WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 19, 726-740. Research Article ISSN 2277– 7105

EVALUATION OF *IN VIVO* ANTIUROLITHIATIC ACTIVITY OF WHOLE PLANT OF *SPERMACOCE HISPIDA* (LINN) K. SCHUM

Prasanth S.* and Veena R.

Department of Pharmacology, Department of Pharmaceutical Sciences Cheruvandoor, Kerala, India.

Article Received on
02 October 2018,

Revised on 23 October 2018,
Accepted on 13 Nov. 2018

DOI: 10.20959/wjpr201819-13594

***Corresponding Author**
Prasanth S.
Department of Pharmacology, Department of Pharmaceutical Sciences Cheruvandoor, Kerala, India.

ABSTRACT

Urolithiasis is a common urological disorder responsible for serious human affliction and cost to the society with a high rate of reoccurrence and treatment failure. **Aim:** The aim of the present study was to systematically evaluate the antiurolithiatic activity of ethanolic extract of whole plant of *Spermacoce hispida* (Linn). **Materials and Methods:** Calcium oxalate urolithiasis was induced in male wistar albino rats using calculi producing diet (5% sodium oxalate and rat pellet feed). The biochemical parameters like calcium, oxalate, creatinine, creatinine clearance, blood urea nitrogen, magnesium, potassium, and sodium were evaluated in urine, serum. At the end of experiment 24hr urine samples were collected for urinary estimation (urinary calcium, oxalate, creatinine) and blood was collected from retro orbital sinus and used for the estimation of BUN, creatinine, and creatinine clearance. **Results:** The increased deposition of stone forming constituents in the kidneys of calculogenic rats was significantly lowered by treatment with ethanolic extract of *Spermacoce hispida* L. **Conclusion:** From this study, we conclude that the treatment with ethanolic extracts of *Spermacoce hispida* L. had an inhibitory effect on crystal growth, with improvement of kidney function.

KEYWORDS: Urolithiasis, *Spermacoce hispida* (Linn), CPD (calculi produced diet), sodium oxalate, antiurolithiatic activity.

INTRODUCTION

Kidney stones, also termed renal calculi, are crystal aggregations formed in the kidneys from dietary minerals in the urine.[1] The incidence of urinary calculi is increasing worldwide and calcium oxalate (CaOx, CaC2O4) is the predominant component of most stones, followed by

struvite, cystine, uric acid and other compounds. Epidemiological data suggests that 60-80% of stone is composed mainly of calcium oxalate (CaOx).[2] The overall probability of forming stones differs in various parts of the world, and is estimated at 1–5% in Asia, 5–9% in Europe, and 13% in North America.[3] Lithiasis is a male- predominant disorder.[4] with a recurrence rate of 70–80% in males and 47–60% in females.[5] Currently, no allopathic medications are available for urolithiasis.[4] The precise mechanism by which kidney stones originate is still not well understood. For quite some time attention was focused toward the relationship between the concentrations of the precipitating substances in urine and the solubility of the salts to be formed.[6] the role of nucleating substances.[7] the role of crystal aggregation and the part played by inhibitors of crystal formation and aggregation.[8] The possible relationship between cell dysfunction and renal stone formation is yet to be explored. It is suggested that stone formation may be a cellular defect in the handling of oxalate, calcium, phosphate, or magnesium or in response by the kidney to hormones such as parathyroid hormone or calcitonin or 25-hydroxy calciferol.[6] Many biochemical changes, notably accumulation of stone substances in kidney or urinary tract membrane fractions by feeding calculi producing diet.[9] or vitamin B6-deficient diet.[10] administration of sodium oxalate.[11] ethelene glycol.[12] etc. There are main three factors which determine stone formation by assessing urinary composition: quantity of inhibitors (glycosaminoglycan, citrate etc), increase in level of stone forming elements and promoter (i.e.-sodium, urates etc.) in urine. Promoters of stone formation are calcium, sodium, oxalate, urates, cystine, low urine pH, Tamm-Horsfall protein and inhibitors of stone formation are divided in two parts, inorganic (magnesium, pyrophosphate and citrate) and organic (nephrocalcin Tamm-Horsfall protein, protease inhibitors, glycosaminoglycans and high urine flow).[13] Hence, there is a need for the search of new drug therapy, which will be cost effective, target multiple etiological risk factors in urolithiasis and reduce the rate of recurrence.[14] Medicicnal plants play a key role in human health care.[15] *Spermacoce hispida* (Linn.) K. Schum. Popularly known as Nattaichuri in Tamil, Tharathaval in Malayalam and Shaggy button weed in English, belongs to the family Rubiaceae. It has been used since ancient times in folk medicine for its many medicinal properties. Recent evidence shows that the leaves and shoots from shaggy weed butter possess several medicinal properties including diuretics and kidney protective activity. But there is no report is available for its antilithiatic activity.[15] The phytochemical analysis of these medicinal plants showed that the terpenoids, steroids, alkaloids, carbohydrate, cardiaic glycoside, flavonoids, saponins, tannins, terpenoids and, antioxidants.[16] *Borreria* and *Spermacoce* species possess a wide variety of medicinal

