An Acad Bras Cienc (2020) 92(4): e20200067 DOI 10.1590/0001-3765202020200067
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

HEALTH SCIENCES

Anti-urolithiatic activity of *Salvia hispanica* L. seeds in ethylene glycol induced urolithiasis rat's model

UZMA SALEEM, NAZIR AHMAD, MUHAMMAD AJMAL SHAH, FAREEHA ANWAR & BASHIR AHMAD

Abstract: Urolithiasis is a disorder of kidneys in which stones formation occur due to the excessive deposition of minerals in the urinary tract. It affects 12% of the population worldwide. *Salvia hispanica* seeds are rich source of quercetin which has preventive role in renal stone formation. The study objective was to explore scientifically the anti-urolithiatic effect of *Salvia hispanica* seed's methanol extract using *in vitro* and *in vivo* urolithiasis models. For *in-vitro* study nucleation, growth and aggregation assays were performed. *In vivo* study was performed on rats and they were divided into six groups (n=6). Group-I was given vehicle only. Group-II was disease control, treated with 0.75% EG in drinking water which triggered urolithiasis. Groups-III received cystone (750 mg/kg, orally). Groups IV–VI were treated with extract at 100, 300 and 700 mg/kg doses orally once daily. Groups III-VI additionally received 0.75% EG in drinking water. *In vitro* study revealed concentration dependent increase in percentage inhibition of crystal's nucleation, growth and aggregation. *In vivo* study revealed anti-urolithiatic activity by lowering oxalate, calcium, phosphate, sodium and potassium levels in the urine and the serum uric acid, blood urea nitrogen, total proteins and total albumin. *Salvia hispanica* seeds are good alterative of allopathic anti-urolithiatic drugs to treat urolithiasis.

Key words: Urolithiasis, *Salvia hispanica,* calcium oxalate, calcium phosphate, ethylene glycol.

INTRODUCTION

Urolithiasis is a type of urinary system disorder in which crystals development occur when minerals get deposited in the renal, urinary bladder or ureter (Mamillapalli et al. 2016a). Urolithiasis develops when inhibitors (e.g. magnesium) and promotors (e.g. uric acid) of kidney stones lose their balance in the kidneys (Gupta et al. 2011, Alelign & Petros 2018). It has been reported that 80% renal stones are composed of calcium oxalate and calcium phosphate, 10% are struvite containing magnesium ammonium phosphate. Whereas, the drug related renal stones are caused by uric acid (9%) and cystine or ammonium acid urate (1%) (Coe et al. 2005, Divakar et al. 2010). The worldwide prevalence of urolithiasis is recorded as 12% and it affects both male and female of age 20 to 49 years (Alelign & Petros 2018). But it is 2 to 4 times less common in women than in men (Sofia & Walter 2016, Alelign & Petros 2018). Data showed that the prevalence of urolithiasis in Pakistan is 40-50% (Desai et al. 2011, Ahmad et al. 2016). The non-pharmacological and pharmacological therapies are available but infertility as major side effect and 50% to 80% relapse rats diverted attention of researchers to find out new drugs having minor side effects and minimum relapse

cases (Mikawlrawng et al. 2014, Raheem et al. 2017, Türk et al. 2013, Erickson et al. 2011).

Many crude drugs have diuretic effect which are considered to be effective in abnormal renal functions, providing a vast scope in the prevention and cure of urolithiasis (Brancalion et al. 2012). The consumption of phytomedicines is increasing day by day because of people believe that natural products have low toxicity or rather no side effect (Mikawlrawng et al. 2014).

Salvia hispanica (*S. hispanica*) belongs to lamiaceae family which is comprised of 900 species and grow in America (natively), northern Guatamala and Maxico and also in steppe regions (Uribe et al. 2011). *S. hispanica* seeds have multiple folklore use including antiarthritis, antidiabetic, antihypertensive, anticancer, antihyperlipidemic, antiaging, antianxiety, laxative, immune-booster and also being used in cosmetics and paints (Muñoz et al. 2013, Sandoval-Oliveros & Paredes-López 2012, Ulbricht et al. 2009, Ullah et al. 2016).

Literature showed *S. hispanica* seeds are the richest source of quercetin which belongs to flavonoid class of compounds which are involved in the prevention of renal stones formation due to its strong anti-oxidant activity (Zeng et al. 2018). However, there is no study available in the literature about anti-urolithiatic activity of *S. hispanica* seeds. Based on the anti-oxidant potential and the presence of flavonoids in the seeds, the current study was aimed to determine physicochemical and phytochemical properties, *in vitro* anti-urolithiatic activity by spectrophotometric method and explore *in vivo* anti-urolithiatic activity of the extract in ethylene glycol induced urolithiasis rats' model.

MATERIALS AND METHODS

S. hispanica seeds collection and preparation of extract

Seeds were obtained from the Botanist of Govt. College University, Faisalabad. After cleaning, washing and drying, seeds were ground to powder. One kg seed's powder was soaked in 5 liters of methanol for five days with occasion shaking. Filtrate was obtained by draining extraction solvent via filter paper. Filtrate was passed through rotary evaporator at 40°C to get semisolid *S. hispanica* methanol extract which was further used in analysis. Percentage yield was calculated with formula:

$$\text{Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} * 100$$

Physiochemical analysis

Total moisture content, total ash content, acid insoluble ash, water insoluble ash, sulphated ash, water and alcohol soluble extractives were quantified (Figure 1) by following methods given in United States Pharmacopoeia-National Formulary (2003).

Phytochemical analysis

Total alkaloid content, phenolic content and flavonoid content in the extract were quantified for phytochemical analysis of *S. hispanica* seeds methanol extract.

