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Research Article

LITHOTRIPTIC AND ANTI-LITHOGENIC EFFECT OF TILA-DHATRI KSHARA ALONG WITH GOKSHURA KASHAYA IN ETHYLENE GLYCOL-INDUCED NEPHROLITHIASIS IN ADULT MALE CHARLES FOSTER RATS

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ABSTRACT

Objectives: Thirty-five adult Charles Foster rats were taken in the study to assess the lithotriptic and anti-lithogenic activity of the Trial drug.

Methods: Thirty-five were divided into seven groups of five animals each. One group was kept as normal control; one group was given ethylene glycol 0.75% alone for 28 days. In the next three groups, Trial drug, kshara with distilled water, and the Standard drug were given, respectively, for 28 days after induction of calculi using ethylene glycol. In the next two groups, to assess the anti-lithogenic activity, simultaneously stone inducing agents and trial/standard drugs were given for 28 days. At the end, blood, urine, and histopathology of rat kidneys were done.

Results: Tila-Dhatri kshara with Gokshura Kashaya and standard drug, Potassium Magnesium Citrate B6 showed equal lithotriptic and anti-lithogenic activity as depicted from blood, urine biochemistry, urine microscopy, and histopathology of rat kidneys. Trial drug-treated group showed more diuresis than other groups. Furthermore, serum LDH values were significantly reduced in trial drug-treated group.

Conclusion: The trial drug showed significant nephroprotective property when compared to the standard drug as depicted from the serum LDH values and histopathological evaluations.

Keywords: Lithotriptic, Anti-lithogenic, Kshara, Gokshura kashaya, Nephrolithiasis, Ethylene glycol.

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INTRODUCTION

Kidney stone disease (KSD) or nephrolithiasis is multifactorial and is strongly related to dietary lifestyle habits or practices. Age, gender, race, geography, climate, occupation, body mass index, water intake, etc. are risk factors of kidney stone formation. The lifetime prevalence of the disease in India is 7.9% [1]. According to Global Burden of Diseases 2019, India had the burden of over one-fifth of global incident cases. The country is also one among those having the greatest number of Urolithiasis associated disability-adjusted life years and deaths [2].

The crystal deposition in kidneys as a consequence of KSD leads to the activation of inflammatory pathways and ultimately kidney dysfunction. The generation of reactive oxygen species (ROS) and lipid peroxidation is outcomes of long-standing inflammation in KSD. This further leads to damage to renal cell membranes which act as a nidus for the crystal deposition. Recurrence rate of nephrolithiasis is 30–50% within 5–10 years after initial stone episode [3]. This can be explained by the *Randalls plaque hypothesis* in which it says that even after the complete dissolution of stone in initial stone formers, randalls plaques in interstitium and tubular walls acts as a nidus and contributes in recurrence of stone episodes after a period of time. Hence, treatment must focus on not only stone dissolution but also prevention of recurrence. Many proven *Ashmarihara*, *mootravirechaneeya,* and Nephro-protective drugs are there in Ayurvedic pharmacopoeia. These drugs cause dissolution of stone and acts as antioxidants which rejuvenates cell death.

Lithotriptic means the ability of a drug to destroy or dissolute the stone formed and *anti-lithogenic* means the ability of a drug to prevent stone formation (helps in checking the recurrence of stones). "*Kshara*" is best known for its alkalizing and chemolysing property. Rasa Tarangini (Ayurveda Pharmaceutics and Indian Alchemy) compiled by Sri Sadananda Sharma is said to be a wonderful guide to those who wish to utilize "products of metals and minerals" into their practice for better and quicker therapeutic results. From this book, Tila-Dhatri kshara along with Gokshura Kashaya [4] has been taken. The indication of the combination is "*nashayet sarkaraa", that is,* the combination destroys the gravels. Thus, an effort was made to peep into the lithotriptic and anti-lithogenic activity of Tila-Dhatri kshara, along with Gokshura Kashaya.

Animal studies are baseline in biomedical research and help in understanding the pathogenesis of disease, its progression, the mode of action of drug, the reversal of pathology after drug administration, etc. Hence in this study, male rats were selected to conduct the study.

METHODS

Plant material and preparation of Tila-Dhatri kshara

All the raw drugs used in the preparation of the trial drug were procured from the Gola Deena Nath Market, Godowlia, Uttar Pradesh, India. The identification of the drug was done in the Department of Dravyaguna, IMS, BHU, Varanasi. Tila panchanga, and Dhatri fruits were made into Paneeya kshara and Gokshura kashaya was prepared as per AFI in the department of Shalyatantra, IMS, BHU.

Animals

35 healthy adult Charles Foster male rats with body weight between 180 and 250 g were procured from the Animal House of IMS, BHU, Varanasi. The rats were maintained under standard laboratory conditions (temp - 24–28°C, relative humidity 60–70% and 12-h dark and light cycles). Rat feeds were purchased from the Animal House BHU (specially prepared under instructions by veterinary doctor) and

given to the rats. Water was given *ad libitum.* The experimental study protocols were approved by the Institutional Animal Ethical Committee (IAEC), (no.Dean/2020/IAEC/2215) and a written permission was obtained from IAEC, IMS, BHU. The study was carried out as per the standard guidelines for the use of experimental animals.

