Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Pharmacology and Toxicology

ISSN 1816-496X
DOI: 10.3923/jpt.2023.98.103

Research Article
Antinephrolithiatic Effect of Plantain Juice from *Musa paradisiaca* Stem on Ethylene Glycol-Induced Renal Calculus in Experimental Wistar Rats

[1]Venkatesan Natarajan, [2]B.A. Vishwanath, [2]K.L.K. Paranjothy, [1]S. Chandan and [1]K. Shivaraj

[1,2]Department of Pharmacology, Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore, Karnataka 560064, India

Abstract

Background and Objective: Renal calculi or kidney stones are crystal concretions that commonly occur in the kidney and are referred to as nephrolithiasis. Ideally, calculi should form in the kidneys and pass out of the body through the urethra painlessly. Larger stones are uncomfortable and can require surgery. Numerous herbal remedies have been utilized to effectively treat urinary stones in both India and other countries. The management of ureteral stones has a variety of options. Because Musa paradisiacal has a long history of use in the treatment of nephrolithiasis. Researchers have observed the potential anti-nephrolithiatic effects of its stem juice. Hence, the purpose of this study is to determine whether plantain juice formulation (PJ), which is collected from *Musa paradisiaca* stems, may protect Wistar rats from ethylene glycol (EG)-induced nephrolithiasis. **Materials and Methods:** Rats with ethylene glycol (EG)-induced nephrolithiasis were used to test the nephroprotective effect of PJ (500 mg kg^{-1}). When EG was administered through drinking water, it caused hyperoxaluria, hypocalcemia and an increase in urea and creatinine output from the kidneys. **Results:** The urine excretion of calcium and oxalate was dramatically reduced with PJ supplementation. Rats given PJ had significantly lower levels of calcium and oxalate as well as less calcium oxalate crystal formations in their renal tissue than rats treated with EG. When PJ was given to rats receiving EG treatment, there was a significant decrease in creatinine, urea and uric acid. **Conclusion:** According to the results of this investigation, PJ supplementation prevented EG-induced nephrolithiasis by slowing the development of kidney stones. The antioxidant, diuretic and decreased amount of stone-forming elements in this substance may be the mechanism underlying this action.

Key words: Ethylene glycol, plantain juice formulation, *Musa paradisiaca*, nephrolithiasis, cysteine, ayurvedic, antinephrolithiatic

Citation: Natarajan, V., B.A. Vishwanath, K.L.K. Paranjothy, S. Chandan and K. Shivaraj, 2023. Antinephrolithiatic effect of plantain juice from *Musa paradisiaca* stem on ethylene glycol-induced renal calculus in experimental wistar rats. J. Pharmacol. Toxicol., 18: 98-103.