properties. So far, a few species have been screened for confirmation of their biological activities. Experimental results have shown some species as antimicrobial.[17] antitumor.[18] antioxidant.[19] anti-inflammatory.[15] hepatoprotective.[17] larvicidal.[20] etc. Hence the present study is undertaken to investigate the antiurolithiatic activity of ethanolic extract of *Spermacoce hispida* (Linn.) in Calculi Producing Diet induced urolithiasis animal model.

MATERIALS AND METHODS

Plant material

Collection and extraction Plant material used in this study was collected from Cheruvandoor, Kottayam district, India. The plant was authentified by Mr. Rojimon P.Thomas, Department of Botany, CMS College, Kottayam. A specimen was deposited with voucher number 1246. The plant were coarsely powdered and used for extraction.

Preparation of ethanolic extract of *Spermacoce hispida* (Linn.)

The shade dried leaves was powdered mechanically and extracted using 95 % ethanol. Extraction was done by successive hot continuous percolation and evaporation by distillation. The crude extract was further used to perform phytochemical screening.

Preliminary phytochemical screening

The preliminary phytochemical screening of ethanolic extract of whole plant of *Spermacoce hispida* (Linn.) was carried out for the presence of alkaloids, carbohydrates, flavonoids, phenolic compounds, saponins, sterols, terpenoids and tannins, using standard procedures.

Animals

Male Wistar albino rats (160-240) were used in the present study to evaluate antiurolithiatic activity. They were housed under controlled conditions of temperature (23 ± 20C), humidity (45- 55 %) and light controlled room under 12 h light and 12 h dark cycles. The rats were fed with rat pellet and water ad libitum for several days before the beginning of experiment. The experimental protocol was approved by the IAEC/M.pharm/DPS/2018-07.

Acute Oral Toxicity Study

The acute oral toxicity study was evaluated as per Organization for Economic Cooperation and Development (OECD) guidelines no. 423. On Wistar albino rats, weighing between 180-220g. Before the experiment, rats were fasted overnight with water ad libitum. Three animals were selected which receives a dose of 2000 mg/kg. All three animals were received a single

dose of 2000 mg/kg body weight of ethanolic extract of *Spermacoce hispida* (Linn.) by oral gavage. Animals were observed individually for any sign of toxicity, behavioral changes, and mortality after dosing, with special attention given during the first 4 hours, and thereafter for 24 hours, for a total period of 7 days.

Experimental Design

***In vivo* Study**

The original protocol was modified and used for in vivo study. Modified version often used to evaluate effect of ethanolic extract of *Spermacoce hispida* L. on Calcium oxalate stones. The experimental animal Wistar albino rats were divided in to seven groups each group consists of 6 animals.

Group I: Received only vehicle (Distilled water) orally for 30 days, serve as normal animals (NC). Group II: Received CPD (from day 1- 15) and vehicle (from day 1 to 15). This group is served as preventive control (PC).

Group III: Received CPD (from day 1- 15) and extract (low dose 200 mg/kg from day 1 to 15). This group is served as Preventive Treatment Group1 (PTG1).

Group IV: Received CPD (from day 1- 15) and extract (High dose 400 mg/kg from day 1 to 15). This group is served as Preventive Treatment Group 2 (PTG2).

Group V: Received CPD (from day 1- 15) and vehicle (from day 16 to 30). This group is served as Curative control (CC).

Group VI: Received CPD (from day 1- 15) and extract (low dose 200 mg/kg from day 16 to 30). This group is served as Curative Treatment Group1 (CTG1).