Chemicals and drugs

Ethylene glycol (0.75% v/v) and Di ethyl ether of analytical grades were purchased from Merck Ltd, Varanasi, India. Reference drug Potassium Magnesium Citrate B6 was purchased from the local medical shop in Varanasi. Double distilled water was made in the ksharasutra laboratory in Shalyatantra Department, Faculty of Ayurveda, IMS, Varanasi.

Drug administration

The trial and reference drugs were administered through stainless steel oral feeding cannulas.

Lithotriptic and anti-lithogenic study

The male rats were divided into seven groups of five animals each and these were randomly selected to receive either the preventive or the curative treatment. Kidney stone was induced in rats by giving 0.75% ethylene glycol.

Group I (Normal control-NC)	Normal control and gave regular feed and drinking water *ad.libitum*.
Ethylene glycol (0.75%) in drinking water was fed for 28 days in Group II – Group VII for induction of urinary calculi	
Group II (Positive control-PC)	Drinking water for subsequent 28 days (29–56th day) (No medicine at all)
Group III (Trial drug treated Group-LT)	Effective dose of Tila – Dhatri kshara with Gokshura Kashaya for subsequent 28 days (29th–56th day) was given
Group IV (Kshara treated Group-LK)	Effective dose of Tila –Dhatri kshara alone in distilled water for subsequent 28 days (29th–56th day) was given
Group V (Standard drug treated Group-LS)	Effective dose of Potassium Magnesium Citate – B6 for subsequent 28 days (29th–56th day) was given
Group VI (Anti-lithogenic Trial Group-ALT)	While giving ethylene glycol, rat dose of Tila – Dhatri kshara with Gokshura kashaya was given for 28 days simultaneously
Group VII (Anti-lithogenic Standard Group-ALS)	While giving ethylene glycol, rat dose of Potassium Magnesium Citate B6 was given for 28 days simultaneously

Collection and analysis of urine

At the end of the experiment, the blood and urine analyses were done. Animals were kept in individual metabolic cages and 24-h urine samples were collected for urine volume, biochemical analysis, and for microscopy (observed under light microscope). After urine collection, the samples were analyzed for calcium, magnesium, citrate, and oxalates.

Serum analysis

After urine collection blood was collected by retro-orbital method (0.5 ml blood) under mild anesthetic condition using diethyl ether. The blood samples were analyzed for serum values of blood urea, creatinine, serum calcium, uric acid, and LDH.

Histopathological study of Kidneys

At the end of the study, rats were sacrificed by giving high-dose anesthesia and dissected to take out the kidneys. The kidneys were washed first in ice-cold normal saline and then kept in separate small containers added with 10% of formalin solution. Thereafter, slides were prepared (processed and embedded in paraffin wax, sectioned at 5µm and stained with Haematoxylin and Eosin) and took under microscope 40X for histology.

Statistical analysis

All data were expressed as mean±standard deviation. The results were analyzed by one-way analysis of variance, followed by a comparison between the groups using *post hoc* BONFERRONI alpha test. Differences between groups were considered significant at $p<0.05$.

RESULTS

Urine analysis

The mean values of various urinary parameters of different groups of experimental rats are given in the Table 1. The mean values of urinary volume, pH, magnesium, and citrate were decreased and that of calcium and oxalate were increased in PC group when compared to NC group.

The trial and standard drugs were effective in increasing the urinary volume. The trial drug showed a significant increase in urine volume in comparison to the Standard drug with $p<0.01$. The inhibitors of crystallization, that is, urinary citrate and magnesium were increased significantly in both the trial and standard groups. Furthermore, the urinary calcium and oxalates were decreased considerably in trial and standard groups in comparison to PC groups.

Serum analysis

The mean values of various blood parameters of different groups of experimental rats are given in Table 2. Blood levels of urea, creatinine, and uric acid have been increased in the positive control group in comparison to the normal control one. Calcium level in blood in the positive control group has been decreased with respect to normal control group. Furthermore, serum LDH is noted high in positive control group.

Trial and standard drug treatment led to a considerable reduction in blood urea, creatinine, and uric acid in both preventive and curative regimen with respect to a positive control group. The serum calcium levels were restored in both trial and standard drug-treated groups. LDH level was increased in the hyperoxaluric positive control group (Table 2). These levels of serum LDH were reduced significantly ($p<0.01$) only in trial drug-treated group.

Observations based on urine microscopy

Urine microscopy of normal animal yielded no crystals or casts in the urine (Fig. 1). The ethylene glycol-induced group/positive control group developed numerous large Calcium oxalate crystals of rectangular shape. Struvite crystals of coffin shape were also noted among them. The crystals were large and were found in aggregates in the urine microscopy (Fig. 2). Compared to the positive control group (Fig. 2), the crystals in trial drug-treated group (Fig. 3) and Kshara-treated groups (Fig. 4) and Standard drug-treated groups (Fig. 5) were very less in number, smaller in size, and tetrahedral (envelope shaped) in shape (marked in arrows). Envelope-shaped tetrahedral crystals denote calcium oxalate dihydrate crystals (COD). Furthermore, crystals were found singly and not adherent. In preventive groups or anti-lithogenic groups (Figs. 6 and 7), the urine showed tiny microliths, having very small sizes as compared with the positive control group and the drug-treated groups**.**

Observations based on histopathological examination

Histological studies revealed that in disease-induced positive control groups, the tubules were filled with crystals and dilated (Fig. 8). Epithelium was flattened and there was sloughing of tubular epithelium. The interstitium had mild lymphocytic infiltration in the positive control group. On the other hand, the trial drug-treated group (Fig. 9) showed scattered tiny crystals here and there in the tubules. Sloughed epithelium, lymphocytes, and dilated tubules were absent in the kidney sections of trial drug-treated group which was suggestive of healed interstitium and tubules. In standard drug-treated group (Fig. 10), tiny crystals were seen scattered in the tubules. Focal dilatation and sloughing of epithelium were evident in some parts of the tubules. The *kshara* only treated group (Fig. 11) showed minimal crystals with some of the tubular dilatation. Histopathology of the preventive group treated with trial drug showed normal glomeruli and tubules (Fig. 12).