Determination of total alkaloid content

The extract; 1 mg/mL; was mixed with (2 N) HCl and filtered to get clear filtrate. Bromocresol green and phosphate buffer (pH 4.7); each 5 mL; were added to filtrate and mixture was shaken well. Trichloromethane was added to this mixture. Different concentrations of standard atropine (0.5, 1, 1.5, 2 and 2.5 µg/mL) solution was prepared in an identical manner as that of sample in order to construct standard curve.

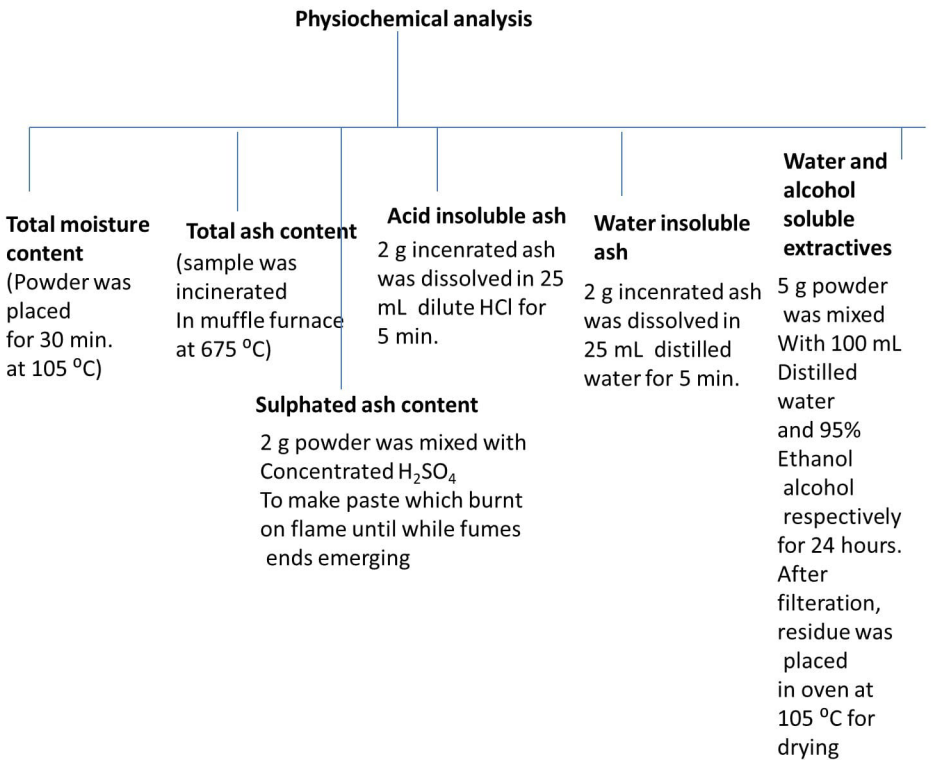

Figure 1. Scheme for physicochemical analysis.

The absorbance of sample and standard were recorded at 470 nm by UV spectrophotometer. Blank was prepared similarly but without sample and standard. Results were displayed as total alkaloid content in the extract as mg of atropine equivalent (AEq/g) (Das et al. 2018, Tabasum et al. 2016).

Determination of total flavonoid content

Saleem et al. study was followed to quantify flavonoid content. Briefly, sample/standard (1 mg/kg, volume: 200 µL) was mixed with 0.1 mL potassium acetate (1 M) and 100 µL aluminum nitrate solution (10%) and distilled water (100 µL). The sample/standard (querecetin; QTN) was incubated at room temperature for 45 minutes. Blank was prepared similarly except analyte. Absorbance was recorded at 415 nm. Linear regression equation was constructed to calculate flavonoid content in the sample. Total flavonoid content were calculated by following equation (Saleem et al. 2014):

$$Total\ flavonoid\ content = \frac{QTN\ equivalents\left(\mu g\ per\ mL\right) X\ extract\ volum}{Sample\left(g\right)}$$

Determination of total phenolic content

Gallic acid was used as standard to draw the standard curve. 200 µL of Folin-Ciocalteu's phenol reagent was added to 200 µL of sample (1 mg/kg) and standard (1 mg/kg) and mixed well. After 4 minutes, 1 mL of Na_2CO_3 (15%) was added and mixture was incubated at room temperature for 2 hours. Absorbance was measure at 760 nm. Blank was prepared similarly but it did not contain analyte. Gallic acid equivalents in the sample were quantified using linear regression line of gallic acid. Total phnolic content in the sample were calculated by putting values in the following equation (Saleem et al. 2014):

$$Total\ Phenolic\ Content = \frac{Gallic\ acid\ equivalents\left(\mu g\ per\ mL\right) X\ extract\ volume}{Sample\left(g\right)}$$

Determination of antioxidant activity of extract by *in vitro* DPPH assay

Sample, control and DPPH were prepared in methanol. DPPH (0.1 mM) 3.5 mL was added to 500 µL of sample and control (ascorbic acid). Sample was prepared in concentration ranging from 0.01 to 1 mM. Blank was prepared identically but 500 µL of methanol was added in place of sample/control. Samples, control and blank were incubated at room temperature for 30 minutes and absorbance was measured in triplicate at 517 nm (Das et al. 2018).

Radical scavenging activity was calculated with equation:

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of Sample} - \text{Absorbance of Blank}}{\text{Absorbance of Blank}} * 100$$

IC_{50} of sample and control were calculated by drawing graph between percent scavenging activity vs sample concentrations/control.