Group VII: Received CPD (from day 1- 15) and extract (High dose 400 mg/kg from day 16 to 30). This group is served as Curative Treatment Group 2 (CTG2).

Assessment of Antiurolithiatic Activity

Collection and analysis of urine

Urine samples were collected on 15^{th} and 30^{th} day for 24 h by keeping the animals in individual propylene metabolic cages. Animals had free access to drinking water during the

urine collection period. The collected urine was analyzed for calcium, oxalate, and creatinine using standard methods.

Serum analysis

After the experimental period, the blood was collected from the retro orbital puncture of rat eye under ether anesthesia. Serum was separated by centrifugation of the blood samples at 10,000 RPM for 10 minutes and analyzed for BUN, creatinine, and creatinine clearance using standard methods.

Effect of whole plant of *Spermacoce hispida* (Linn.) extracts on the kidney weight

The weights of the kidney of normal, induced, and extract treated group rats were weighed. In addition, the wet weight of kidneys were taken and compared between the groups.

Histopathological examination

For microscopic evaluation, the kidney was fixed in 10 % formalin solution. The tissues were cleared in xylene and embedded in paraffin. Tissue section was stained with hematoxylin-eosin. Each kidney slide was examined for renal tubular necrosis, lymphocyte infiltration, and tubular dilation.

Data analysis

The data expressed are mean ± standard error of mean (SEM). All statistical comparisons between the groups are made by means of *t* test or one way analysis of variance (ANOVA) with Turkeys test using GraphPad Prism (GraphPad Software, San Diego, CA, USA). *p* value <0.05 is regarded as significant.

RESULTS

***In vivo* study**

CPD- induced urolithiasis The CPD induced urolithiasis showed a marked changes in animal body weight, urinary volume, urinary parameters (calcium, oxalate, and creatinine), haematological parameters (BUN, creatinine, and creatinine clearance) and in the histopathology of kidney.

Effect of *Spermacoce hispida* L. extraction animal body weight (g)

The preventive control groups on 15th day and curative control groups on 30th day showed an increase in body weight when compared to negative controls. There was a decreasing body weight in case of treatment groups (preventive and curative treatment groups) with 200 and

400 mg/kg of ethanolic extract of *Spermacoce hispida* L. when compared to preventive and curative controls.

Urinary parameters

Effect of ethanolic extract of *Spermacoce hispida* L. on Urine volume (ml)

At the end of experiment the urine output in the ethanolic extract of *Spermacoce hispida* L. treated groups was increased in comparison to preventive and curative controls. When the treatment groups 2 compared with treatment groups 1 (both preventive and curative) the results showed that there was an increase in urine output in case of treatment groups 2 (400 mg/kg).

Effect of ethanolic extract of *Spermacoce hispida* L. on Urinary calcium (mg/dl)

Urinary excretion of calcium was significantly increased in case of both preventive and curative controls when compared with negative control. On treatment with extract showed that there is a reduction in urinary excretion of calcium in treatment groups.

Effect of ethanolic extract of *Spermacoce hispida* L. on Urinary oxalate (mg/dl)

Urinary excretion of Oxalate was significantly increased in case of both preventive and curative controls when compared with negative control. On treatment with extract showed that there is a reduction in the urinary excretion of Oxalate in treatment groups (both preventive and curative).

Effect of ethanolic extract of *Spermacoce hispida* (L.) on urinary creatinine (mg/dl)

Urinary creatinine level significantly decreased both preventive and curative control compared with negative control. The ethanolic extract of *Spermacoce hispida* (L.) showed increase in urinary excretion of creatinine in treatment groups. When the preventive treatment groups compared with preventive control there was comparable increase in urine creatinine level. When the curative treatment groups compared with curative control there was comparable increase in urine creatinine level.

Haematological parameters

Effect of ethanolic extract of *Spermacoce hispida* L. on Blood Urea Nitrogen

(BUN) There is a significant increase in BUN was observed in both preventive and curative controls when compared with negative control. On treatment with extract shows a reduction

in excretion of BUN in treatment groups (both preventive and curative). The extract is more effective at high concentration.

Effect of ethanolic extract of *Spermacoce hispida* L. on Serum creatinine (mg/dl)

There is a significant increase in serum creatinine was observed in case of both preventive and curative controls when compared with negative control. On treatment with extract shows a reduction in Serum Creatinine in treatment groups (both preventive and curative). The extract is more effective at high concentration.