Table 1: Urinary parameter mean values±SD

Parameters	NC	PC	LT	LK	LS	ALT	ALS
Volume (ml/24 h)	13.40±1.517	5.60±1.140	18±2.121	12.40±1.817	13.60±1.517	17±2.121	15±1.414
Ph	7.22±0.242	6.18±0.154	6.97±0.119	6.83±0.377	6.77±0.227	7.05±0.262	7.01±0.297
Calcium (mmol/L)	0.74±0.020	2.32±0.114	1.06±0.123	1.07±0.112	1.11±0.141	0.89±0.073	1.02±0.100
Magnesium (mmol/L)	1.94±0.050	0.90±0.055	2.39±0.166	2.14±0.147	2.62±0.089	2.35±0.294	2.35±0.138
Citrate (mg/dl)	50.07±1.741	24.46±2.736	57.54±2.107	58.10±4.621	56.18±1.238	52.66±2.887	52.15±3.762
Oxalate (mg/dl)	1.38±0.111	5.57±0.560	2.18±0.171	2.30±0.223	2.08±0.144	1.77±0.236	1.67±0.166

NC: Normal control, PC: Positive control, LT: Trial drug treated Group, LK: Kshara treated Group, LS: Standard drug treated Group, ALT: Anti-lithogenic Trial Group, ALS: Anti-lithogenic Standard Group

Table 2: Blood parameters - Mean values±SD

Parameters	NC	PC	LT	LK	LS	ALT	ALS
Blood urea (mg/dL)	32.16±2.228	47.34±4.367	33.72±1.570	37.24±1.556	36.92±1.066	34.96±1.291	36.40±1.976
Creatinine (mg/dL)	0.84±0.034	1.66±0.135	0.89±0.124	0.97±0.045	0.97±0.071	0.89±0.104	0.96±0.066
Calcium (mg/dL)	8.52±0.258	7.18±0.571	8.06±0.486	8.95±0.307	9.08±0.558	8.49±0.450	8.49±0.218
Uric acid (mg/dL)	1.94±0.665	4.08±0.563	2.21±0.442	2.46±0.400	2.18±0.536	1.98±0.649	1.97±0.663
LDH (IU/L)	526±161	2050±425.574	833±73.855	1135±114.254	1351±100.229	955±75	1065±50.333

NC: Normal control, PC: Positive control, LT: Trial drug treated Group, LK: Kshara treated Group, LS: Standard drug treated Group, ALT: Anti-lithogenic Trial Group, ALS: Anti-lithogenic standard Group