Corresponding Author: Venkatesan Natarajan, Department of Pharmacology, Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore, Karnataka 560064, India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nephrolithiasis, commonly referred to as kidney stones or renal calculi, is the presence of stones inside the kidneys. It is among the most prevalent kidney conditions in adults. With a few efficient medications, kidney stone disease is becoming more widespread globally. About 12% of people worldwide are affected by this nephrological condition[1]. Oxalate calcium makes up around 80% of all kidney stones, while, calcium phosphate kidney stones, which also form as a result of elevated calcium, make up about 20% of all kidney stones[2]. Medication can also be used to treat it but doing so takes time and has drawbacks. Herbal medicine presents a practical alternative due to the high cost of conventional pharmaceuticals. While, only a small number of relatively infrequent kidney stones contain cystine, which makes up about 1% of kidney stones, calcium oxalate makes up more than 80% of kidney stones[3]. Due to the expensive cost and negative side effects of conventional treatment, the public is becoming more interested in using herbal remedies to treat nephrolithiasis disease[4].The usage of herbal products has recently received increased attention due to the exorbitant prices and negative side effects of instrument implantation and urinary tract surgery[5]. Humans and even animals have long relied on medicinal plants as a significant source of food and medicine[6]. Nowadays, a lot of researchers base their studies on natural remedies and a range of antiquated techniques, like those used in India. There are several ayurvedic preparations for the treatment of nephrolithiasis now on the market. One well-known tropical fruit is the banana. By roughly 600 BC, the banana plant had made its way from its original Southwestern Pacific Region to India and eventually, it had spread throughout the entire tropical world. It may be the oldest crop still grown today. As early as 200-300 BC, it even reached Asian countries[7]. A herbaceous plant called *Musa paradisiaca* (Musaceae) has a maximum length of 9 m. It has a sturdy, pseudo-treelike stem and a crown of enormous, long, oval, deep-green leaves that can reach lengths of up to 365 cm and widths of 61 cm and large, successively opening ovate bracts, 15-20 cm long, concave, dark red and somewhat fleshy. Each plant produces a single inflorescence that resembles a fruits are rectangular, fleshy and range in length from 5-7 cm in the wild to longer in cultivated forms. The large flower has a reddish brown bract and is used to make veggies[8]. An anti-nephrolithiatic property of PJ from the *Musa paradisiaca* stem, however, has not yet been the subject of a published scientific investigation. This study uses male Wistar albino rats to examine the preventive effect of PJ against ethylene glycol (EG) induced nephrolithiasis and any potential underlying mechanisms. Hence the objective of this study is to determine the anti-nephrolithiatic activity of plantain juice formulation (PJ), which is collected from *Musa paradisiaca* stems and may protect Wistar rats from ethylene glycol (EG)-induction.

MATERIALS AND METHODS

Study area: The studies were carried out in the Department of Pharmacology, Aditya Bangalore Institute of Pharmacy Education and Research (IAEC Approval No: 81/1611/CPCSEA) at Central animal house in the period of March-July, 2022. The animals were kept in a room with controlled temperature (25±1EC) and lighting (light/dark 12:12 hrs in polypropylene cages with sufficient food and water), which were carried out in all series of tests.

Plantain juice formulation: *Musa Paradisiaca* (Banana tree) stem was chosen and an incision above 2 feet from the ground level was made of the plant. A 15 cm well has been created by scooping out the fibrous material inside, to which jeera and fenugreek seeds were added and covered with white nylon cloth for 24 hrs. After 24 hrs the liquid that accumulated inside the well was collected. The trivial name is called plantain juice, the formulation was prepared by adding other agents like sucralose (0.2 g) as a sweetener, sodium benzoate (2 g) as an antimicrobial agent, citric acid (3 g) as a flavouring and preservative agent, sorbitol (100 mL) as laxative and glycerin (500 mL) as a moisturizer.

Animals: In this investigation, 24 adult male Wistar rats weighing 200-250 g were employed. The rats were kept in strict hygienic conditions and provided with unlimited access to normal rat pellets as well as water. They were housed in typical conditions with temperature and humidity controls of 22°C±2°C, 55%±5% and 12 hrs light/dark cycles. Aditya Bangalore Institute of Pharmacy Education and Research (Reg No: 1611/CPCSEA) institutional animal ethics committee gave its approval to all experimental protocols, which were conducted following the guidelines established by the Committee for Control and Supervision of Experiment on Animals within the Ministry of Social Justice and Empowerment of the Government of India. The experiment was carried out in compliance with generally acknowledged standards for the treatment and utilization of animals in scientific research.

Acute toxicity study: According to OECD guidelines 423, a study on the acute oral toxicity of plantain juice formulation

(PJ) was conducted. Male Wistar rats were used in the study on the acute toxicity of PJ. After a 12 hrs fast, increasing dosages of PJ (50, 200, 400, 1000 and 2000 mg kg^{-1}) were given orally to groups of 3 animals for each dose. After 0, 30, 60, 120, 180 and 240 min, the signs and symptoms of PJ were noticed and then once daily for the following 14 days. For 14 days, the animals were regularly monitored for any signs of mortality, including general motor activity, convulsions, writhing and drowsiness. After the observation period, the number of survivors was reported.