Effect of ethanolic extract of *Spermacoce hispida* L. on Creatinine Clearance (ml/min)

The Creatinine Clearance was significantly reduced in both preventive and curative controls when compared with negative control. On treatment with extract shows a reduction in urinary excretion of Oxalate in treatment groups (both preventive and curative). The extract is more effective at high concentration.

Effect of ethanolic extract of *Spermacoce hispida* L. on Kidney Weight (g/100g wt.)

Kidney weight was seems to be increased in case of both preventive and curative controls when compared with negative control. On treatment with extract shows a reduction in Kidney weight in treatment groups (both preventive and curative). The extract is more effective at higher concentration on Kidney Weight (g/100g wt.).

Histopathological evaluation

Administration of CPD caused glomerular destruction, tubular dilation and lymphocyte infiltration in both preventive and curative controls. Treatment with extract reduced renal tubular damage, tubular dilation and lymphocyte infiltration when compared to control kidney sections. The effect was more observed in preventive treatment groups when compared to curative treatment groups. The Preventive treatment group 2 was almost similar to that of normal control. Hence more effective was seemed to be higher concentration (400 mg/kg).

Table 1: Effect of Ethanolic Extract of *Spermacoce hispida* L. on urinary parameters

Groups	Calcium (Mg/dl)	Oxalate (mg/dl)	Creatinine (mg/dl)
Normal	1.656 ± 0.074	1.504 ± 0.176	4.409 ± 0.160
Preventive control	5.333 ± 0.401	4.075 ± 0.173	2.477 ± 0.306
Preventive treatment group 1	2.947 ± 0.269	3.151 ± 0.198	2.785 ± 0.265
Preventive treatment group 2	2.284 ± 0.096	2.68 ± 0.149	3.309 ± 0.375
Curative control	5.887 ± 0.146	5.355 ± 0.345	1.422 ± 0.139
Curative treatment group1	4.328 ± 0.115	4.022 ± 0.335	2.237 ± 0.205
Curative treatment group 2	3.555 ± 0.135	3.252 ± 0.203	3.04 ± 0.167

The values are expressed as mean ± SEM

Comparison were made between: Control vs preventive control & curative control. Preventive control vs preventive treatment group 1 & preventive treatment group 2. Curative control vs curative treatment group 1 & curative treatment 2. Statistical significant test for comparisons was done by ANOVA, followed by tukey- t test.

Table 2: Effect of Ethanolic Extract of *Spermacoce hispida* L. on serum parameters

Groups	BUN (mg/dl)	Creatinine (mg/dl)	Creatinine clearance (mg/min)
Normal	19.74 ± 1.771	0.935 ± 0.184	0.847 ± 0.023
Preventive control	57.56 ± 2.341	2.475 ± 0.163	0.122 ± 0.014
Preventive treatment group 1	43.3 ± 1.254	2.062 ± 0.258	0.431 ± 0.043
Preventive treatment group 2	31.41 ± 1.299	1.372 ± 0.154	0.639 ± 0.041
Curative control	68.59 ± 1.209	3.009 ± 0.133	0.099 ± 0.020
Curative treatment group1	55.39 ± 1.969	2.192 ± 0.079	0.162 ± 0.034
Curative treatment group 2	42.6 ± 2.564	1.744 ± 0.132	0.307 ± 0.066

The values are expressed as mean ± SEM

Comparison were made between: Control vs preventive control & curative control. Preventive control vs preventive treatment group 1 & preventive treatment group 2. Curative control vs curative treatment group 1 & curative treatment 2. Statistical significant test for comparisons was done by ANOVA, followed by tukey- t test.

