by a microplate reader (in triplicates) at a wavelength of 240 nm.

**ELISA:** ELISA was used to test TNF- $\alpha$  and IL-1 $\beta$  contents in the serum. A total of 50  $\mu\text{l}$  diluted standard substance were added into each well to establish standard curve. Next, 50  $\mu\text{l}$  sample was added into well (in triplicates) in the plate and washed for five times. Then 50  $\mu\text{l}$  conjugate reagent was added into each well and incubated at  $37^\circ\text{C}$  for 30 min. After washed for five times, 50  $\mu\text{l}$  color agent A and B was added and incubated at  $37^\circ\text{C}$  for 30 min at dark. At last, 50  $\mu\text{l}$  stop buffer was added to stop the reaction and then measured by a microplate reader at a wavelength of 450 nm to obtain the OD value. The OD value of standard substance was used to prepare the linear regression equation, which was adopted to calculate the concentration of tested samples.

**Real-time PCR:** Total RNA was extracted from renal tissue by Trizol and reversely transcribed into cDNA. The primers were designed using PrimerPremier 6.0 software and synthesized by

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**Table 2.** General index analysis

Index	Control	Model group	Potassium citrate group	Combined group
Weight (g)	497.6±45.3	311.9±32.6*	381.3±41.2*.#	381.3±41.2*.#,&
Urine volume (ml)	11.5±3.2	26.3±3.1*	20.1±4.1*.#	16.3±3.4*.#,&
Water consumption (ml)	22.5±0.9	45.8±1.8*	36.8±0.6*.#	26.5±1.6*.#,&

\*P < 0.05, compared with control; #P < 0.05, compared with model group; &P < 0.05, compared with potassium citrate group.

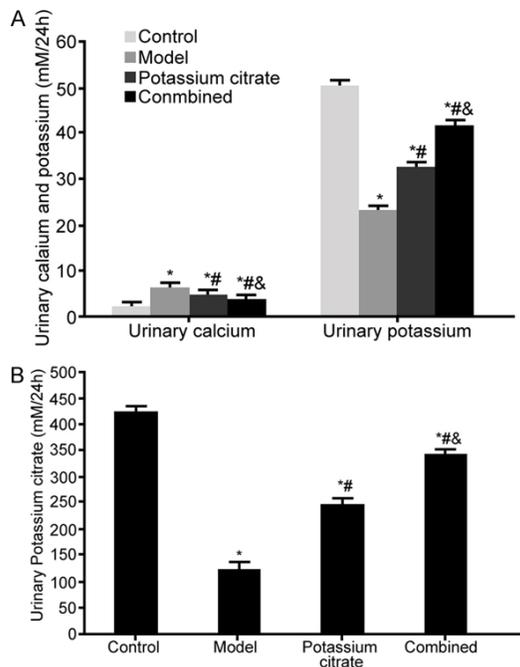
**Table 3.** Renal function detection

Index	Control	Model group	Potassium citrate group	Combined group
Kidney/body weight (mg/g)	2.4±0.3	5.6±0.8*	4.1±0.7*.#	3.5±1.2*.#,&
Scr (μmol/L)	84.5±14.7	1561.6±51.5*	1191±79.6*.#	791±69.2*.#,&
BUN (mmol/L)	7.1±0.8	14.7±1.6*	12.5±1.4*.#	10.1±0.6*.#,&
UAib (mg/24h)	0.4±0.3	1.7±0.6*	1.1±0.2*.#	0.8±0.2*.#,&

\*P < 0.05, compared with control; #P < 0.05, compared with model group; &P < 0.05, compared with potassium citrate group.

expression of mRNA was calculated by 2<sup>-ΔCt</sup> method.

**Statistical analysis:** All data analyses were performed on SPSS19.0 software and presented as mean ± standard deviation (SD). Comparison of difference among different groups was performed by one-way ANOVA Newman-Keuls multiple comparison post-hoc analysis. P < 0.05 was depicted as statistical significance.



