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Antilithiatic Potential Of Herbo-Mineral Formulations *Sindhuvallathy Mezhugu(Svm)* And *Kalladaippu Kudineer* (*Kk*) Against Ethylene Glycol Induced Nephrolithiasis.

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Abstract: Kidney stones are hard masses formed at anywhere in the urinary system, leads to blood in the urine, urinary iritation and pain in the abdomen, flank, or groin by multiple causes like concentrated urine, excessive excretion of stone forming agents etc by improper food and lifestyle. Urolithiasis, nephrolithiasis, renal stones, kalladaippu are different terminologies used to mention this multifactorial disorder which has been tormenting mankind from the dawn of civilization. Present day conventional medications and measures for this disease remain unfriendly to mediocre population as they are highly expensive and not free from side effects. The Siddha system of medicine uniquely stands as an ancient heritage of south India and offers enormous herbal and herbo-mineral formulations for the prevention and management of kalladaippu. In this present study, the Siddha formulations Sindhuvallathy mezhugu (SVM), kalladaippu kudineer (KK) and a combination of both (SVM+KK) were evaluated for antilithiatic activity against Ethylene glycol (EG) induced hyperoxaluric Sprague Dawley rats. The study results supported that Sindhuvallathy mezhugu (SVM) and kalladaippu kudineer (KK) are safe and pharmacologically effective Siddha formulations and also they complement each other in the prevention and alleviation of nephrolithiasis.

Keywords: Siddha, urolithiasis, , kidney stones, nephrolithiasis**,** kalladaippu. Herbs, Traditional medicine.

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I. INTRODUCTION

Kidney stones are hard masses formed at anywhere in the urinary system, leads to blood in the urine, urinary iritation and pain in the abdomen, flank, or groin by multiple causes like concentrated urine, excessive excretion of stone forming agents etc by improper food and lifestyle. Urolithiasis, nephrolithiasis, renal stones, kalladaippu are different terminologies used to mention this condition.

Urinary stones are a multi-factorial disorder which occurs due to combined influences of environmental, biochemical or genetic factors[1]. Nearly 5-15% of population in industrialized countries are affected by this condition worldwide [2,3]. The incidence has been more in developing countries including India and more predominantly affecting 30-50 years age group. Previous studies indicated that men were more prone for this disease and the male-to-female ratio was approximately 3:1 [4,5]. Recent epidemiological survey on nephrolithiasis suggested that the changing socio economic condition in women resulting in life style related risk factors like obesity has generated changes in the sex distribution and the present ratio is almost equal in both sexes consequently reducing the gender gap [6,7]. The search for potential medication for treating calculi formation is still elusive due to short recurrence interval and subsequent increase in relapse rate. In traditional Siddha system of medicine this disorder is mentioned as *kalladaippu* and various herbs, metals, minerals and marine products are indicated for its treatment.

Siddha formulations are most often prepared with single or many herbs or herbo-mineral compounds, which may act in an agonistic, synergistic, complementary and antagonistic ways. *Sindhu Vallathy mezhugu (SVM)* and *Kalladaippu Kudineer(KK)* are classical *Siddha* formulations that have longstanding literature evidences for the treatment of kidney stones. Even though not scientifically evaluated, Some ingredients of SVM and KK have been pharmacologically reported for their anti-lithiatic activities. In clinical practice using combinations of herbs and minerals to harmonize the drug potential is common in *Siddha* system of medicine.

In this present study an attempt has been made to identify the antilithiatic potentials of SVM and KK as single as well as in combination and also to evaluate the synergistic effects of these formulations that complement each other against ethylene glycol induced nephrolithiasis in experimental Sprague Dawley rats.