Ethylene glycol-induced nephrolithiasis in rats: Male albino Wistar rats were tested for anti-nephrolithiatic activity using a model of EG-induced nephrolithiasis. By giving EG (0.75% w/v, p.o.) in drinking water for 28 days at a time, nephrolithiasis was brought on[9].

Experimental design: There were four groups of six rats each and group I (control) received carboxyl methyl cellulose (5 mL kg^{-1} of 0.5% w/v) for 28 days. For 28 days, the group II EG treatment group received only EG (0.75% v/v) in water. The marketed herbal formulation (500 mg kg^{-1}, p.o.) was given to group III (EG treated) for 28 days. The PJ formulation (500 mg kg^{-1}, p.o.) was administered to the animals in group IV (EG-treated) for 28 days. After 28 days, rat's retro-orbital sinuses were exposed to light diethyl ether anaesthesia while blood samples were taken using glass capillaries for biochemical analysis. The kidneys were taken out, dissected, measured for antioxidant enzymes and kept in a 10% formalin solution for future research.

Collection of serum: Under a light ether anaesthetic, glass capillaries were used to draw blood from the rat's retro-orbital plexus. A total of 2 mL Eppendorf tubes were used to collect the blood. It was centrifuged at 5000 rpm for 20 min to separate the serum after allowing it to clot in the open for 15 min. The collected serum was kept at 20°C to estimate additional biochemical parameters like calcium, creatinine, uric acid, urea and blood urea nitrogen (BUN)[10].

Kidney homogenate analysis: The rats were anaesthetized (Ketamine 90 mg kg^{-1} and Xylazine 10 mg kg^{-1}) and sacrificed at the end of the experiment and the kidneys were removed and isolated. They received a cold physiological saline washing as well as tissue removal from excess tissue. Crushed ice was used to preserve half of the isolated kidney. They were divided into thin pieces, crosswise and cooled in 0.25 M sucrose before being immediately blotted with filter paper. 10% (w/v) of the tissues were homogenized at a speed of 2500 rpm in 0.1 M Tris hydrochloride buffer (pH 7.4). Using a cooling centrifuge, the homogenate was centrifuged at 5000 rpm for 20 min (around 4°C). The estimate of several marker enzymes was done using the supernatant that was left over after centrifugation[11]. To measure malondialdehyde (MDA), glutathione (GSH) and catalase (CAT), clear supernatant were separated and employed.

Histopathological studies: One kidney from each group of animals was removed during the sacrifice process, preserved in 10% formalin after being washed with normal saline and then histological parameters were examined. The tissue was cleaned, alcohol was used to dehydrate it, xylene was used to clarify it and paraffin blocks were created. Using a rotary microtome, 5 m thick serial slices were made. With the use of xylene, the sections were deparaffinized and then hydrated in falling alcohol grades. The slides were then exposed to hematoxylin for 10 min before being thoroughly washed with water. These were examined, counterstained stained with eosin, rinsed with water, dried with escalating levels of alcohol, cleaned with xylene and mounted after drying[12].

Statistical analysis: The results were shown as a mean and standard error of the mean. One-way ANOVA was used to statistically examine the differences between the data and the Dunnett multiple comparison tests were used to establish the degree of significance (Prism, GraphPad Version 5, GraphPad Software, Inc). At $p<0.05$, differences between the data were deemed significant.

RESULTS

Acute toxicity study: There was no mortality for 14 days following oral treatment of the PJ formulation to rats at doses up to 2000 mg kg^{-1}.

Effect of PJ formulation on various serum and urinary parameters in ethylene glycol-induced nephrolithiasis: In comparison to the control group, the EG-treated group's urine pH and kidney weight significantly increased. However, treatment with the PJ formulation (500 mg kg^{-1}, p.o.) revealed significant differences in kidney weight ($p<0.05$) and urine pH ($p<0.05$) when compared to the marketed formulation shown in Table 1.