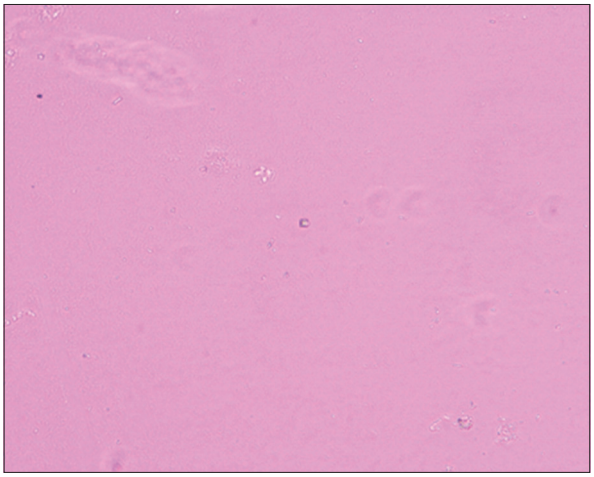

Fig. 1: Urine microscopy – Normal control - ×40

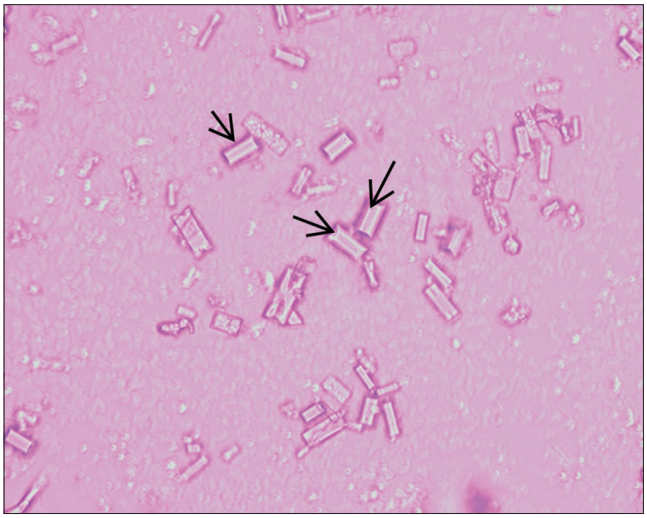

Fig. 2: Urine microscopy – Positive control -×40

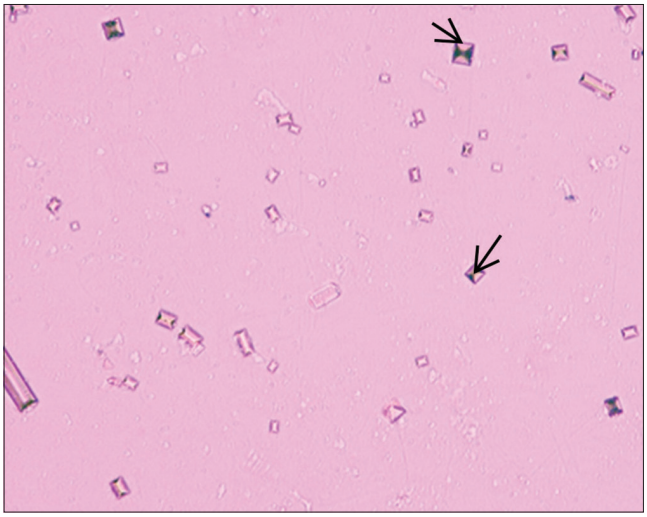

Fig. 3: Urine microscopy – Trial drug - ×40

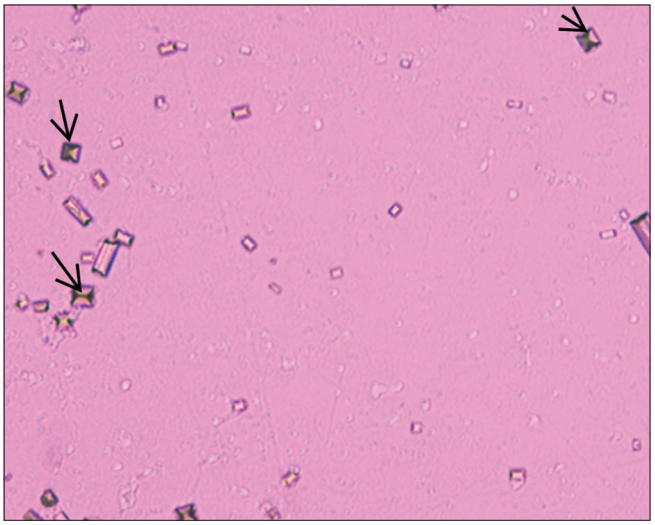

Fig. 4: Urine microscopy - Kshara - ×40

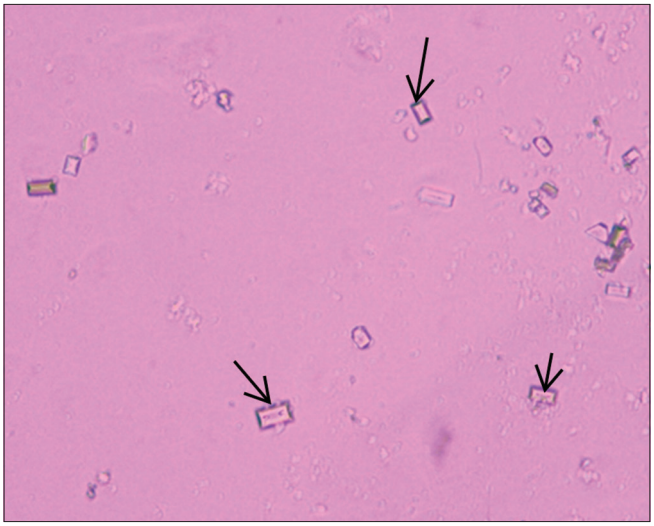

Fig. 5: Urine microscopy – Standard drug - ×40

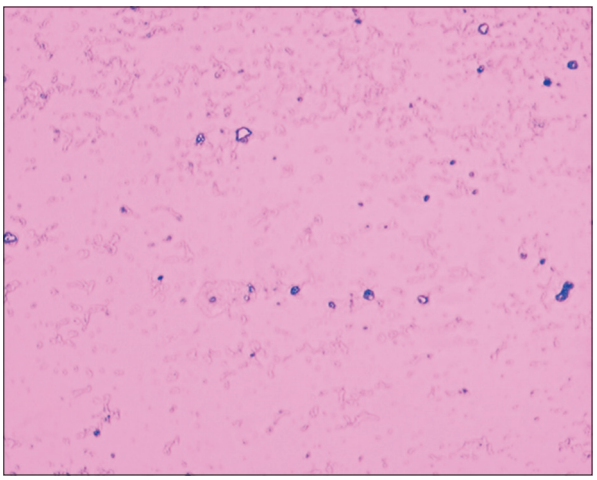

Fig. 6: Urine microscopy – Anti-lithogenic Trial - ×20

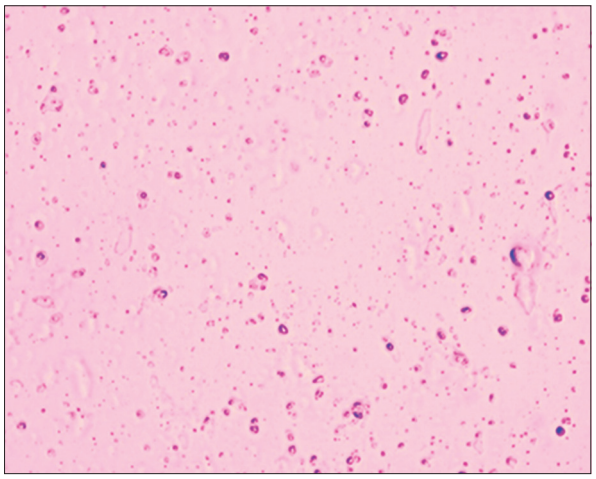

Fig. 7: Urine microscopy – Anti-lithogenic standard - ×20