II. MATERIAL AND METHODS

Study material

The study drug *Sindhuvallathagi mezhugu*[8] consists of herbo mineral ingredients - *Semicarpus anacardium*(Fruit), *Tamarindus indicus*(fruit pulp), Borax, Potassium nitrate and Sodium chloride. The drug *Kalladaippu kudineer*[9] has various herbal ingredients – *Tribulus terrestris*(fruit), *Aerva lanata*(root), *Crataeva religiosa*(bark),*Pavonia odorata*(root). All the herbals and salts of study drugs were identified and authenticated by Research officers of the department of Pharmacognosy and chemistry, Siddha Central Research Institute, Arumbakkam, Chennai. Then the trial drugs *SVM* and *KK* were prepared as per the Siddha pharmacopoea and GMP guidelines.

Experimental Animals

Male Sprague dawley rats (200-250g) were used to evaluate the anti-lithiatic effects in 1% ethelene glycol – induced urolithiasis model. Rats were housed (5per cage) and maintained at 25 ± 2^0C in 12-hrs dark/12-hrs light cycles, with both standard pelleted diet and water *ad libitum* in accordance with the CPSCEA guidelines. Rats were acclimatized, at least for a period of 7 days, to laboratory conditions prior to experimental study. 36 healthy male Sprague dawley rats were randomly selected and then divided into six groups with 6 rats in each group. All experimental animals except normal control received ethylene glycol (1 %) orally in drinking water (Aqua guard on-line water filter-cum-purifier) for a period of 28 days [10,11,12]. The Institutional Animal Ethics Committee of Sri Ramachandra University, Chennai has approved this experimental work by its notification number IAEC/XXXIII/ SRU/267/2013.

Group-I: Normal control (G1)
Group-II: 1% Ethylene glycol - Urolithiasis induction (G2)
Group-III: Urolithiasis induction + SVM (400 mg/kg b.wt) (G3)
Group-IV: KK (10ml/kg) + 1% EG via drinking water (G4),
Group-V: SVM (400 mg/kg) +KK (5 ml) + 1% EG via drinking water (G5)
Group-VI: SVM (400 mg/kg) +KK (10 ml) + 1% EG via drinking water (G6).

Assessment of antiurolithiatic activity

Assessment of antiurolithiatic activities was performed after collection of urine. Urine analysis, hematological parameters, biochemical parameters, body weight changes and histopathological changes were also documented. At the end of the experimental period, after blood collection the animals were sacrificed, the liver, kidney ureter and urinary bladder were excised and weighed.

Collection of Urine samples

The 24 hour urine samples were collected by housing rats in individual metabolic cages on 14^{th} and 28^{th} day using sodium azide as preservative. They are further used for the following assays in which the collected urine samples were measured to assess the Volume / Urine output. The pH of the urine was measured using a glass electrode pH meter. The Specific gravity urine sample was determined using URISCAN 11 SG strip, YD Diagnostics, Korea. The concentration of calcium [13], oxalate [14] inorganic phosphorus [15], and magnesium [16] in urine were estimated using standard methods. Uricacid, urea and creatinine were analyzed using Accurex kit in semiautoanalyze**r**

Collection of Blood samples

Blood was collected at the end of study through retro orbital puncture from the experimental animals in a tube containing 0.2 ml of 11% TSC for plasma separation. The plasma separated was used for the biochemical assays. Aspartate amino transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), was analyzed in plasma using Accurex kits, Accurex Biomedical Pvt. Ltd., Mumbai. Creatinine (Cr), uric acid (UA), total bilirubin and total protein (TP) in plasma were estimated using Accurex kits, Accurex Biomedical Pvt. Ltd., Mumbai in semiautoanalyzer. The concentration of calcium, oxalate, inorganic phosphorus and magnesium in plasma were estimated using standard methods.

Liver and kidney

From the whole liver and kidney, 500mg was utilized for 10% homogenate preparation using 10% KCl and was used for the following biochemical study. Antioxidant profile such as TBARS[17], SOD [18], GSH

[19], GPx [20] were measured using standard methods. Membrane stabilizing enzymes such as NaK ATPase[21], Ca ATPase[22] and Mg ATPase [23] were measured using standard methods. The amount of phosphorus liberated was estimated using Fiske and Subbarow method.