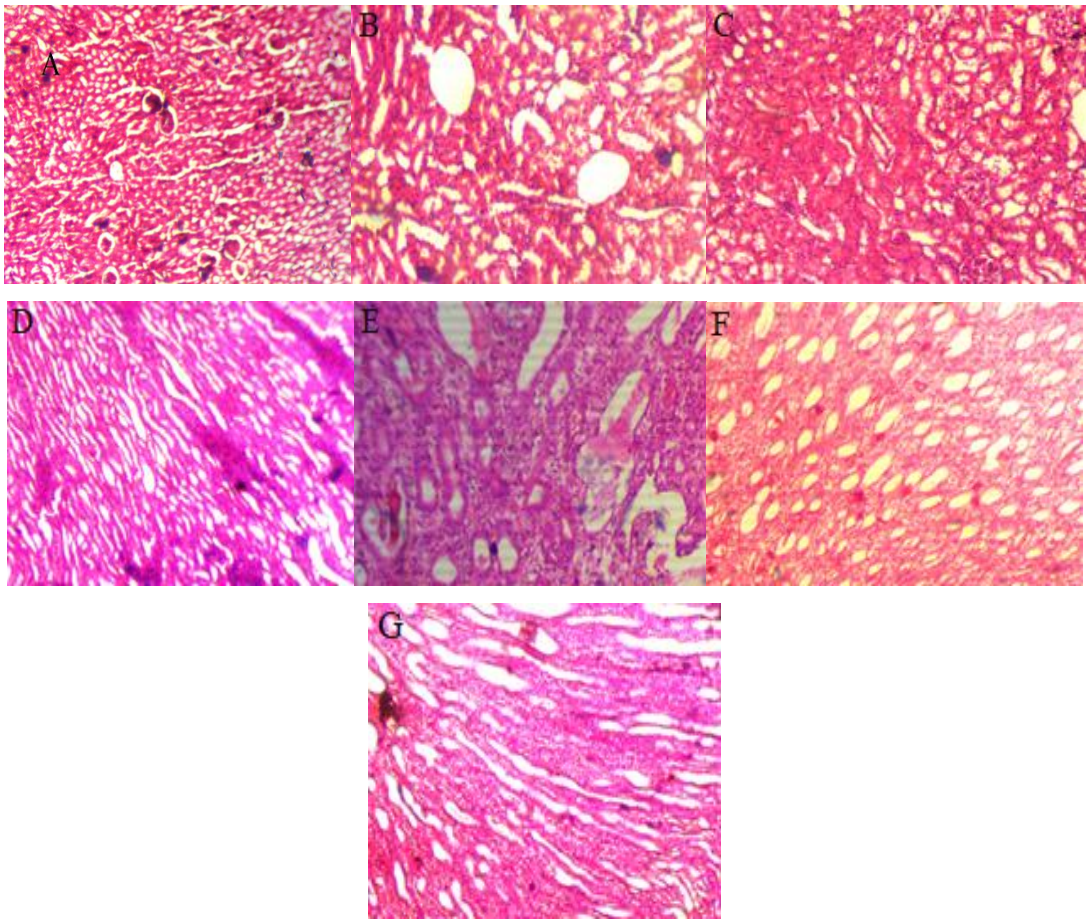
Table 3: Effect of Ethanolic Extract of *Spermacoce hispida* L. on urinary volume and kidney weight.

Groups	Urinary volume	Kidney weight
Control	21.17 ± 0.856	0.523 ± 0.014
Preventive control	10.28 ± 0.351	0.701 ± 0.023
Preventive treatment group 1	15.93 ± 0.585	0.635 ± 0.011
Preventive treatment group 2	17.3 ± 0.672	0.540 ± 0.015
Curative control	7.91 ± 0.397	0.744 ± 0.024
Curative treatment group 1	11.82 ± 0.408	0.612 ± 0.021
Curative treatment group 2	12.63 ± 0.625	0.573 ± 0.018

The values are expressed as mean ± SEM

Comparison were made between: Control vs preventive control & curative control. Preventive control vs preventive treatment group 1 & preventive treatment group 2. Curative control vs curative treatment group 1 & curative treatment 2. Statistical significant test for comparisons was done by ANOVA, followed by tukey- t test.

Histopathological Evaluation



DISCUSSION

Hyperoxaluria can provoke calcium oxalate urolithiasis in both human and rats. Oxalate metabolism is considered to be almost identical between rat and humans. Thus, a rat model of calcium oxalate urolithiasis can be used to investigate the mechanism involved in human kidney stone formation and also for screening new agents with anti urolithiatic activity.[5]

300g of dried plant was subjected to hot continuous extraction and obtained a percentage yield of 12.6%. The preliminary phytochemical investigation suggested that extracts contain saponins, carbohydrate, sterols, tannins, flavonoids, and alkaloids etc. different activities observed in the crude extract might be due to the presence of these phytochemicals.