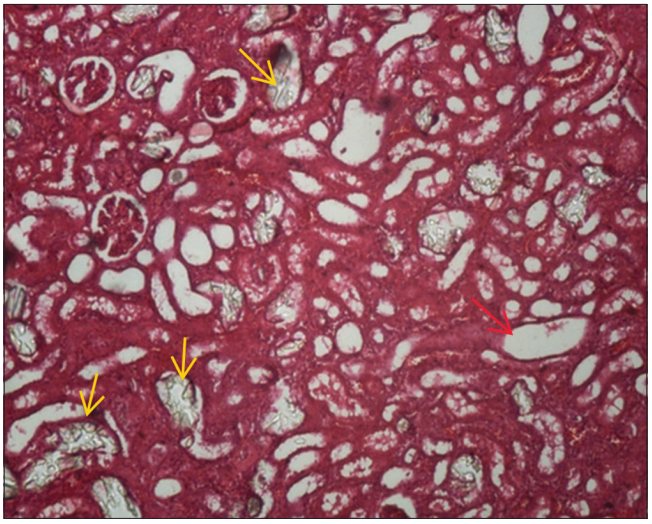

Fig. 8: Histopathological examination – Positive control

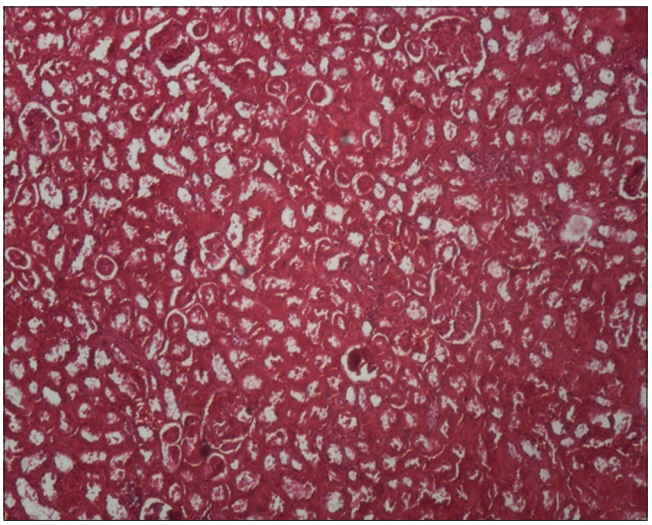

Fig. 9: Histopathological examination – Trial drug

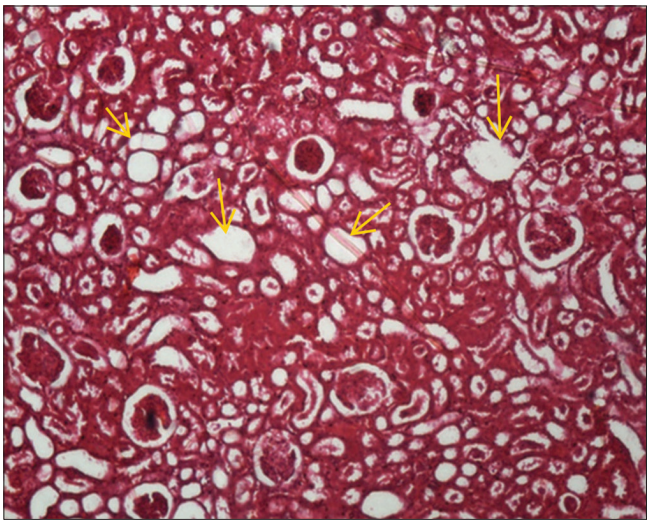

Fig. 10: Histopathological examination – PMCB6-treated group

No crystals were seen deposited in their tubules. There were no signs of inflammation. All features suggested the normal appearance of kidney tissues. In the preventive standard drug group, histopathology revealed normal glomeruli (Fig. 13). However, tubules were seen dilated slightly suggestive of mild inflammation.