Histopathology

Part of kidney collected from all the animals were preserved and fixed in 10% buffered neutral formalin. They were sliced adequately wherever necessary. After a minimum of 24 h fixation, the samples were processed by conventional methods, paraffin blocks were made and 6 µm paraffin sections were stained with Hematoxylin and Eosin. They were examined under a light and polarised microscope. All deviations from normal histology were recorded and compared with the corresponding controls.

Statistical analysis

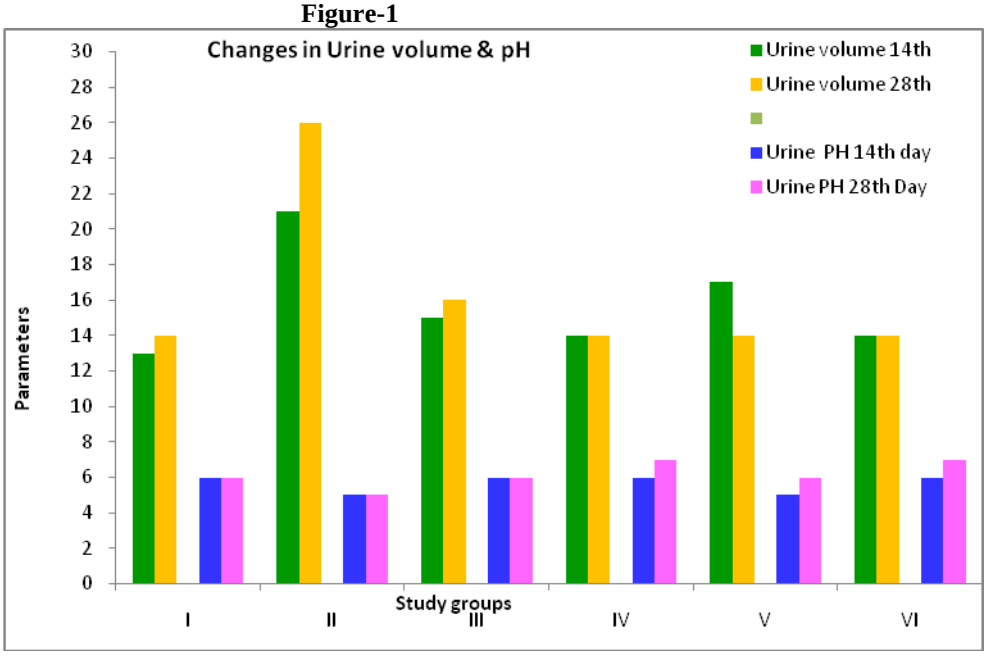
Results were expressed as mean ± SEM. Mean difference between the groups were compared by one way Analysis of Variance (ANOVA) followed by unpaired‘t’ test and Tukey post hoc multiple comparison test. $P < 0.05$ and 0.01 were considered to be significant. Statistical analyses were performed using Graph pad prism 4.0 version.

III. RESULTS AND DISCUSSION

In an effort to evaluate the anti-lithiatic potential of siddha formulations SVM and KK, we conducted pre-clinical study on anti-urolithiatic effect of these formulations on ethylene glycol induced nephrolithiasis. In the pathogenesis of renal stones hyperoxaluria is the major risk factor than hypercalciuria and the changes in urinary oxalate levels are relatively more important than those of calcium [24]. Hence Calcium oxalate stones are the most common type of stones as a result of hypercalciuria and hyperoxaluria [25].