**Figure 1.** Urinary calcium, urinary potassium, and urinary citric acid changes. A: Urinary calcium and urinary potassium detection; B: Urinary citric acid detection. \*P < 0.05, compared with control; #P < 0.05, compared with model group; &P < 0.05, compared with potassium citrate group.

Invitrogen (Table 1). Real-time PCR was performed including 35 cycles of 92°C for 30 s, 58°C for 45 s, and 72°C for 35 s. GAPDH was selected as an internal reference. The relative

## Results

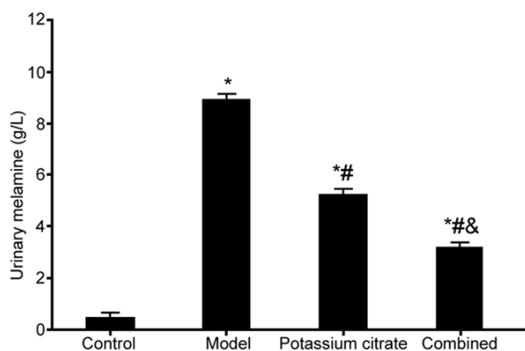
### General conditions and urinary calculus formation

Rat general conditions and urinary calculus formation rate were observed. The rats in control exhibited good mental state, glossy hair, and normal eating, drinking, activity, as well as urine output. 8 out of 10 rats formed urinary calculus after Mel induction. Rats in model group presented significant spirit drooping, hair removal, water quantity increase, urine output elevation, and weight loss compared with those in control group (P < 0.05). Potassium citrate single or combined estradiol obviously reduced calculus formation rate (40% and 20%, respectively), improved general conditions, perfected hair color, increased weight, as well as declined water consumption and urine volume compared with rats from model group (P < 0.05) with combined treatment showing better effects (Table 2).

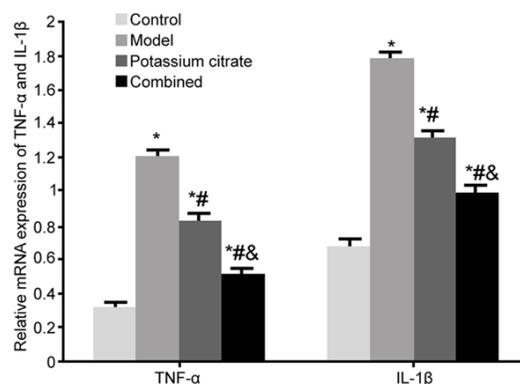
### Renal function analysis

Ratio of kidney to body weight, Scr, BUN, and UAib levels were markedly increased in model group compared with rats in control (P < 0.05). Potassium citrate single or combined estradiol significantly reduced Scr, BUN, and UAib levels compared with model group (P < 0.05) with better effects observed in combined treatment group (Table 3).

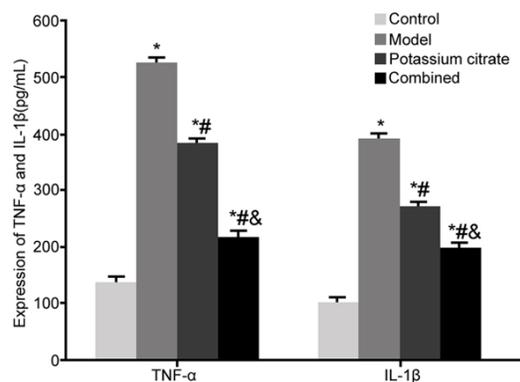
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**Figure 2.** Urine Mel content changes. \* $P < 0.05$ , compared with control; # $P < 0.05$ , compared with model group; & $P < 0.05$ , compared with potassium citrate group.



**Figure 3.** The impact of potassium citrate combined estradiol on inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  expressions in renal tissue. \* $P < 0.05$ , compared with control; # $P < 0.05$ , compared with model group; & $P < 0.05$ , compared with potassium citrate group.



**Figure 4.** The impact of tamsulosin on inflammatory cytokine expressions in the serum. \* $P < 0.05$ , compared with control; # $P < 0.05$ , compared with model group; & $P < 0.05$ , compared with potassium citrate group.