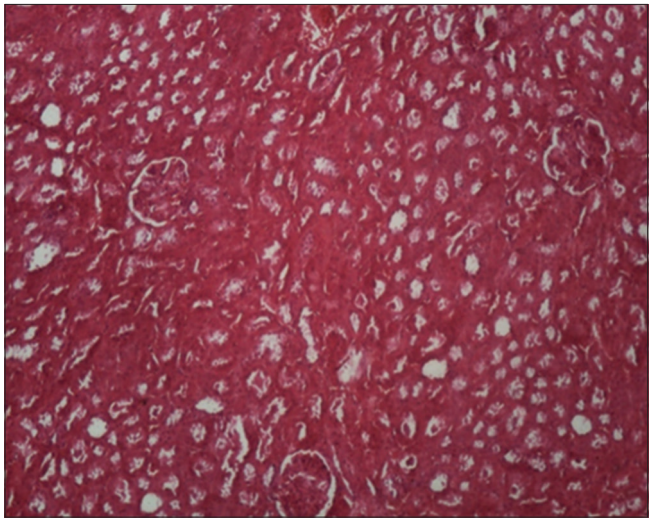

Fig. 11: Histopathological examination – Kshara-treated group

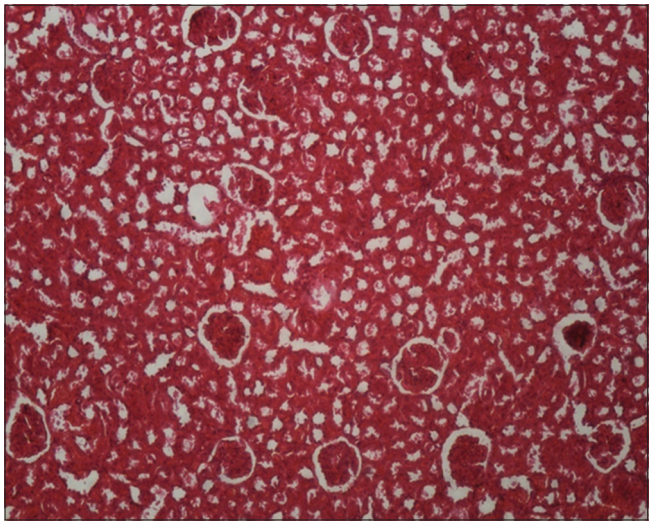

Fig. 12: Histopathological examination – Anti-lithogenic trial

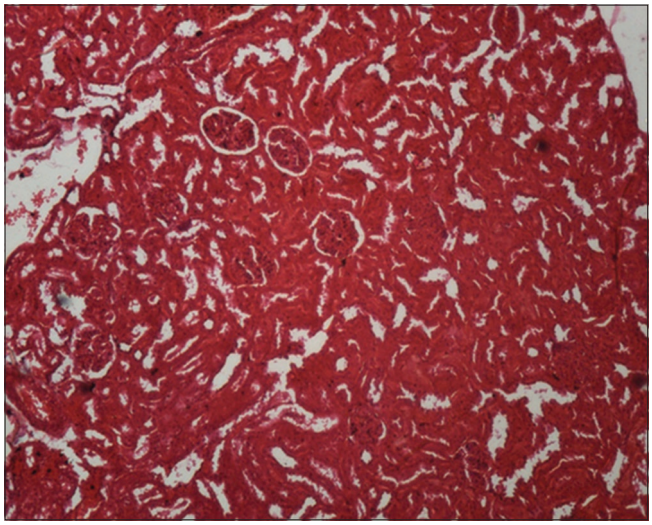

Fig. 13: Histopathological examination – Anti-lithogenic standard

DISCUSSION

Drugs that are *ashmarihara* are used in various forms in a number of studies in urolithiasis. *Gokshura* (Tribulus terrestris Linn.) is a frequently used drug as it has *mutrala* (diuretic) and *ashmarihara* (lithotriptic) property. Kshara is a unique preparation in Ayurveda which is highly alkaline and has *chedana, bhedana and lekhana, and ashmarihara properties.* Hence, in search for a novel drug for research, a combination of these two was taken from Rasatarangini. In Contemporary science Potassium Magnesium Citrate B6, which is a proven litholyser, is the mainstream in managing uncomplicated urinary calculi <10 mm. Hence, this was taken as the reference drug to compare the lithotriptic action and their ability in modulating crystal morphology and their property of rejuvenating renal epithelial cells.

As a preclinical study, an experimental study was conducted and male rats were selected to induce calculi because the urinary system of male rats resembles that of humans and earlier studies have shown that kidney stone formation in female rats was significantly less than male rats [5]. Ethylene glycol 0.75% v/v was given for induction of calculi in rats. Ethylene glycol when ingested gets absorbed in the body. The end product of ethylene glycol metabolism is oxalic acid. This leads to precipitation of calcium oxalate monohydrate (COM) crystals in the tubules of kidney. These crystals eventually attach to tubular cell membrane through endocytosis and cause structural damage in cell membrane producing free radicals and lipid peroxidation. The end event is renal tubular necrosis and apoptosis, ultimately leading to acute kidney injury [6].

For evaluating the stone attenuating property of drugs, curative groups were designed (groups-LT, LK, and LS). Preventive groups (ALT and ALS) were designed to assess the ability to slow down or inhibit the process of stone formation in an environment that is conductive to stone formation otherwise.

Looking into the results of urine microscopy, there is an evident reduction in stone size and number in curative groups. Morphology of stones was also altered. COM crystals in PC groups were converted into COD crystals in curative groups. Envelope-shaped tetrahedral crystals denote Calcium oxalate dihydrate crystals (COD) which are less adhesive and cause less injury to the renal epithelium in comparison to CaOx monohydrate (COM) crystals [7]. This reveals anti-crystallization and lithotriptic activity of the trial drug, standard drug, and Kshara. Furthermore, the lekhana and bhedana property of kshara and the presence of saponins and Kaempferol in Gokshura which inhibits crystallization, contribute to the lithotrptic activity. Saponins and Flavonoids are found to have stone disintegrating property and CaOx stone dissolution potency respectively [7]. Furthermore, Tannins exhibit anti-crystallization effect by aiding calcium complexation and thus deterring CaOx crystal formation [7]. All these chemical constituents, that is, saponins, tannins, and flavonoids in the Trial drugs may have contributed to the stone disintegrating property. The antiurolithic activity of Gokshura is attributed to its GOX inhibition. Quercetin and kaempherol, the active components of Gokshura, were found to be non-competitive and competitive inhibitors of GOX, respectively [8]. All have stone disintegrating and dissolution properties.