Flavonoids are known to possess antispasmodic and calcium channel blocking, antioxidant and diuretic activity. From the earlier studies it has been reported that flavonoids have diuretic activity and tannins have protective effect on kidney.[21] Saponins are known to possess anti crystallising by disaggregating the suspension of mucoproteins, promotors of crystallization.[16] Presence of these phytochemical might be responsible for the anti urolithiatic activities of the plant *Spermacoce hispida* (Linn).

Kidney stones are hard, and solid particles and occur due to several factors like excess amount of stone forming constituents (Calcium oxalate, calcium phosphate, uric acid struvite, and cysteine), imbalance between promotors (eg. Sodium urateset) and inhibitors like citrate, glycosaminoglycans, etc. There are different sizes of stones. In many cases, the stone are very small and can pass out of the body without any problem. However, if a stone blocks the flow of urine, excruciating pain occurs and prompt medicinal treatment is needed.[15]

When the stone forming constituents are excess in urine it become supersaturated. Urinary supersaturation is generally considered to be one of the causative factors in calculogenesis. The supersaturation of urine with calcium oxalate may be an important factor in crystallization. Calcium oxalate monohydrate crystals are considered to be more harmful than calcium oxalate dihydrate because of their tendency to attach with the membrane to form aggregates and are more likely to attach with the kidney epithelial cells than calcium oxalate dihydrate, resulting in the formation of kidney stones. The mechanism of calcium oxalate renal calculi formation has attracted the attention of medical scientists because of its widespread clinical occurrence and the difficulty of treatment. Thus if supersaturation or later steps in crystallization can be prevented, then lithiasis can be avoided.[23]

The extract of the plant causes fewer numbers of crystals in solution, thereby reduced supersaturation and the size of the particles. These results are of much great interest since supersaturation may be lowered and small crystals can be easily flushed out through urinary tract. This property of the extract is therefore, advantageous, preventing urinary stone formation by inducing the excretion of small particles from the kidney and reducing the chance of retention in the urinary tract.

There for in the present study, CPD was preferred to induce lithiasis. Administration of CPD to *wistar* albino rats in a particular group up to 15 (preventive) and 15 (curative) days significantly shows the renal stones which were mainly composed of calcium oxalate in renal calculi. The sodium oxalate accelerates lithiasis through urinary acidification and also disturb oxalate metabolism by increasing the substrate availability thus lead to hyperoxaluria.

In *in vivo* study the lithiatic rats showed increase in body weight in preventive and curative controls may be due to edema. On treatment with extract showed a decrease in animal body weight. It might be due to reduction in edema and recovery from urolithiasis. The results supports the protective activity of plant extract.

The present study showed an increased in urine output of ethanolic extract of *Spermacoce hispida* (Linn) treated animals which dilutes the concentration of urinary electrolytes. As a result, calcium and oxalate are flushed out via the urine and there are lesser chances of precipitation, decreased formation as well as the growth of urinary stone. The excretion of oxalate and calcium were progressively increased in calculi-induced animals which is consistent with the previous reports. Most calculi in the urinary system arise from a common component of urine such as calcium oxalate (CaOx) and hypercalciuria, representing up to 80% of analyzed stones. Increased urinary calcium is a factor favoring the nucleation and precipitation of calcium oxalate from urine and subsequent crystal growth. However, ethanolic extract of *Spermacoce hispida* (Linn) lowered the levels of oxalate as well as calcium excretion, which is beneficial in preventing calculi formation.

The biochemical mechanism of calculi produced diet induced lithiasis are related to an increase in the urinary concentration of oxalate. This causes precipitation of oxalate in urine as calcium oxalate due to its poor solubility. High oxalate levels and calcium oxalate crystals especially in nephron damages epithelial cells, leading to heterogeneous nucleation followed by causing aggregation of calcium oxalate crystals in the renal tubules of experimental

animals. Male rat were selected to induce urolithiasis because the urinary system of male rats has more resemblance to that of humans. Furthermore, earlier studies have reported that the amount of stone deposition in female rats was significantly less compared to male rats. The enhanced urine volume is expected to remove urine stone especially in early stage.[24] In this study, ethanolic extract of *Spermacoce hispida* (Linn.) in preventive and curative treatment group produced significant increase in urine volume as compared preventive and curative control group. And also ethanolic extract of *Spermacoce hispida* (Linn.) in preventive and curative treatment group produced significant decrease in urinary oxalate excretion as compared to preventive and curative control group. This effect is might be due to interference of ethanolic extract of *Spermacoce hispida* (Linn.) with oxalate metabolism.