When compared to control animals, the EG-treated group had a significantly higher body weight at days 0 and 28 as well as a higher kidney weight. However, compared to the

Table 1: Effect of PJ formulation on kidney weight and urine pH in ethylene glycol-induced nephrolithiasis

Groups	Relative weight of kidney	Urine pH
Control	1.20±0.4	7.6±0.34
Ethylene glycol induced	1.91±1.2	6.6±0.28
Marketed formulation	1.62±1.7	7.0±0.32
Plantain juice	1.31±0.5*	7.6±1.1*

All values are Mean±SEM (n = 6) and *p<0.05 when compared to control

Table 2: Effect of PJ formulation on body weight on 0 day and 28th day in ethylene glycol-induced nephrolithiasis

Groups	Body weight (g) of rats in 0 days	Body weight (g) of rats on the 28th day
Control	180.0± 2.5	202.3±1.3
Ethylene glycol Induced	185.3±7.9	265.5±2.5
Marketed formulation	178.5±5.6	246.8±3.2
Plantain juice	170.4±2.9	201.7±4.5*

All values are Mean±SEM (n = 6) and *p<0.05 when compared to control

Table 3: Effect of PJ formulation on urea, creatinine, uric acid and calcium in ethylene glycol-induced nephrolithiasis

Groups	Serum blood urea (mg dL^{-1})	Serum creatinine (mg dL^{-1})	Serum uric acid (mg dL^{-1})	Serum calcium (mg dL^{-1})
Control	41.12±3.6	0.9±0.01	4.5±1.2	8.7±1.3
Ethylene glycol induced	65.23±2.5	2.9±0.3	8.3±2.1	15.8±2.1
Marketed formulation	54.87±2.9	1.5±0.1	7.6±3.2	10.1±2.9
Plantain juice	43.85±3.1*	1.0±0.02*	5.5±2.1*	7.3±0.8*

All values are Mean±SEM (n = 6) and *p<0.05 when compared to control

Table 4: Effect of PJ formulation on MDA, GSH and catalase in ethylene glycol-induced nephrolithiasis

Groups	MDA (nmol mg^{-1} of protein)	GSH (nmol mg^{-1} of protein)	Catalase (μmol/H_2O_2/min)
Control	0.49±0.12	6.57±0.54	38.15±1.56
Ethylene glycol induced	4.63±0.34	4.94±0.12	15.85±0.74
Marketed formulation	2.54±0.81	4.59±1.45	29.30±0.64
Plantain juice	1.43±0.23*	5.98±0.93*	36.64±0.59*