The preventive property of a drug in terms of stone formation can be confirmed if a drug is able to arrest the progression of stone size by intruding in the stages of stone formation [9]. Here, in preventive groups or anti-lithogenic groups (Figs. 6 and 7), the urine showed tiny microliths, having very small size as compared with the positive control group and the drug-treated groups. Thus, it can be concluded that the trial drug may have inhibited the crystal growth or nucleation of stones due presence of Quercetin and kaempherol (active principles in Gokshura) which are inhibitors of GOX (Glycolate oxidase) [10]. Potassium magnesium

citrate B6 is also a strong inhibitor of crystallization [11]. This shows that both the trial and standard drug are equally effective in terms of the prevention of formation of kidney stones.

The increased BUN, serum creatinine, uric acid in the PC group marks tubular and glomerular damage in the kidney causing significant impairment of renal functions. In the urine microscopy, it was evident that the trial (Fig. 3), standard (Fig. 5), and Kshara (Fig. 4) treated groups reduced the urine crystals in terms of its number and size when compared to positive control group (Fig. 2). Thus, due to reduced crystal size and number, pathogenesis in kidney tissues may have reversed, ultimately leading to reduced blood urea, uric acid, and creatinine.

Serum calcium has been decreased in the positive control group (Table 2). This may be because of the metabolism of ethylene glycol [12]. The end product of EG metabolism is oxalate. Calcium in blood readily binds to this oxalate which ultimately results in decreased serum levels of calcium. Thus, more calcium in the blood binds with oxalates leading to hypocalcemia.

In this study, serum LDH values have been found to be highly increased in positive control group (Table 2). It is regarded as the enzyme marker for cellular damage. The EG increases the activity of LDH which is an oxalate synthesizing enzyme present in the liver and kidney [13]. This may give a clue that the kidney tissues are being injured due to calcium oxalate crystal deposition in the tubules of the positive control group damaging the epithelial lining of renal tubules which leads to adhesion and retention of crystals [14]. In the trial drug-treated group, LDH has been significantly reduced when compared to standard drug-treated group (Table 2). This suggests the nephroprotective action (defeating the oxidative stress) of the trial drug – Tila, Dhatri, and Gokshura as they are powerful antioxidants which decrease the oxidative stress in the kidneys [15-17].

In the standard group, serum LDH was reduced than in the positive group, but not as much as that of the trial group (Table 2). That is, even after administering standard drug for 28 days, standard drug contains only inhibitors of crystallization which prevents the further growth of stone This point toward the fact that standard drug do not have any nutraceuticals agents that may rejuvenate the kidney. Hence, it may be inferred that the Standard drug could break the pathology of KSD but not have any nephroprotective activity.

Calculi develop due to imbalance between the promoters and inhibitors of stone formation in the urine. Hyper calcinuria, hyper oxaluria, hypocitraturia, and hypo magnesuria are the findings in CaOx crystallization [7]. This is evident in positive control group (Table 2). Standard and trial drug-treated groups significantly reduced urinary calcium and oxalate and restored diminished urinary levels of magnesium and citrate (Table 2) and thus restored the equilibrium between promoters and inhibitors of stone formation. The trial drug Tila-Dhatri kshara has a pH of 10.17 which act as a strong alkaliser. Kshara or plant alkalis are rich in potassium and magnesium that act as stone inhibitors.

Potassium and magnesium citrate when given orally produce an alkaline load due to absorbed citrate. This causes an increase in urinary pH along with urinary citrate, potassium, and magnesium excretion. As a result of this, urine becomes less conductive to the crystallization of salts such as calcium oxalate, calcium phosphate, and uric acid [12]. Urinary citrate complexes with calcium ion, thus decrease calcium ion activity and decrease saturation of CaOx. Increased Magnesium in urine complexes with oxalate and decreases oxalate ion activity which decreases saturation of CaOx [12].

Urine volume has significantly decreased in the positive control group when compared to normal control (Table 1). This is because, due to stone formation, GFR becomes reduced and hence urine becomes highly concentrated which results in less excretion of urine. In positive control group, the urine was very concentrated and yellowish in color and very less compared to other groups. In all other treated groups, decreased urine volume was *not* noted. This may be due to the reversal of disease pathology, leading to greater GFR and eventual normal urine volumes. Other one striking thing noted in the study was that the trial drug-treated group had an increased urinary volume than that of the normal control group. This may be due to the diuretic action of Gokshura.