In this study, decreased urinary calcium excretion was observed in lithiatic preventive and curative controls rats. It reported that hyperoxaluria leads to precipitation of the oxalate in the urine as calcium oxalate and this might be reason of observed decrease in urinary calcium excretion. This calcium oxalate precipitation further result in damage to the renal architecture ar variable range and there by induces functional damage too. Precipitation and long term deposition of calcium oxalate make the system more vulnerable and elicit onset of complications.[24] However, the preventive and curative treatment group with ethanolic extract of *Spermacoce hispida* (Linn.) caused significant improvement in urinary calcium excretion. Out of all these fractions, ethanolic extract of *Spermacoce hispida* (Linn.) was found to most effective in this regard. The improvement in urinary calcium excretion indicating potential of ethanolic extract of *Spermacoce hispida* (Linn.) to prevent damage and thereby halts possible functional abnormality which is most widely expected result from any therapy. The efficient might be due to either prevention of hyperoxaluria or inhibition of binding of calcium with oxalate by the ethanolic extract of *Spermacoce hispida* (Linn.). In urolithiasis management, prevention of stone formation is considered to be first step especially during recurrence which can avoid further symptoms and complications.

The increase in BUN and serum creatinine and reduction in urinary creatinine is due to the disturbance in the out flow of urine. The result impaired GFR. Treatment with extract significantly decreased the plasma creatinine, BUN and increased the urine creatinine levels. The significant increase in urine creatinine and the significant decrease in plasma creatinine in treatment groups is a strong indication of the positive impact on treatment with extract on the glomerular filtration rate. Thus these results support its antiurolithiatic activity. Creatinine

levels in blood and urine are usually used to calculate the creatinine clearance which reflects the glomerular filtration rate. GFR is clinically important because it is a marker of the renal function. Renal dysfunction diminishes the ability to filter creatinine, thus decreases creatinine clearance.[5] In this study showed that the creatinine clearance was restored by using ethanolic extract.

Histopathological studies revealed congested blood vessels, tubular changes, glomerular necrosis, renal tubular dilation and inflammatory infiltration in both the diseased control groups. Treatment with ethanolic extract of *Spermacoce hispida* (Linn.). Prevented these histological changes causing a significant decrease in the damage index of renal tissue. All the results obtained were support the antiurolithiatic activity of ethanolic extract of *Spermacoce hispida* (Linn.).

CONCLUSION

The results of the present study have shown that the urinary stone could be dissolved with ethanolic extract of *Spermaoce hispida* L. in conclusion, the present data agree with the popular use of the *Spermacoce hispida* L. for the treatment of urolithiasis. The effect of ethanolic extract was confirmed by its ability to maintain kidney function, prevent the formation of urinary stones, reduce retention crystals in the kidney tissue and stimulate their extretion in urine. It also appears that the cure effect is less effective than preventive.

REFERENCES

1. Zhang H, Li N, Li KUN, Li P. Protective effect of Urtica dioica methanol extract against experimentally induced urinary calculi in rats, 2014; 31: 57–62.
2. Kokilavani R, Sathya M. Preventive effect of sacchrum spontaneum linn. agaainst glycolic acid-induced urolithiasis in male wistar albino rats. Int J Pharma Bio Sci., 2013; 4(1): 1–10.
3. Saha S, Verma RJ. Inhibition of calcium oxalate crystallisation in vitro by an extract of Bergenia ciliata. Arab J Urol [Internet], 2013; 11(2): 187–92. Available from: http://dx.doi.org/10.1016/j.aju.2013.04.001
4. Shah JG, Patel BG, Patel SB, Patel RK. Antiurolithiatic and antioxidant activity of Hordeum vulgare seeds on ethylene glycol-induced urolithiasis in rats, 2012; 44(6).
5. Alba M, Animal LIN, Maya S, Pramod C. Evaluation of anti-Nephrolithiatic activity of ethanolic leaf extract of Morus alba L. in animal models. Int Res J Pharm, 2014; 5(5): 427–33.