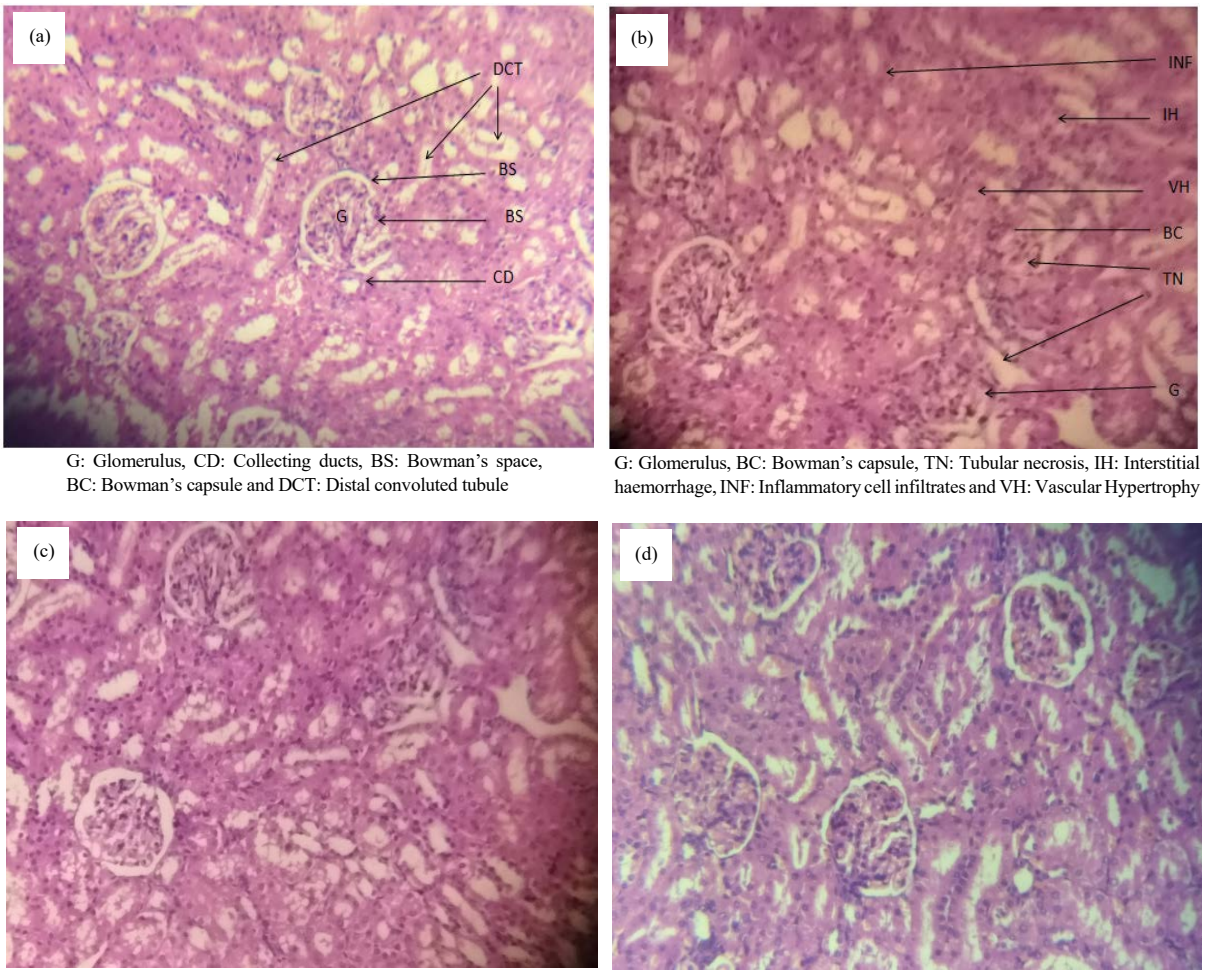
All values are Mean±SEM (n = 6) and *p<0.05 when compared to control

marketed formulation shown in Table 2, treatment with PJ formulation (500 mg kg^{-1}, p.o.) resulted in substantial changes in animal body weight on days 0 and 28 ($p<0.05$).

Comparing the EG-treated group to the control animals, urea, creatinine, uric acid and calcium levels significantly increased. However, compared to the commercial formulation shown in Table 3, therapy with the PJ formulation (500 mg kg^{-1}, p.o.) resulted in a substantial decrease in urea ($p<0.05$), creatinine ($p<0.05$), uric acid ($p<0.05$) and calcium ($p<0.05$).

As compared to the control group, there was a significant increase in MDA and a drop in GSH and catalase levels in the EG-treated group. When compared to the control group, the PJ formulation (500 mg kg^{-1}, p.o.) treatment significantly (p 0.05) decreased MDA levels and increased GSH and catalase levels are shown in Table 4. Marketed formulation therapy does not significantly alter the MDA, GSH and Catalase levels.