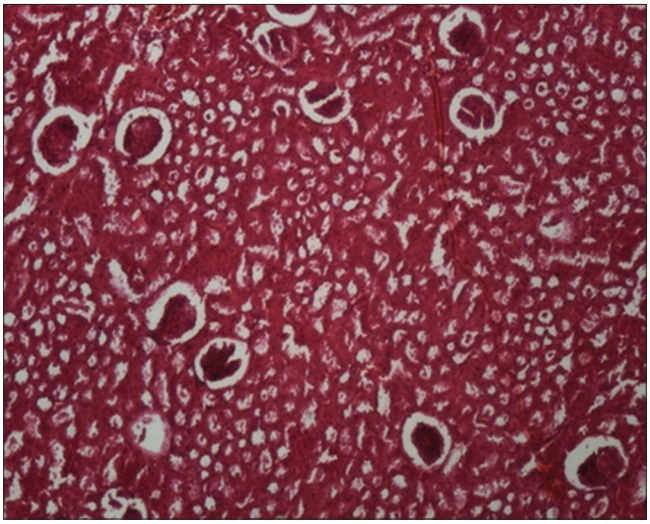

Fig. 14: Histopathological examination – Normal control

Histopathology of the rat kidneys was done to assess the microtubular crystallization, interstitium, glomerulus, and vascular status in each group. The kidney sections of normal control (Fig. 14) group when examined under the light microscope showed normal kidney cells with no signs of inflammation or crystallization in the tubules. Ethylene glycol administered group (Fig. 8) showed signs of acute tubular necrosis. The tubules were filled with crystals and dilated. Epithelium was flattened and there was sloughing of tubular epithelium. Some of them showed epithelial casts. The interstitium had mild lymphocytic infiltration. Some of the tubules were dilated and there were numerous refractive crystals in the lumen of tubules. The end product of ethylene glycol metabolism is oxalic acid. This leads to precipitation of calcium oxalate monohydrate (COM) crystals in the tubules of kidney. These crystals eventually attach to tubular cell membrane through endocytosis. This leads to structural damage in cell membrane producing free radicals and lipid peroxidation. The end event is renal tubular necrosis and apoptosis ultimately leading to acute kidney injury [6]. This is the pathology behind formation of tubular crystals in positive control group. Sloughing of epithelium, lymphocytic infiltration, and tubular dilatation are all signs of acute inflammation following the formation of stone in the kidneys.

In standard drug-treated group (Fig. 10), tiny crystals were seen scattered and showed focal dilatation of tubules. Sloughing of epithelium was also evident in some parts of the tubules. Glomerulus and vascular findings were within normal limits. However overall, the findings were very positive compared to the positive control group (Fig. 8). Inhibition of Crystallization by PMCB6 led to the reduction in number and size of COM crystals. This eventually led to less tubular injury which promoted the healing of urothelium and injured cells leading to the above findings. The trial drug-treated group (Fig. 9) showed tiny crystals scattered here and there in the tubules. However, the interstitium, vessels, tubules, and glomerulus were found unremarkable. Following administration of the trial medication for 28 days, marked histological alterations caused by CaOx crystal deposition in the hyperoxaluric group were significantly reduced.

Agarwal *et al*. (2013) in his paper- "*The Role of Natural Antioxidants as Potential Therapeutic Agent in Nephrolithiasis*" conveys that crystal nephropathies have been linked to ROS induced damage and

in hyperoxaluric conditions, ROS-induced renal damage leads to an alteration in the levels of antioxidant enzymes, an increase in lipid peroxidation, along with impairment in glutathione metabolizing enzyme activity. This can be restored by antioxidant treatment [18]. All the three drugs of Trial group – Tila, Dhatri, and Gokshura are potent antioxidants. This may be the reason for the significant reduction of CaOx-induced renal damage in rats when treated with the trial drug in comparison to standard and positive control group. The kshara-treated group (Fig. 11) showed minimal crystals with some of the tubular dilatation in comparison to positive Control. Kshara has *ashmarihara, chedana and bhedana* properties. Ethylene glycol-induced rat models give rise to formation of acidic urine which facilitates calcium oxalate crystal formation. Tila-Dhatri kshara has a pH of 10.17. This is highly basic. Hence, it alkalises the urine and thus inhibits calcium oxalate crystal formation and aggregation.

In the preventive groups, the anti-lithogenic trial (Fig. 12) and standard groups (Fig. 13) showed focal tubular dilatation only. This shows that there is only mild inflammation present. There were no crystals within the tubules showing the efficacy of both the drugs in prevention of stones.

Urine microscopy of normal animal – no crystals or casts in the urine (Fig. 1). Positive control group large calcium oxalate crystals of rectangular shape (Fig. 2). Less number of crystals in tetrahedral shape-in trial (Fig. 3); Kshara (Fig. 4) and standard drug (Fig. 5) treated groups. No crystals in tubules in preventive groups (Figs. 6 and 7).

Normal kidney cells no signs of inflammation or crystallization in the tubules (Fig. 14). The tubules filled with crystals (yellow arrows) and dilated (Red arrow) – in positive control group (Fig. 8). The trial (Fig. 8) and Kshara (Fig. 9) treated groups almost showing normal tissue with the presence of tiny crystals here in there. Standard drug-treated group (Fig. 10) showing focal dilatation of tubules.

CONCLUSION

Lithotriptic and anti-lithogenic activity of Tila-Dhatri kshara, along with Gokshura Kashaya and the reference drug potassium magnesium citrate B6 is well evident from the urine microscopy, HPE, and blood and urine examination of the rats. In addition, the serum LDH values and histopathology point toward the nephroprotective property or renal cell regenerative property of the Tila-Dhatri kshara and Gokshura Kashaya combination. However for more evidence for the nephroprotective activity, advanced studies like scanning electron microscopy of the renal crystal deposits, enzymatic estimation of renal tissues, and gene expression studies are to be conducted as further research.

CONFLICTS OF INTEREST

There are no conflicts of interest.

AUTHORS CONTRIBUTION

The corresponding author collected all the study material, analyzed it, and prepared the complete manuscript. Professor Lakshman Singh and Dr Lalit Kumar provided guidance throughout the study and played a key role in manuscript editing and revision.

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