6. Selvam R, Bijikurien T. Methionine feeding prevents kidney stone deposition by restoration of free radical mediated changes in experimental rat urolithiasis, 1991; 2: 644–51.
7. Salman A, Mohtasheem H, Zafer alam mahmood. Antiurolithiatic plants : Multidimensional pharmacology Antiurolithiatic plants : Multidimensional pharmacology. Res gate, 2016; (March).
8. Ratkalkar VN, Kleinman JG. Mechanisms of stone formation. Clin Rev Bone Miner Metab, 2011; 9(3–4): 187–97.
9. Chitme HR, Rathod NM, Chandra R. In vivo and In vitro Models for Evaluating Anti-urolithiasis Activity of Herbal Pharmaceutical Research And Bio-SciencE. Res gate, 2014; (October).
10. Therapeutic THE, Review A, Technical JN, Pradesh A, Kuchala U, Nuxvomica S, et al. Strychnos nux-vomica seeds: Pharmacognostical standardization, extraction, and antidiabetic activity. Homeopathy [Internet], 2014; 19(2): S63–S63. Available from: http://www.jaim.in/ text.asp?2012/3/2/80/96523%5Cnhttp://www.nature.com/doifinder/10.1038/sj.bhj.5800401
11. Ilhan M, Ergene B, Süntar I, Ozbilgin S, Saltan Çitoğlu G, Demirel MA, et al. Preclinical Evaluation of Antiurolithiatic Activity of Viburnum opulus L. on Sodium Oxalate-Induced Urolithiasis Rat Model. Evid Based Complement Alternat Med [Internet], 2014; 2014(August): 578103. Available from: http://www.ncbi.nlm.nih.gov/pubmed/ 25165481.
12. Yasir F, Iqbal WM. Protective effect of dietary polyphenol caffeic acid on ethylene glycol ‐ induced kidney stones in rats. Urolithiasis, 2017.
13. Tiwari P, Kothiyal P, Ratan P. Antiurolithiatic effect of some polyherbal formulations used in experimentally induced urolithiasis. Int Res jouranl Pharm, 2017; 8(5): 14–22.
14. Namburu SL, Dodoala S, Koganti B. Antiurolithiatic activity of Phaseolus vulgaris seeds against ethylene glycol-induced renal calculi in wistar rats. Int J green Pharm, 2017; 11(4): 7–11.
15. V. R, G A, R M. screening of phytochemicals from different solvent extracts in borreria hispida linn. world J Sci Res., 2016; 1(3): 16–20.
16. P T, Dr poonguzhali t v, B J. phytochemical screening and antioxidant activivty of borreria hispida l. an anticancer plant. Int J Mod trends Eng Res., 2016; (6): 168–73.
17. Studies on Borreria hispida. In, 215–308.
18. Rupachandra S, Sarada DVL. Anticancer activity of methanol extract of the seeds of

Borreria hispida and Momordica dioica. JOPR J Pharm Res [Internet], 2013; 6(5): 565–8. Available from: http://dx.doi.org/10.1016/j.jopr.2013.04.027.

19. Sivaelango G, Kumaran PS, Kumaravel P, Revathi P, Jaswant A, Sivaelango G. Antihyperlipidaemic Activity of Spermacoce Hispida Ethanolic Extract in Triton WR-1339 Induced Hyperlipidaemic Rats. J Appl Pharm Sci., 2012; 02(02): 95–8.
20. Conserva LM, Costa J, Júnior F. Borreria and Spermacoce species (Rubiaceae): A review of their ethnomedicinal properties, chemical constituents, and biological activities. Pharmacogn Rev., 2012; 6(11).
21. Kumar S, Pandey AK. Chemistry and Biological Activities of Flavonoids : An Overview. Sci world J., 2013; 2013.
22. Ram J, Moteriya P, Chanda S. An overview of some promising medicinal plants with in vitro anti-urolithiatic activity. IOSR J Pharm, 2015; 5(5): 23–8.
23. Urolithiasis ARON. A review on urolithiasis and phytotherapy. world J Pharm Res, 2015; 4(8): 861–70.
24. Pawar AT, Vyawahare NS. Journal of Traditional and Complementary Medicine Protective effect of ethyl acetate fraction of Biophytum sensitivum extract against sodium oxalate-induced urolithiasis in rats. J Tradit Chinese Med Sci [Internet], 2017; Available from: http://dx.doi.org/10.1016/j.jtcme.2016.12.012.