Histopathological studies: In the histopathological findings of the present study, the renal tissue and glomerulus in the normal control (group-A) had normal architecture. The collecting ducts, Bowman's capsule and distal convoluted tubule were also clear and the parenchyma did not exhibit any abnormalities. Bowman's capsule is somewhat expanded in the ethylene glycol-treated (group-B), which also had glomerulus congestion and shrinkage with infiltration, vacuolar degeneration in the tubular epithelium and glomerulus lesions. Haemorrhage inside the interstices, tubular necrosis Vascular hypertrophy and mild chronic inflammatory cell infiltrate. Marketed (group-C) and plantation juice treated (group-D): Within the renal corpuscles, there was a retraction of the glomerular flocculus along with a slight vacuolar degeneration of the tubular epithelium. The glomerular density significantly increased as compared to the other groups, however, the tissue of the kidneys did not significantly change. In contrast to the animals in the ethylene glycol-induced (group-B), the animals in the PJ formulation treated (group-D) displayed normal histological traits, according to the results of the histological study. The outcomes were shown in Fig. 1a-d.



G: Glomerulus, CD: Collecting ducts, BS: Bowman's space, BC: Bowman's capsule and DCT: Distal convoluted tubule

G: Glomerulus, BC: Bowman's capsule, TN: Tubular necrosis, IH: Interstitial haemorrhage, INF: Inflammatory cell infiltrates and VH: Vascular Hypertrophy

Fig. 1(a-d): Histopathological studies of kidney, (a) Normal control, (b) Ethylene glycol induced, (c) Marketed formulation and (d) PJ formulation

DISCUSSION

In this experiment, EG-induced nephrolithiasis in male rats. Because compared to male rats, female rats had less stone accumulation, according to past studies[13]. When EG (0.75%) was administered to male Wistar rats, hyperoxaluria developed. Treatment with PJ formulation (500 mg kg^{-1}) considerably reduced it. Rats treated with the PJ formulation had significantly more urine produced than those treated with EG. Because urinary system stones were obstructing the flow of urine, the EG-treated group displayed a decreased glomerular filtration rate. Waste products including urea, creatinine, BUN and uric acid accumulate in the blood as a result, signalling severe kidney impairment[14]. The MDA levels were much higher in the group that received EG treatment and antioxidant capability was significantly lower in the renal tissue. The polyunsaturated fatty acids in the cell membrane react with elevated oxalate concentration in urine to trigger lipid peroxidation and harm the kidneys[15]. The higher serum levels of the glomerular and tubular damage indicators creatinine and uric acid in EG-treated rats showed that there was significant renal damage. Treatment of the PJ formulation decreased MDA levels and stopped these markers' serum levels from rising. Following treatment with PJ formulation, kidney weight, MDA levels and GSH and catalase levels all significantly decreased, demonstrating PJ formulation's protective impact against EG-induced oxidative stress. Due to the presence of active ingredients in PJ formulation, which appear to have anti-nephrolithiasis-preventing activity in rats treated with EG, this activity may have been caused. It was demonstrated that the PJ formulation treatment groups reduced intracellular calcium. This could be a result of enhanced nitric oxide bioavailability, which in turn activates 3, 5'cyclic guanosine monophosphate, which in turn regulates

the rise in intracellular calcium levels[16]. Histopathological studies suggested that the animals in the PJ-treated group showed normal histological features compared to the nephritis-induced group.

CONCLUSION

These findings suggested that using the PJ formulation reduced and stopped the development of kidney stones. To stop the growth of stones in their early stages, PJ formulation was useful. The mechanism underlying this effect may have been mediated by a potent antioxidant, nephroprotective function and a decrease in urinary stone-forming ingredient concentration brought on by the synergistic interaction of the components used in the PJ formulation.

SIGNIFICANCE STATEMENT

This investigation uncovered the rationale behind the use of an unspecified quantity of a *Musa paradisiaca* formulation in conventional medicine without any toxicity symptoms being noted. The reason for this is that it has a high mean Lethal Dosage (LD_{50}), indicating a high level of relative safety that may be helpful in the treatment of renal problems. This investigation supported its application in Indian traditional medicine.