In this present study 1% Ethelene Glycol (EG) is used to induce lithiasis because calcium oxalate crystalluria formation in animals primarily depends on dosage of administration of EG [26]. Male Sprague Dawley rats were used as suitable animal model because the resemblance of urinary system and oxalate metabolism in the male SD rats are nearly identical to that in humans [27].The urinary risk factors like the increased amount of Calcium, oxalate, pH, and reduced volume of urine leads to chemical risk factor favoring the supersaturation of urine leading to the formation of calcium oxalate crystalluria which further progress to form calcium oxalate or calcium phosphate stones [28,29]. Hence measurement of volume, PH, Specific gravity, sodium, calcium, phosphorous, oxalate, uric acid in a 24 hour urine collection identifies the potential risk factors of supersaturation.

Figure-1



The specific gravity of urine may be a better indicator of urinary dilution and a stronger predictor of stone formation. Increase in the volume of urine favors increase in frequency of micturation and thereby reduces the super saturation and reduces the risk of crystal formation. The formation of various types of renal calculi is strongly influenced by urinary PH. An alkaline pH favours the crystallization of calcium and phosphate

containing stones. Whereas acidic pH promotes uric acid or cystine stones [30,31]. In this study SVM and KK increased the volume of urine and showed its effect in altering the acidic PH of the EG induced group. This may be due to the diuretic property of Potassium nitrate [32] and borax [33] present in the drug SVM and the diuretic effect of the individual ingredients of KK namely tribulus terrestris, Aerva lanata, Crateva religiosa and Pavonia odorata studied by Kamboj et al [34]., Venkatesh same et al [35]., and Suresh patnakar et al [36]., and Kashima et al [37] respectively.

Table -1 : Effect of Sindhu vallathey melugu (SVM) and Kaladaippu kudineer (KK) on renal markers in plasma and urine

Group	Parameters in plasma				Parameters in Urine			
	Calcium (mg/dl)	**oxalate (g/dl)**	**Magnesium (mg/dl)**	**Phosphorus (mg/dl)**	**Calcium (mg/dl)**	**oxalate (g/dl)**	**Magnesium (mg/dl)**	**Phosphorus (mg/dl)**
I	10.36±0.80	4.45±0.20	1.16±0.10	14.32±1.07	0.28±0.02	9.97±0.21	0.41±0.02	3.86±0.26
II	15.18±0.53	6.79±0.65	0.68±0.03†	8.62±0.68†	0.71±0.03††	24.67±2.79††	0.15±0.02††	1.04±0.28††
III	13.83±1.89	5.77±0.64	0.88±0.14	12.92±0.60	0.28±0.05**	11.93±1.34**	0.30±0.09**	2.82±0.46*
IV	12.91±2.05	5.29±0.39	0.95±0.07	13.40±1.34	0.25±0.04**	10.84±1.44**	0.28±0.08*	2.71±0.56
V	12.49±0.83	5.18±0.68	0.85±0.15	14.47±0.80	0.28±0.05**	11.93±1.34**	0.34±0.08**	2.82±0.46*
VI	12.46±0.34	5.11±0.40	0.97±0.08	15.21±0.36*	0.30±0.06**	11.79±0.93**	0.31±0.07**	2.96±0.60*

The results are expressed in mean ± SEM (n =6); Statistical analysis was done using prism 4.0 Version, One way ANOVA, Tukey and p values † (0.05) & †† (0.01) compared with group I; * (0.05) & ** (0.01) Compared with Group II

In urolithiasis the glomerular filtration rate (GFR) decreases due to obstruction in the outflow of urine by stones in the urinary system. This leads to accumulation of waste products particularly nitrogenous substances such as urea, creatinine and uric acid in the blood [38]. Uric acid is known to promote calcium oxalate stone growth[39]·The treated groups suggested that the study drugs showed significant decrease in creatinine, urea and uric acid levels near to the normal. However the diuretic property of SVM and KK hastened the process of dissolving the stones and increased the urinary excretion of Urea, creatinine and uric acid, thus normalizing the kidney functionTable-1).