Determination of anti-urolithiatic activity: *in vitro* study

Nucleation assay

$CaCl_2$ (0.5 g/L) and sodium oxalate (0.75 g/L) solutions were prepared in Tris HCl buffer (5 g/L; pH 6.5). Cystone was used as standard drug. Two fold serial dilutions were prepared for standard and sample. 100 µg of extract at different concentrations was added to 950 µL $CaCl_2$ solutions. Next, solution of sodium oxalate (950 µL) was added to it and crystal aggregation started immediately. Then this solution was incubated at 37 °C for 30 minutes. The mixture's optical density (OD) was measured at 620 nm. The percentage inhibition of nucleation by the extract was calculated against standard using following formula (Mamillapalli et al. 2016b, Nirmaladevi et al. 2012, Bawari et al. 2018):

$$\text{Percentage inhibtion} = 1 - \frac{\text{OD of sample}}{\text{OD of standard}} * 100$$

Growth assay

NaC_2O_4 (4 mM) and $CaCl_2$ (4 mM) solutions (1 mL each) were added to 1.5 mL of Tris HCL (10 mM) and NaCl (10 mM) buffer having pH 7.2. CaOx crystals (30 µL) were added to this solution. Two fold serial dilutions (200-100 µg/mL) of sample and standard were mixed with mixture having CaOx crystals and incubated for 30 minutes at 37 °C. Absorbance was recorded at 240 nm. The absorption of sample was compared with absorption of standard. The percentage inhibition of growth assay was calculated using formula (Chaudhary et al. 2008):

$$\text{Percentage inhibtion} = 1 - \frac{\text{OD of sample}}{\text{OD of standard}} * 100$$

Aggregation assay

Aggregation assay was performed according to method described by Bawari et al. NaC_2O_4 and $CaCl_2$ (each 50 mmol/L) were mixed and placed in water bath at 60 °C for one hour then incubated at 37 °C for 12 hours. Next mixture was evaporated to yield CaOx crystals. One mL Tris HCL (0.05 mol/L) and NaCl (0.15 mol/L) buffer having pH 6.5 was added to 1 mg of CaOX crystals. CaOx solution (3 mL) was added to each concentration (20-80 mg/mL) of extract/standard (cystone) and incubated at 37 °C for 30 minutes. Absorbance of sample and standard was measured at 620 nm. The percentage inhibition of CaOx crystals aggregation was calculated by formula given in the above mentioned nucleation assay (Bawari et al. 2018).

Determination of anti-urolithiatic activity: *In vivo* study on rats

Animals

Healthy albino rats both sexes, weighing 150-200 g, were used for *in-vivo* anti-urolithiatic

activity. Rats were housed in polypropylene cages as six rats/cage (n=6) in the animal house of Govt. College University, Faisalabad-Pakistan. The temperature was maintained at 23 ± 2 ºC following a 12 h light & dark cycle. All animals were provided chao and water *ad libitum*. Rats were placed in the animal house seven days before the start of experimental work in order to acclimatize them with the environment.

Ethical Approval

Ethical approval for *in vivo* study was obtained from Institutional Review Board of Govt. College University Faisalabad. Study reference was GCUF/ERC/2034.

Induction of urolithiasis in rats

Ethylene glycol (0.75% v/v) and ammonium chloride (1% w/v) were given to animals in drinking water for the initial three days followed by ethylene glycol (0.75% v/v) alone for the next eighteen days for induction of urolithiasis in rats (Iman et al. 2020a, b).

Study design

Thirty six rats were classified into six groups (n=6). Group-I was normal control, receiving vehicle only. Group-II was served as disease control group, receiving ethylene glycol (0.75% v/v). Group-III served as standard and was given Cystone® (750 mg/kg) orally for three weeks. Groups IV-VI were treatment groups, administered with extract at doses (100, 300 and 700 mg /kg, orally), respectively for 21 days.

Urine collection and measurement of urine parameters

The urine samples were collected at 22nd day of the study for biochemical analysis, such, as oxalate, phosphate, magnesium, sodium and calcium which were analyzed using standard diagnostic kits.

Determination of the serum parameters

The blood specimens were collected by cardiac puncture at 22nd day of study under anesthesia and serum was separated for biochemical analysis (serum creatinine, serum uric acid, blood urea nitrogen, serum protein and serum albumin) using standard diagnostic kits.

Histopathological analysis of kidneys

The rats were killed humanely by cervical dislocation after blood samples collection. Both kidneys were removed by incising the abdominal part of the each rat and each kidney was weighed on the weighing balance. Isolated kidneys were preserved in formalin 10% solution. Slides were prepared with hematoxylin and eosin staining solutions. Slides were observed under light microscope and images were captured at 10X.

Statistical analysis

Results were expressed as mean ± S.E.M. Data were analyzed by applying two way ANOVA followed by Bonferooni posthoc test by using Graph pad prism version 5.0.

$p < 0.05$ was set as statistically significant value.

Table I. Physical properties and percentage yield of the *S. hispanica* seeds extract.

Properties	Extract
Viscosity	Highly viscous
Odor	Characteristic
Color	Deep green
Percentage yield	3.68

Table II. Solubility of the *S. hispanica* seeds extract.

Vehicles	Extract
Vegetable oil	Soluble
Normal saline 0.9%	Soluble
Tween 20, 60, 80	Slightly soluble
Distilled water	Soluble
Ethanol	Completely soluble

RESULTS

Physical properties and percent yield of the extract

The extract was highly viscous with deep green color and specific odor. The percent yield of the extract was found 3.68% (Table I).

Solubility of the extract

The solubility of the extract was assessed in different types of vehicles. The extract was soluble when it was dissolved in vegetable oil, normal saline, distilled water, tween 20-, 60- and 80, volatile solvents like ethanol and methanol (Table II).