### Urinary calcium, urinary potassium, and urinary citric acid changes

Urinary calcium was increased, while urinary potassium and urinary citric acid was reduced in model group compared with control ( $P < 0.05$ ). Potassium citrate single or combined estradiol markedly declined urinary calcium as well as elevated urinary potassium and urinary citric acid compared with model group ( $P < 0.05$ ). However, combined treatment demonstrated better effects (**Figure 1**).

### Urine mel content changes

Mel content was obviously elevated in the urine of rats from model group compared with rats in control group ( $P < 0.05$ ). Potassium citrate single or combined estradiol treatment significantly declined Mel level compared with model group ( $P < 0.05$ ) with combined treatment demonstrating better effects (**Figure 2**).

### The impact of potassium citrate combined estradiol on the secretion of TNF- $\alpha$ and IL-1 $\beta$ expressions in renal tissue

As TNF- $\alpha$  and IL-1 levels were demonstrated to be significantly increased in patients with stones in the urinary tract [18], we also test TNF- $\alpha$  and IL-1 $\beta$  mRNA expressions in renal tissue by Real-time PCR. TNF- $\alpha$  and IL-1 $\beta$  mRNA expressions were markedly upregulated in renal tissue of rats from model group compared with control ( $P < 0.05$ ). Potassium citrate single or combined estradiol treatment significantly inhibited TNF- $\alpha$  and IL-1 $\beta$  mRNA expressions compared with model group ( $P < 0.05$ ). However, combined treatment demonstrated better effects (**Figure 3**).

### The impact of tamsulosin on TNF- $\alpha$ and IL-1 $\beta$ expressions in the serum

ELISA was used to detect TNF- $\alpha$  and IL-1 $\beta$  contents in the serum. TNF- $\alpha$  and IL-1 $\beta$  contents were obviously enhanced in the serum of rats from model group compared with control ( $P < 0.05$ ). Potassium citrate single or combined estradiol treatment apparently reduced TNF- $\alpha$  and IL-1 $\beta$  contents compared with model group ( $P < 0.05$ ) with combined treatment demonstrating better effects (**Figure 4**).

## Discussion

As a common disease in urinary surgery, the incidence of urinary calculus is gradually increased, while its specific pathogenic factors and mechanisms have not been elucidated. Because of high incidence and recurrence rate, it is required to identify novel approaches on the treatment and prevention of urinary calculus [19]. Normal renal environment is acidity, thus in favor of Mel dissolution and self-assembly. Small molecular substance with weak acidity delays in the urine, thus forming urinary calculus and renal injury [19, 20]. Through administration of 3% Mel into SD rat, this study established urinary calculus rat model (90% success rate) with increased water intake and urine volume, Scr, BUN, UA1b, and ratio of kidney to body weight. Crystal inhibiting factor can block crystal growth, nucleation, or aggregation by adsorbing on the surface of crystal. Some crystallization inhibitors can also reduce the saturation level of stone materials in the urine [20]. Citric acid and potassium are important crystallization inhibitors existing in urine that can affect urine supersaturation. Therefore, potassium citrate may influence calcium oxalate, calcium phosphate, and urine pH to regulate urinary stone formation [21]. Since the urinary tract is in inflammatory state during calculus formation, it produces a large amount of inflammatory cytokines, thus accelerating calculus formation, leading to damage of renal function [22, 23]. On the other hand, estrogen is confirmed to inhibit urinary calculus [24]. However, the effect of combined application of potassium citrate and estradiol on the prevention and treatment of urinary calculus still remains poorly understood. It was showed that potassium citric is conducive to the excretion of urinary calculus [11, 12]. In this study, potassium citric suppressed inflammatory cytokines synthesis and secretion, improved general conditions, increased weight, reduced 24 h urine volume and water intake, declined Mel, Scr, BUN, UA1b, accelerated urine calcium discharge, elevated urine potassium and urine citrate acid, decreased ratio of kidney to weight, as well as declined calculus formation rate. However, combined treatment exhibited better improvement on the renal function by inhibiting inflammatory cytokines secretion and reducing calculus formation. This study for the first time reported that potassium

citric combined estradiol restrained calculus formation rate, suppressed inflammatory cytokines secretion, and improved renal function. However, the exact mechanism by how potassium citric combined estradiol reduced calculus formation remains unclear and requires further investigations.