Stone markers

The biochemical mechanisms which enhance the process of crystallization are related to an increase in calcium, oxalate, inorganic phosphate levels and decrease in magnesium levels in urine and serum. Supersaturation of these stone forming agents in urine leads to crystal growth aggregation and stone formation [40,41]. In the present study oxalate and calcium excretion nprogressively increased in calculi induced group animals G2 (Table-2). However SVM, KK and the combination of both the formulations lowered the levels of oxalate as well as calcium excretion. The capacity of lowering calcium and oxalate are almost equal in single and combination groups

Table-2: Effect of Sindhu vallathey melugu (SVM) and Kaladaippu kudineer (KK) on Stone markers in plasma and Urine

Group	Plasma parameters			Urinary parameters		
	urea(mg/ml)	Creatinine(mg/ml)	Uricacid(mg/ml)	Urea(mg/ml)	Creatinine (mg/ml)	Uric acid (mg/ml)
I	37.14±1.79	0.70±0.03	1.12±0.20	25.64±1.35	0.55±0.01	2.13±0.30
II	88.36±9.68††	1.49±0.07††	0.57±0.03†	46.27±2.46††	1.61±0.09††	0.55±0.08††
III	69.78±8.55	0.87±0.12**	0.90±0.10	27.58±1.16**	0.72±0.07**	1.19±0.07
IV	60.77±10.85*	0.86±0.11**	0.98±0.17	27.64±2.13**	0.72±0.09**	1.63±0.20**
V	53.97±8.80**	0.73±0.08**	0.96±0.09	27.58±1.97**	0.72±0.07**	1.19±0.07
VI	51.52±5.32**	0.71±0.08**	0.88±0.09	25.83±0.33**	0.68±0.08**	1.46±0.13*

The results are expressed in mean ± SEM (n =6); Statistical analysis was done using prism 4.0 Version, One way ANOVA, Tukey and p values † (0.05) & †† (0.01) compared with group I; * (0.05) & ** (0.01) Compared with Group II

An increase in urinary phosphorus along with oxalate stress seems to provide a favorable condition for the formation of calcium phosphate crystals which ultimately induces calcium oxalate crystals. Hence the reduction of phosphorus level reduces the risk of stone formation. In this study, a decrease in urinary phosphorus in treatment group compared with normal control group (G1) was observed (Table-2) .Magnesium is a divalent cation and it is complexed with calcium as well as oxalate and decreases its excretion. Magnesium inhibits oxalate absorption and excretion and thus prevents supersaturation [42]. Administration of SVM and KK significantly increased serum magnesium level in the blood.

Renal and Hepatic markers in plasma

Hepatic marker enzymes in plasma such as ALT, AST, ALP and LDH are considered to be sensitive indicators of hepato-cellular damage and their limits can provide a quantitative assessment of damage to the liver[43]. In the present study, the hepatic enzyme markers were found to be uninfluenced by the oral administration of SVM, KK and SVM+KK(Table-3)The results revealed that these formulations and also their combinations did not provoke any impairment on the hepatic functions. Thereby the safety of the drug targeting the liver function was confirmed.

Table-3: Effect of Sindhu vallathey melugu (SVM) and Kaladaippu kudineer (KK) on Hepatic markers in plasma

group	ALP (U/L)	GPT (ALT) (U/L)	GOT(AST) (U/L)	GGT (U/L)	LDH (U/L)
I	321.72±13.03	37.80±2.04	58.35±4.68	5.03±0.69	235.68±8.22
II	495.28±41.18††	62.34±2.83	101.03±12.68	12.23±1.49	512.30±11.55††
III	308.33±18.37**	46.91±6.31	74.24±3.00	8.43±1.25	286.97±24.26**
IV	315.79±10.20**	40.10±3.69	65.28±4.28	7.94±1.12	314.13±27.74**
V	326.22±35.83**	52.59±4.99	73.81±3.13	8.10±1.17	244.40±21.90**
VI	320.01±19.09**	38.23±7.38	65.36±2.87	8.01±0.72	228.58±25.06**