Table III. Physicochemical analysis of crude powder of *S. hispanica* seeds.

Physicochemical parameters	Percentage value
Moisture content	12.50
Total ash content	95.83
Acid insoluble ash content	0.26
Water insoluble ash content	95.12
Sulfated ash content	22.5
Alcohol soluble extractives	0.56
Water soluble extractives	0.02

Physicochemical analysis of the seeds powder

Pulverized material contained 12.50% moisture content, 95.83% total ash content, 0.26% acid insoluble ash content, 95.12% water insoluble ash content, 22.50% sulfated ash content, 0.56% alcohol soluble extractives and 0.02% water soluble extractives (Table III).

Phytochemical analysis

The extract contained 119.08 mg of quercetin equivalents/g of total flavonoid content which were quantified using linear regression equation $y = 0.0009x + 0.5989$ ($R^2 = 0.9711$) of the quercetin standard curve. Total alkaloid content in extract were 48.55 mg of atropine equivalents/g of total alkaloid content which were calculated using linear regression equation $y = 0.0009x + 0.3215$ ($R^2 = 0.9748$) of the atropine standard curve. Moreover, extract had 7.45 mg of gallic acid equivalents/g of total phenolic content which were determined applying linear regression equation $y = 0.0014x + 0.4462$ ($R^2 = 0.9764$) of gallic acid standard curve (Figure 2; Table IV).

Determination of antioxidant activity of extract by *in vitro* DPPH assay

The extact and standard (ascorbic acid) showed dose dependent increase in percent scavenging effect but the results of ascorbic acid were more pronounced as compared to extract. IC_{50} value of extract was 214.01 µg/mL which was higher than ascorbic acid value i.e. 163.73 µg/mL indicating antioxidant potential of extract is less as compared to ascorbic acid (Table V).

Determination of anti-urolithiatic activity: *in vitro* study

Percentage inhibition of nucleation assay, growth assay and aggregation assay was increased dose dependently with extract and standard. Maximally significant percentage inhibition was noted at 1000 mg/kg of extract and standard.

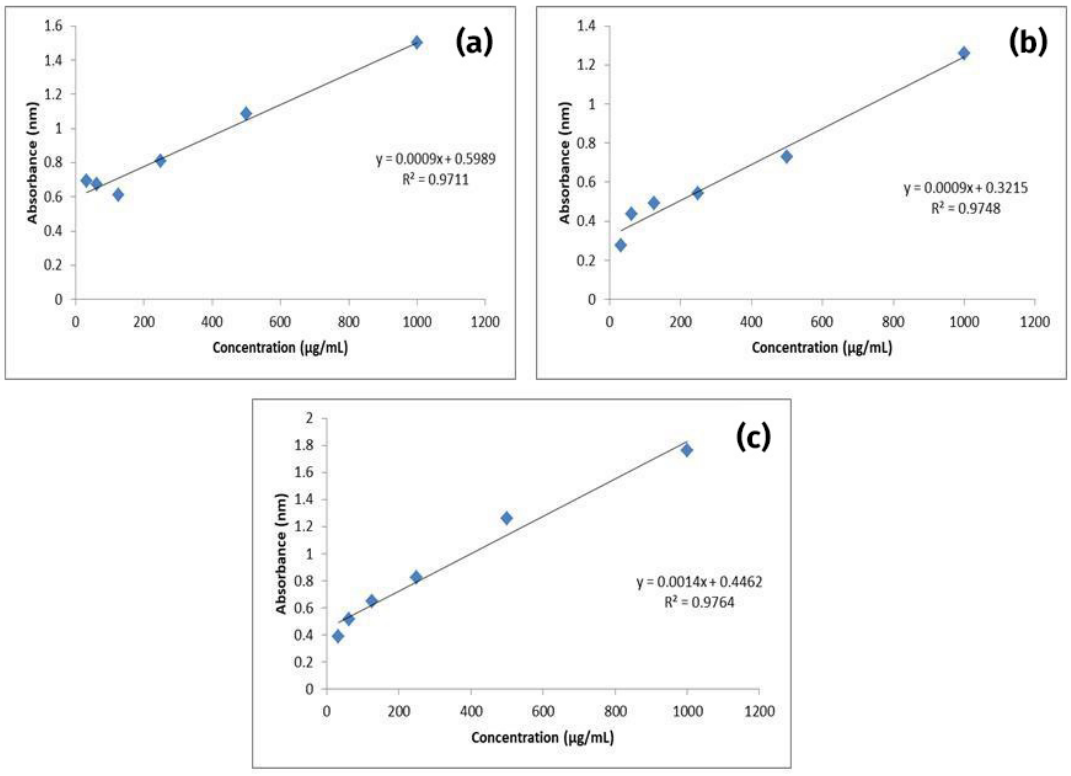

Figure 2. **Standard curves (a): Quercetin; (b): atropine; (c): gallic acid.**

The results of extract in nucleation and growth assays at 1000 mg/kg were comparable with that of standard values but extract had more aggregation inhibition than standard at highest selected concentration i.e. 1000 mg/kg (Table VI).

Determination of anti-urolithiatic activity: *In vivo* study on rats

Effect of extract on the urine parameters

In disease control group a significant increase ($p < 0.001$) in the levels of urinary oxalate, calcium, magnesium, phosphate, sodium, potassium and urine volume (19.04 ± 0.01 mg/24 h, 38.05 ± 0.02 mg/L, 38.08 ± 0.02 mmol/L, 0.25 ± 0.02 mg/L, 36.07 ± 0.03 mmol/L, 17.06 ± 0.02 mmol/L and 4.05 ± 0.02 mL respectively) except magnesium and urine volume as compared to normal control (11.05 ± 0.01 mg/24 h, 27.06 ± 0.02 mg/L, 46.06 ± 0.02 mmol/L, 0.15 ± 0.02 mg/L, 32.05 ± 0.02 mmol/L, 10.05 ± 0.02 mmol/L and 8.07 ± 0.02 mL respectively). Conversely, in standard and extract treating groups, levels of urinary oxalate, calcium, magnesium, phosphate, sodium, potassium and urine volume were parallel to normal control values as shown in Table VII.