REFERENCES

1. Al-Mamoori, F., T. Aburjai and D.M. Al-Tawalbe, 2022. *In-vitro* anti-nephrolithiatic activity of selected medicinal plants. Trop. J. Nat. Prod. Res., 6: 1426-1429.
2. Fink, H.A., T.J. Wilt, K.E. Eidman, P.S. Garimella and R. MacDonald *et al*., 2013. Medical management to prevent recurrent nephrolithiasis in adults: A systematic review for an American college of physicians clinical guideline. Ann. Intern. Med., 158: 535-543.
3. Sakhaee, K., 2009. Recent advances in the pathophysiology of nephrolithiasis. Kidney Int., 75: 585-595.
4. Butterweck, V. and S.R. Khan, 2009. Herbal medicines in the management of urolithiasis: Alternative or complementary? Planta Med., 75: 1095-1103.
5. Li, X., Q. Liang, Y. Sun, L. Diao and Z. Qin *et al*., 2015. Potential mechanisms responsible for the antinephrolithic effects of an aqueous extract of fructus aurantii. Evidence-Based Complementary Altern. Med., Vol. 2015. 10.1155/2015/491409.
6. Daudon, M., O. Traxer, E. Lechevallier and C. Saussine, 2008. Lithogenesis. Progrès Urologie, 18: 815-827.
7. Kaleeswaran, B., S. Ramadevi, R. Murugesan, S. Srigopalram, T. Suman and T. Balasubramanian, 2019. Evaluation of anti-urolithiatic potential of ethyl acetate extract of *Pedalium murex* L. on struvite crystal (kidney stone). J. Traditional Complementary Med., 9: 24-37.
8. Imam, M.Z. and S. Akter, 2011. *Musa paradisiaca* L. and *Musa sapientum* L. : A phytochemical and pharmacological review. J. Appl. Pharm. Sci., 1: 14-20.
9. Moriyama, M.T., K. Suga, K. Miyazawa, T. Tanaka and M. Higashioka *et al*., 2009. Inhibitions of urinary oxidative stress and renal calcium level by an extract of *Quercus salicina* blume/*Quercus stenophylla* makino in a rat calcium oxalate urolithiasis model. Int. J. Urol., 16: 397-401.
10. Doizi, S. and O. Traxer, 2018. Flexible ureteroscopy: Technique, tips and tricks. Urolithiasis, 46: 47-58.
11. Gainetdinov, I.V., S.A. Kondratieva, Y.V. Skvortsova, M.V. Zinovyeva and E.A. Stukacheva *et al*., 2016. Distinguishing epigenetic features of preneoplastic testis tissues adjacent to seminomas and nonseminomas. Oncotarget, 7: 22439-22447.
12. Kiernan, J.A., 2008. Nuclear stains. CSH Protoc., Vol. 3. 10.1101/pdb.top50.
13. Karadi, R.V., N.B. Gadge, K.R. Alagawadi and R.V. Savadi, 2006. Effect of *Moringa oleifera* Lam. root-wood on ethylene glycol induced urolithiasis in rats. J. Ethnopharmacol., 105: 306-311.
14. Selvam, R., P. Kalaiselvi, A. Govindaraj, V.B. Murugan and A.S.S. Kumar, 2001. Effect of *A. lanata* leaf extract and vediuppu chunnam on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria. Pharmacol. Res., 43: 89-93.
15. Huang, H.S., M.C. Ma, J. Chen and C.F. Chen, 2002. Changes in the oxidant-antioxidant balance in the kidney of rats with nephrolithiasis induced by ethylene glycol. J. Urol., 167: 2584-2593.
16. Iyú, D., N.M. Atucha, C. Martínez-Prieto, M.C. Ortiz and J. García-Estañ, 2004. Altered calcium signaling in platelets from nitric oxide-deficient hypertensive rats. Cell Commun. Signaling, Vol. 2. 10.1186/1478-811X-2-1.