The results are expressed in mean ± SEM (n =6); Statistical analysis was done using prism 4.0 Version, One way ANOVA, Tukey and p values † (0.05) & †† (0.01) compared with group I; * (0.05) & ** (0.01) Compared with Group II

Oxidative stress markers

Recent research has also proved that kidney stone formation is significantly contributed by oxidative stress in humans and an increase in lipid peroxidation and decrease in antioxidant potential have been reported in ethylene glycol induced urolithiatic rats. The present study results on renal and hepatic oxidative stress markers suggests that SVM and KK significantly reduces these oxidative stress markers when compared with EG induced group G2(Table-4) [44].The increase in TBARS in liver and kidney as observed in G2 may contribute to the production of Reactive oxygen species (ROS) due to the destruction of tissues. And the increase in antioxidants in SVM and KK treated groups may be due to the increase in the antioxidant enzyme synthesis or due to the presence of phyto chemical comstituents in the given Siddha formulations which may behave as scavengers of free radicals[45].

Table-4 : Effect of Sindhu vallathey melugu (SVM) and Kaladaippu kudineer (KK) on hepatic and renal oxidative stress marker

group	Hepatic parameters				Renal parameters			
	GSH (µm/g tissue)	TBARS (nm/g tissue)	SOD (U/min/mg ptn)	GPx (µM of GSH consumed /mt/mg ptn)	GSH (µm/g tissue)	TBARS (nm/g tissue)	SOD (U/min/mg ptn)	GPx (µM of GSH consumed /mt/mg ptn)
I	4.09±0.19	106.60±10.73	12.77±0.32	55.06±3.11	6.04±0.18	83.75±6.85	74.64±6.46	79.09±9.28
II	3.38±0.13	201.14±5.58††	9.10±0.88††	33.83±2.40††	4.04±0.18††	137.81±3.35††	29.40±1.40††	46.63±4.47†
III	3.68±0.36	152.68±3.39**	12.44±0.66**	38.79±1.76	4.59±0.35	118.27±5.83	49.47±2.06*	55.53±8.04
IV	3.73±0.24	136.74±6.88**	12.87±0.47**	37.93±2.86	4.88±0.52	129.07±11.34	56.03±4.50**	59.94±4.40
V	3.83±0.19	157.26±12.95*	11.64±0.42*	41.48±3.78	4.04±0.21	136.24±4.79	53.15±7.00**	54.99±5.28
VI	3.96±0.30	150.82±3.37**	13.04±0.63**	43.41±2.49	4.78±0.31	110.03±3.85	67.51±5.46**	70.75±4.24

EG -Ethylene glycol; The results are expressed in mean ± SEM (n =6); Statistical analysis was done using prism 4.0 Version, One way ANOVA, Tukey and p values † (0.05) & †† (0.01) compared with group I; * (0.05) & ** (0.01) Compared with Group II