Effect of extract on the body and kidney weights

Body weight of rats in extract and standard treated groups increased significantly ($p < 0.001$) during study as compared to disease control whereas body weight of disease control was decreased significantly ($p < 0.001$) during experiment with reference to normal control (Table VII).

Effect of extract on the serum parameters

In disease control group, the levels of serum uric acid, creatinine, blood urea nitrogen, total protein and albumin (4.56 ± 0.02 mg/dL, 0.84 ± 0.02 mg/dL, 14.17 ± 0.02 mg/dL, 6.14 ± 0.02 g/dL

Table IV. Phytochemical analysis of the *S. hispanica* seeds extract.

Phytochemical parameters	Weight (w/w)
Total flavonoid content	119.08 mg QTNE/g
Total alkaloid content	48.55 mg APE/g
Total phenolic content	7.45 mg GAE/g

w/w: weight/weight, QTNE: quercetin equivalent, APE: atropine equivalent, gallic acid equivalent, g: gram.

Table V. % scavenging activity and IC_{50} values of *S. hispanica* seeds extract and control in DPPH assay.

Concentrations (µg/mL)	*S. hispanica* seeds extract (%)	Control (ascorbic acid) (%)
1000	69.11	86
500	61.97	78
250	48.82	66
125	47.49	60
62.5	46.72	56
31.25	44.02	53
IC_{50}		
S. hispanica seeds extract (µg/mL)		Ascorbic acid (µg/mL)
214.01		163.73

and 3.74 ± 0.03 g/dL respectively) were increased significantly ($p < 0.001$) than normal control values (2.75 ± 0.01 mg/dL, 0.56 ± 0.02 mg/dL, 9.14 ± 0.02 mg/dL, 5.44 ± 0.02 g/dL and 3.06 ± 0.02 g/dL respectively). But in standard and extract treated groups, serum uric acid, creatinine, blood urea nitrogen, total protein and albumin were parallel to normal control values as shown in Table VIII.

Effect of extract on histopathology of kidneys

The results of histopathological examination of the kidneys of all the six groups have been summarized in figure 3. No calcium oxalate aggregates and interstitial inflammation were seen in normal control (Figure 3a). Prominent calcium oxalate stone deposition and interstitial inflammation were observed in the disease control (Figure 3b). Standard treated group had histology picture identical to that of normal control (Figure 3c). Extract at 100 mg/kg showed moderate tubular injury with inflammation (Figure 3d). Extract at 300 mg/kg showed slight interstitial inflammation and tissue damage (Figure 3e). Extract at 700 mg/kg revealed no cellular injury and no interstitial inflammation (Figure 3f). Dissolution of calcium oxalate crystals was clear at all concentrations of extracts.

DISCUSSION

In current study, physicochemical and phytochemical analyses of *S. hispanica* seeds powder and extract were performed in order to characterize the seeds. Extract was enriched with flavonoid content followed by alkaloid content and phenolic content which prevent occurrence of urolithiasis by protecting the CaOx crystals formation (Nirumand et al. 2018).

Mostly CaOx crystals aggregate and grow in the kidney and resulted in stone formation (Aggarwal et al. 2013). Nucleation, aggregation and growth are three main steps in the formation of CaOx crystals. *In vitro* study showing dose related increase in percentage inhibition of these three main steps of CaOx crystal formation which is indicative of antiurolithiatic activity of the extract. Our result is in agreement with Bawari et al. study on Daucus carota (Bawari et al. 2018).

For *in vivo* study, ethylene glycol induced urolithiasis rats model was used. The addition of ammonium chloride with ethylene glycol accelerates the stone formation. Extract treated

Table VI. Effect of *S. hispanica* seeds extract and standard on percentage inhibition of crystal nucleation, growth and aggregation assays.

Concentration (µg/mL)	Extract (Nucleation)	Standard (Nucleation)	Extract (Growth)	Standard (Growth)	Extract (Aggregation)	Standard (Aggregation)
1000	94.18***	95.35	90.58***	92.15	97.87***	95.21
800	61.38***	86.48	63.35***	83.45	68.62***	89.36
600	54.50***	61.43	48.69***	69.59	51.06***	76.06
400	21.69*	35.32	23.56**	39.27	24.47**	40.95
200	0.53^{ns}	9.54	4.7^{ns}	13.61	6.39^{ns}	15.43

ns: non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to the standard.

Table VII. Effect of the *S. hispanica* seeds extract on the urine parameters, body and kidney weights.