## Conclusion

Potassium citrate combined estradiol treatment suppresses urinary calculus formation which is induced by Mel, inhibits inflammatory cytokines secretion, reduces urine calcium, as well as improves urine potassium and urine citric acid to improve renal function. It could be used to treat urinary calculus especially in female in menopause that require estrogen supplement.

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## Disclosure of conflict of interest

None.

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## References

- [1] Yu Y, Li J, Guo L, Gu T, Xiao R, Ye Y, Pan Q, Zhang Y and Huang H. Bilateral duplex urinary collecting systems accompanied with horseshoe kidneys deformity and right renal ureteral calculi and hydronephrosis: diagnosis in magnetic resonance urography. *J Xray Sci Technol* 2017; [Epub ahead of print].
- [2] Anjum M, Moorani KN, Sameen I, Mustufa MA and Kulsoom S. Functional and structural abnormalities of the kidney and urinary tract in severely malnourished children - A hospital based study. *Pak J Med Sci* 2016; 32: 1135-1140.
- [3] Peng J, Li D, Chan YK, Chen Y, Lamb JR, Tam PK and El-Nezami H. Effects of water uptake on melamine renal stone formation in mice. *Nephrol Dial Transplant* 2012; 27: 2225-2231.
- [4] Wu W, Yang Z, Xu C, Gu X, Yang S, Liao S, Wang R, Gao W, Ye Z and Zeng G. External physical