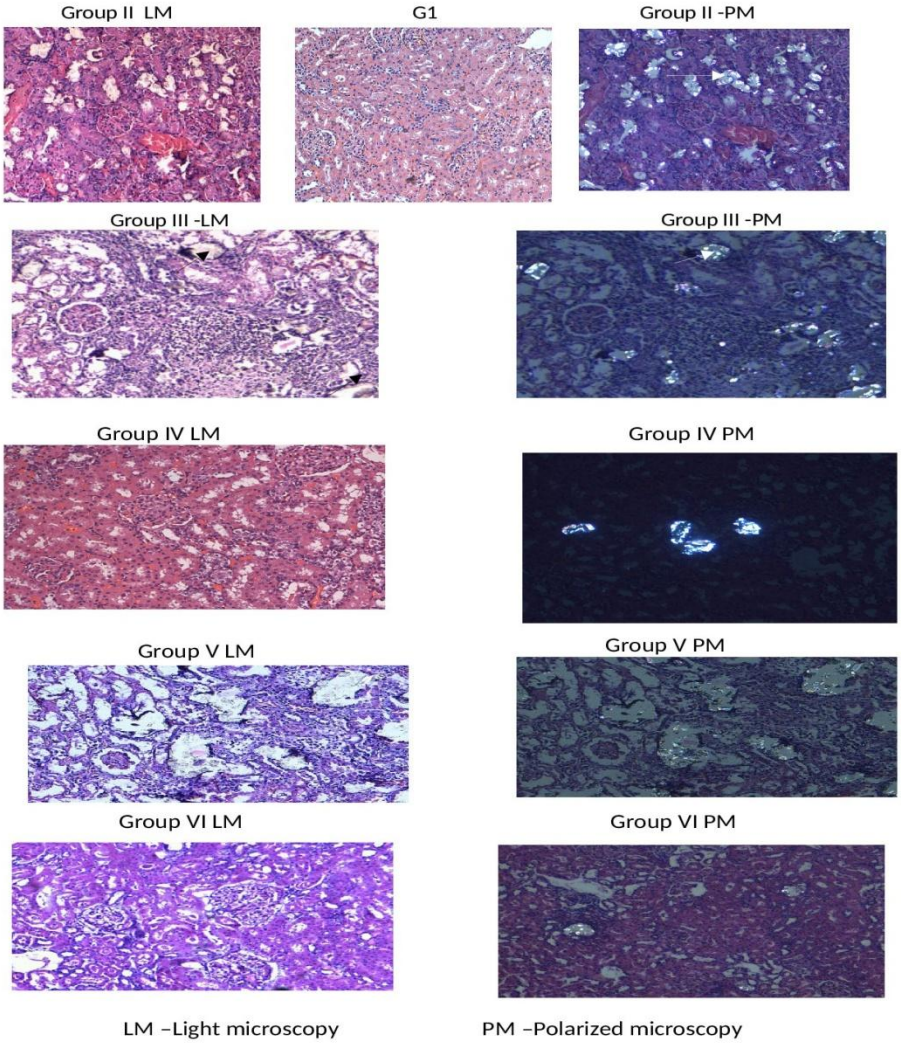
Body weight changes

Body weight changes depend upon the intensity of pain as intense pain may lead to decrease in the food consumption which may further result in the decrease in body weight. Administration of Siddha formulations in this study resulted in increased body weight suggesting that the drug SVM and KK may also reduce renal colic[46].This effect may be due to the analgesic and anti inflammatory effect of semicarpus anacardium[47] and Moringa oleifera[48] of SVM and Tribulus terrestris [49] of KK. No significant change in the organ weight was observed.

Histopathology

Histopathological studies on EG induced kidneys showed destruction of tubules with more number of crystals and blood vessel congestion with severe inflammation in glomeruli. Administration of SVM and KK reduced the crystal formation as shown by both light microscope and polarized microscope with considerable improvement in the renal architecture.

Figure : 1 Histopathological photomicrographs of experimental animals treated with SVM, KK SVM +KK – Kidney (20x magnification)



IV. CONCLUSION:

The pharmacological studies of Siddha formulations *Sindhu Vallathy mezhugu (SVM)* and *Kalladaippu Kudineer (KK)* on ethylene glycol induced urolithiasis model showed their safety and efficacy in reducing the plasma and urinary stone markers levels in single (SVM/KK) or in combination (SVM+KK). Both the formulations SVM and KK exhibited almost equal efficacy on stone stone markers. The combination of these two formulations produced mild synergistic effect on urolithiasis. Results revealed that they also have prophylactic properties on liver and renal functions. The histopathological studies showed crystal growth reduction and reversibility of tissue damage caused by ethylene glycol induced urolithiasis. Therefore this invitro pharmacological study provides a lead data for the progress of further clinical trials and molecular level studies on target action of SVM and KK is needed to justify the literature claim of these Siddha formulations as potent anti urolithiatic agents to the contemporary world.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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