Groups Oxalate (mg/24 h)		Urine parameters							Body and kidney weights (g)	
		Calcium (mg/L)	Magnesium (mmol/L)	Phosphate (mg/L)	Sodium (mmol/L)	Potassium (mmol/L)	Urine volume (mL)	Body weight	Kidney weight	
Normal control		11.05 ± 0.01	27.06 ± 0.02	46.06 ± 0.02	0.15 ± 0.02	32.05 ± 0.02	10.05 ± 0.02	8.07 ± 0.02	210.05 ± 0.02	0.68 ± 0.01
Disease control		19.04 ± $0.01^{###}$	38.05 ± $0.02^{###}$	38.08 ± $0.02^{###}$	0.25 ± $0.02^{###}$	36.07 ± $0.03^{###}$	17.06 ± $0.02^{###}$	4.05 ± $0.02^{###}$	155.46 ± $0.53^{###}$	0.98 ± 0.01
Standard		12.07 ± 0.01***	31.03 ± 0.01***	44.05 ± 0.02***	0.16 ± 0.01**	32.05 ± 0.02***	10.05 ± 0.01***	7.57 ± 0.06***	212.58 ± 0.13***	0.70 ± 0.01
Extract (mg/kg)	100	18.35 ± 0.33	35.05 ± 0.01***	40.06 ± 0.02***	0.16 ± 0.02**	34.12 ± 0.04***	16.11 ± 0.04***	5.34 ± 0.21***	170.48 ± 0.24***	0.93 ± 0.01
	300	17.06 ± 0.01***	29.04 ± 0.02***	42.05 ± 0.02***	0.15 ± 0.02**	34.05 ± 0.02***	14.04 ± 0.02***	6.41 ± 022 ***	188.50 ± 0.13***	0.85 ± 0.01
	700	13.06 ± 0.02***	27.05 ± 0.02***	43.05 ± 0.02***	0.13 ± 0.01***	33.15 ± 0.02***	12.06 ± 0.02***	7.61 ± 0.14***	209.36 ± 0.27***	0.69 ± 0.01

The values are expressed as mean ± SEM, (n=6), $^{###}$ $p < 0.001$ as compared to the normal control, ** $p < 0.01$, *** $p < 0.001$ as compared to the disease control.

groups had low levels of calcium, phosphate and oxalate in the urine and also lower the levels of serum creatinine, urea and uric acid, protein excretion indicating that extract possessed antiurolithiatic activity. The result is in consistence with Kishore et al., Makasana et al. and Sharma et al. studies (Sharma et al. 2017, Makasana et al. 2014, Kishore et al. 2014). Magnesium is an inhibitor of renal stone formation. It forms complex with calcium oxalate crystals and dissolves them. The urinary magnesium level in disease control was high as compared to that of extract treated groups. The decrease in urinary magnesium level promotes urolithiasis whereas

Table VIII. Effect of *S. hispanica* seeds extract on the serum parameters. The values are expressed as mean ± SEM, (n=6), ### $p < 0.001$ as compared to the normal control, ** $p < 0.01$, *** $p < 0.001$ as compared to the disease control group.

Groups Uric acid (mg/dL)		Serum parameters				
		Creatinine (mg/dL)	BUN (mg/dL)	Serum protein (g/dL)	Serum albumin (g/dL)	
Normal control		2.75 ± 0.01	0.56 ± 0.02	9.14 ± 0.02	5.44 ± 0.02	3.06 ± 0.02
Disease control		4.56 ± 0.02###	0.84 ± 0.02###	14.17 ± 0.02###	6.14 ± 0.02###	3.74 ± 0.03###
Standard		3.05 ± 0.02***	0.64 ± 0.01***	10.08 ± 0.04***	5.45 ± 0.02***	3.13 ± 0.02***
Extract (mg/kg)	100	4.15 ± 0.01***	0.75 ± 0.2**	13.05 ± 0.02***	5.94 ± 0.01***	3.45 ± 0.01***
	300	3.85 ± 0.02***	0.74 ± 0.02**	12.15 ± 0.02***	5.74 ± 0.02***	3.44 ± 0.02***
	700	3.44 ± 0.02***	0.65 ± 0.02***	11.05 ± 0.02***	5.55 ± 0.02***	3.24 ± 0.02***

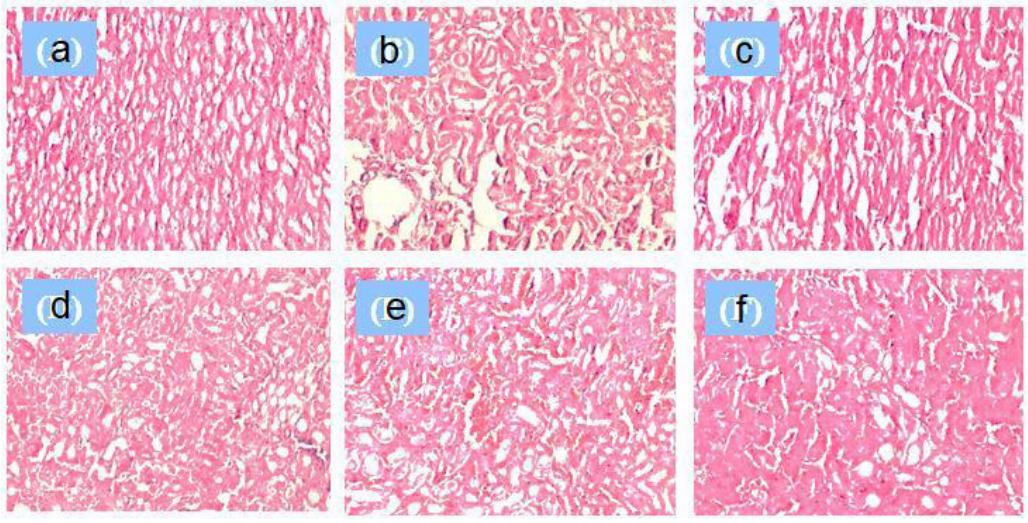

Figure 3. Histopathological analysis of kidney at 10X (a) Normal control; (b) Disease control; (c) Standard; (d-f) Extract treated groups at 100, 300 and 700 mg/kg concentrations.

increased urinary magnesium level indicating dissolution of CaOx crystals (Dinnimath et al. 2017, Ramaswamy et al. 2015).