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- vibration lithotripsy promotes the clearance of upper urinary stones after retrograde intrarenal surgery: a prospective, multicenter, randomized controlled trial. *J Urol* 2017; 197: 1289-1295.
- [5] Cancian M, Brito J, Renzulli J 2nd and Pareek G. Endourologic and open ureterolithotomy and common sheath reimplant for large bladder and distal ureteral calculi. *J Endourol Case Rep* 2016; 2: 209-211.
- [6] Kang DH, Cho KS, Ham WS, Chung DY, Kwon JK, Choi YD and Lee JY. Ureteral stenting can be a negative predictor for successful outcome following shock wave lithotripsy in patients with ureteral stones. *Investig Clin Urol* 2016; 57: 408-416.
- [7] He Z, Zhang C and Zeng G. Minimally invasive percutaneous nephrolithotomy guided by ultrasonography to treat upper urinary tract calculi complicated with severe spinal deformity. *Int Braz J Urol* 2016; 42: 960-966.
- [8] Jin C, Fan Y, Zhang Q, Wang Y, Wu S and Jin J. Removal of foreign bodies embedded in the urinary bladder wall by a combination of laparoscopy and carbon dioxide cystoscopic assistance: case report and literature review. *Investig Clin Urol* 2016; 57: 449-452.
- [9] Zanetti SP, Boeri L, Catellani M, Gallioli A, Trinchieri A, Sarica K and Montanari E. Retrograde intrarenal surgery (RIRS), regular and small sized percutaneous nephrolithotomy (PCNL) in daily practice: European association of urology section of urolithiasis (EULIS) survey. *Arch Ital Urol Androl* 2016; 88: 212-216.
- [10] Al-Kharashi A, Azimzadeh AA, Leung J, Radomski S, Radomski L and Lam WC. Anterior segment optical coherence tomography changes with introduction and discontinuation of tamsulosin. *Saudi J Ophthalmol* 2016; 30: 150-156.
- [11] Guan X and Deng Y. Melamine-associated urinary stone. *Int J Surg* 2016; 36: 613-617.
- [12] Kosilov K, Loparev S, Kuzina I, Shakirova O, Zhuravskaya N and Lobodenko A. The effective tool for self-assessment of adherence to treatment in patients with benign prostatic obstruction and overactive bladder symptoms. *Aging Male* 2017; 20: 1-6.
- [13] Yuruk E, Tuken M, Gonultas S, Colakerol A, Cakir OO, Binbay M, Sarica K and Muslumanoglu AY. Retrograde intrarenal surgery in the management of pediatric cystine stones. *J Pediatr Urol* 2017; [Epub ahead of print].
- [14] Postnikova GB and Shekhovtsova EA. Hemoglobin and myoglobin as reducing agents in biological systems. Redox reactions of globins with copper and iron salts and complexes. *Biochemistry (Mosc)* 2016; 81: 1735-1753.
- [15] Naghii MR, Babaei M and Hedayati M. Androgens involvement in the pathogenesis of renal stones formation. *PLoS One* 2014; 9: e93790.
- [16] Zhao Z, Mai Z, Ou L, Duan X and Zeng G. Serum estradiol and testosterone levels in kidney stones disease with and without calcium oxalate components in naturally postmenopausal women. *PLoS One* 2013; 8: e75513.
- [17] Ko IG, Moon BM, Kim SE, Jin JJ, Hwang L, Ji ES, Kim CJ, Kim TH, Choi HH and Chung KJ. Effects of combination treatment of alpha 1-adrenergic receptor antagonists on voiding dysfunction: study on target organs in overactive bladder rats. *Int Neurourol J* 2016; 20: S150-158.
- [18] Carrasco-Valiente J, Anglada-Curado FJ, Aguilar-Melero P, Gonzalez-Ojeda R, Muntane-Relat J, Padillo-Ruiz FJ and Requena-Tapia MJ. [State of acute phase markers and oxidative stress in patients with kidney stones in the urinary tract]. *Actas Urol Esp* 2012; 36: 296-301.
- [19] Albert A, Tiwari V, Paul E, Ganesan D, Ayyavu M, Kujur R, Ponnusamy S, Shanmugam K, Saso L and Govindan Sadasivam S. Expression of heterologous oxalate decarboxylase in HEK293 cells confers protection against oxalate induced oxidative stress as a therapeutic approach for calcium oxalate stone disease. *J Enzyme Inhib Med Chem* 2017; 32: 426-433.
- [20] Jhagroo RA, Wertheim ML and Penniston KL. Alkali replacement raises urinary citrate excretion in patients with topiramate-induced hypocitraturia. *Br J Clin Pharmacol* 2016; 81: 131-136.
- [21] Phillips R, Hanchanale VS, Myatt A, Somani B, Nabi G and Biyani CS. Citrate salts for preventing and treating calcium containing kidney stones in adults. *Cochrane Database Syst Rev* 2015; CD010057.
- [22] Oh WS, Kim YS, Yeom JS, Choi HK, Kwak YG, Jun JB, Park SY, Chung JW, Rhee JY and Kim BN. Developing a model to estimate the probability of bacteremia in women with community-onset febrile urinary tract infection. *J Infect Dev Ctries* 2016; 10: 1222-1229.
- [23] Chhiber N, Kaur T and Singla S. Rottlerin, a polyphenolic compound from the fruits of *Malotus philippensis* (Lam.) Mull.Arg., impedes oxalate/calcium oxalate induced pathways of oxidative stress in male wistar rats. *Phytomedicine* 2016; 23: 989-997.
- [24] Prezioso D, Strazzullo P, Lotti T, Bianchi G, Borghi L, Caione P, Carini M, Caudarella R, Ferraro M, Gambaro G, Gelosa M, Guttilla A, Illiano E, Martino M, Meschi T, Messa P, Miano R, Napodano G, Nouvenne A, Rendina D, Rocco F, Rosa M, Sanseverino R, Salerno A, Spatafora S, Tasca A, Ticinesi A, Travaglini F, Trinchieri A, Vespasiani G, Zattoni F; CLU Working Group. Dietary treatment of urinary risk factors for renal stone formation. A review of CLU Working Group. *Arch Ital Urol Androl* 2015; 87: 105-120.