In this study, urine samples were collected and body weight along with kidney weight (separately) were measured and both of these parameters in extract treated groups were found as that of normal control values. Urine volume was increased in extract treated groups that revealed diuretic activity of the understudied seeds. The weight of kidneys was higher in disease control group due to CaOx crystal precipitation as compared to extract treated groups (Dinnimath et al. 2017). Histopathological analysis also supported whole this data.

CONCLUSION

The present study unveiled anti-urolithiatic activity of *S. hispanica* seeds methanol extract in EG induced urolithiasis rat model. The extract prevented the formation of calcium oxalate stones by inhibiting early steps of stone formation i.e. nucleation, aggregation and growth. Moreover, the extract displayed anti-urolithiatic activity by decreasing oxalate, calcium, phosphate, total proteins, albumin, blood urea nitrogen and uric acid levels.

Acknowledgments

The author is thankful to the G.C. University Faisalabad for providing research facilities.

REFERENCES

AGGARWAL KP, NARULA S, KAKKAR M & TANDON C. 2013. Nephrolithiasis: molecular mechanism of renal stone formation and the critical role played by modulators. Biomed Res Int 2013: DOI: 10.1155/2013/292953.

AHMAD S, ANSARI TM & SHAD MA. 2016. PREVALENCE OF RENAL CALCULI. The Professional Medical Journal 23: 389-395.

ALELIGN T & PETROS B. 2018. Kidney Stone Disease: An Update on Current Concepts. Advances in urology 2018: DOI: 10.1155/2018/3068365.

BAWARI S, SAH AN & TEWARI D. 2018. Antiurolithiatic Activity of Daucus carota: An *in vitro* Study. Pharmacognosy Journal 10.

BRANCALION APS, OLIVEIRA RB, SOUSA JPB, GROPPO M, BERRETTA AA, BARROS ME, BOIM MA & BASTOS JK. 2012. Effect of hydroalcoholic extract from Copaifera langsdorffii leaves on urolithiasis induced in rats. Urol Res 40: 475-481.

CHAUDHARY A, SINGLA SK & TANDON C. 2008. 171: Calcium oxalate crystal growth inhibition by aqueous extract of Tamarindus indica. Indian J Urol 24 p.

COE FL, EVAN A & WORCESTER E. 2005. Kidney stone disease. J Clin Invest 115: 2598-2608.

DAS K, DANG R, SIVARAMAN G & ELLATH RP. 2018. Phytochemical Screening for Various Secondary Metabolites, Antioxidant, and Anthelmintic Activity of Coscinium fenestratum Fruit Pulp: A New Biosource for Novel Drug Discovery. Turk J Pharm Sci 15.

DESAI M, DE LISA A, TURNA B, RIOJA J, WALFRIDSSON H, D'ADDESSI A, WONG C & DE LA ROSETTE OBOTCPSG J. 2011. The clinical research office of the endourological society percutaneous nephrolithotomy global study: staghorn versus nonstaghorn stones. J Endourol 25: 1263-1268.

DINNIMATH BM, JALALPURE SS & PATIL UK. 2017. Antiurolithiatic activity of natural constituents isolated from Aerva lanata. J Ayurveda Integr Med 8: 226-232.

DIVAKAR K, PAWAR A, CHANDRASEKHAR S, DIGHE S & DIVAKAR G. 2010. Protective effect of the hydro-alcoholic extract of Rubia cordifolia roots against ethylene glycol induced urolithiasis in rats. Food Chem Toxicol 48: 1013-1018.

ERICKSON S, VRTISKA T & LIESKE J. 2011. Effect of Cystone® on urinary composition and stone formation over a one year period. Phytomedicine 18: 863-867.

GUPTA M, BHAYANA S & SIKKA S. 2011. Role of urinary inhibitors and promoters in calcium oxalate crystallisation. Int J Research in Pharmacy and Chemistry 1: 793-798.

IMAN S, SALEEM U & AHMAD B. 2020a. Preclinical Assessment of Antiurolithiatic Activity of Mangifera indica Seeds on Ethylene Glycol Induced Urolithiasis Rat Model. Pak Vet J 10.29261/pakvetj/2020.028.

KISHORE RN, MANGILAL T, ANJANEYULU N, ABHINAYANI G & SRAVYA N. 2014. Investigation of anti-urolithiatic activity of Brassica oleracea gongylodes and Desmostachya bipinnata in experimentally induced urolithiasis in animal models. Int J Pharm Pharm Sci 6: 602-604.

MAKASANA A, RANPARIYA V, DESAI D, MENDPARA J & PAREKH V. 2014. Evaluation for the anti-urolithiatic activity of Launaea procumbens against ethylene glycol-induced renal calculi in rats. Toxicol Rep 1: 46-52.

MAMILLAPALLI V, KHANTAMNENI PL, MOHAMMAD Z, MATHANGI A, NANDIGAM N, NAMBURI SM & KATTA V. 2016a. Phytochemical & *in vitro* antiurolithiatic studies on the leaf extracts of *Bauhinia variegata linn*. Int J Pharm Sci Res 7: 4074-4084.

MAMILLAPALLI V, KHANTAMNENI PL, MOHAMMAD Z, MATHANGI A, NANDIGAM N, NAMBURI SM & KATTA V. 2016b. Phytochemical & *in vitro* antiurolithiatic studies on the leaf extracts of *Bauhinia variegata linn*. Development 542: 21.

MIKAWLRAWNG K, KUMAR S & VANDANA R. 2014. Current scenario of urolithiasis and the use of medicinal plants as antiurolithiatic agents in Manipur (North East India): a review. Intern Jour Herb Med 2: 1-12.

MUÑOZ LA, COBOS A, DIAZ O & AGUILERA JM. 2013. Chia seed (Salvia hispanica): an ancient grain and a new functional food. Food Rev Int 29: 394-408.

NIRMALADEVI R, KALPANA S, KAVITHA D & PADMA P. 2012. Evaluation of antilithiatic potential of Hibiscus rosa-sinensis Linn, *in vitro*. J Pharm Res 5: 4353-4356.

NIRUMAND MC, HAJIALYANI M, RAHIMI R, FARZAEI MH, ZINGUE S, NABAVI SM & BISHAYEE A. 2018. Dietary plants for the prevention and management of kidney stones: preclinical and clinical evidence and molecular mechanisms. Int J Mol Sci 19: 765.

RAHEEM OA, KHANDWALA YS, SUR RL, GHANI KR & DENSTEDT JD. 2017. Burden of urolithiasis: trends in prevalence, treatments, and costs. Eur Urol Focus 3: 18-26.

RAMASWAMY K, KILLILEA DW, KAPAHI P, KAHN AJ, CHI T & STOLLER ML. 2015. The elementome of calcium-based urinary stones and its role in urolithiasis. Nat Rev Urol 12: 543.

SALEEM U, HUSSAIN K, AHMAD M, IRFAN BUKHARI N, MALIK A & AHMAD B. 2014. Physicochemical and phytochemical analysis of Euphorbia helioscopia (L.). Pak J Pharm Sci 27.

SANDOVAL-OLIVEROS MR & PAREDES-LÓPEZ O. 2012. Isolation and characterization of proteins from chia seeds (Salvia hispanica L.). J Agric Food Chem 61: 193-201.

SHARMA I, KHAN W, PARVEEN R, ALAM M, AHMAD I, ANSARI MHR & AHMAD S. 2017. Antiurolithiasis Activity of Bioactivity Guided Fraction of Bergenia ligulata against Ethylene Glycol Induced Renal Calculi in Rat. Biomed Res Int 2017.

SOFIA NH & WALTER TM. 2016. Prevalence and risk factors of kidney stone. Global Journal For Research Analysis 5 (3): 183-187.

TABASUM S, KHARE S & JAIN K. 2016. Spectrophotometric quantification of total phenolic, flavonoid and alkaloid contents of Abrus precatorius L. seeds. Asian J Pharm Clin Res 9: 371-374.

ULBRICHT C, CHAO W, NUMMY K, RUSIE E, TANGUAY-COLUCCI S, IANNUZZI CM, PLAMMOOTTIL JB, VARGHESE M & WEISSNER W. 2009. Chia (Salvia hispanica): a systematic review by the natural standard research collaboration. Rev Recent Clin Trials 4: 168-174.

ULLAH R, NADEEM M, KHALIQUE A, IMRAN M, MEHMOOD S, JAVID A & HUSSAIN J. 2016. Nutritional and therapeutic perspectives of Chia (Salvia hispanica L.): a review. J Food Sci Technol 53: 1750-1758.

URIBE JAR, PEREZ JIN, KAUIL HC, RUBIO GR & ALCOCER CG. 2011. Extraction of oil from chia seeds with supercritical CO2. J Supercit Fluids 56: 174-178.

ZENG X, XI Y & JIANG W. 2018. Protective roles of flavonoids and flavonoid-rich plant extracts against urolithiasis: a review. Crit Rev Food Sci Nutr 1-11.

How to cite

SALEEM U, AHMAD N, SHAH MA, ANWAR F & AHMAD B. 2020. Anti-urolithiatic activity of *Salvia hispanica* L. seeds in ethylene glycol induced urolithiasis rat's model. An Acad Bras Cienc 92: e20200067. DOI 10.1590/0001-3765202020200067.

Manuscript received on February 18, 2019; accepted for publication on May 21, 2020

UZMA SALEEM[1]
https://orcid.org/0000-0002-1541-4236

NAZIR AHMAD[1]
https://orcid.org/0000-0003-3151-0276

MUHAMMAD AJMAL SHAH[2]
https://orcid.org/0000-0003-3471-184X

FAREEHA ANWAR[3]
https://orcid.org/0000-0001-5097-8128

BASHIR AHMAD[3]
https://orcid.org/0000-0003-0083-3750

[1]Department of Pharmacology, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad 38000, Pakistan

[2]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad 38000, Pakistan

[2]Riphah Institute of Pharmaceutical Sciences, Riphah International University, Lahore 54000, Pakistan

Correspondence to: **Dr. Uzma Saleem**
E-mail: uzma95@gmail.com

Author contributions

U.S. and M.A.J. supervised the project. U.S. and B.A. drafted the manuscript. N.A. and F.A. performed the experiments.

ERRATUM

In the article **Anti-urolithiatic activity of *Salvia hispanica* L. seeds in ethylene glycol induced urolithiasis rat's model,** with DOI number: **http://doi.org/10.1590/0001-3765202020200067**, published in the journal **Anais da Academia Brasileira de Ciências**, 92(4): e20200067, page 12

Reads:
MUHAMMAD AJMAL SHAH[1]
FAREEHA ANWAR[2]
BASHIR AHMAD[2]

Should read:
MUHAMMAD AJMAL SHAH[2]
FAREEHA ANWAR[3]
BASHIR AHMAD[3]

Reads:
[2]Riphah Institute of Pharmaceutical Sciences, Riphah International University, Lahore 54000, Pakistan

Should read:
[2]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad 38000, Pakistan
[3]Riphah Institute of Pharmaceutical Sciences, Riphah International University, Lahore 54000, Pakistan

An Acad Bras Cienc (2021) 93(1): e20200067e