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Effect of Sarva Noi Linga Chenduram for anti urolithiatic activity in ethylene glycol induced animal model.

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Abstract

The aim of the study is to evaluate AntiUrolithiatic activity of "Sarva Noi Linga Chenduram" in Ethylene Glycol induced animal model. Sarva Noi Linga Chenduram was prepared by standard operative procedure mentioned in the siddha text. Urolithiasis was induced in Wistar albino rats by 0.75% ethylene glycol in drinking water for 28days. Administration of Ethylene Glycol in drinking water resulted in hyperoxaluria, hypocalcemia as well as an increased renal excretion of Phosphate.Urinevolume,sodium,potassium,chloride,calcium,oxalate,phosphorous,BUN,creatinine,uric acid was evauluated. Total protein, Citrate, Magnesium were also studied. Histopathological examination was also performed. Treatment with sarva noi linga chenduram(50mg/kg,100mg/kg) significantly reduced the urinary calcium, oxalate, and phosphate excretion dose-dependently. There was a significant reduction in the levels of calcium, oxalate as well as a number of calcium oxalate crystals deposits in the kidney tissue of rats administered with Sarva Noi Linga Chenduram in Ethylene Glycol treated rats. There was a significant reduction in creatinine, urea, uric acid, and blood urea nitrogen when Sarva Noi Linga Chenduram was administered in Ethylene Glycol treated rats. From this study, it was concluded that the Sarva Noi Linga Chenduram protected Ethylene Glycol induced urolithiasis as it reduced the growth of urinary stones. The mechanism underlying this effect might be due to its diuretic, and reduction in stone-forming constituents.

Keywords: Siddha, Sarva Noi Linga Chenduram, Anti- Urolithiatic Activity.

Introduction

Renal calculus, one of the most common urological disorder. Archeological findings give profound evidence that humans have suffered from kidney and bladder stones for centuries. Bladder stones were more prevalent during older ages, but kidney stones became more prevalent during the past 100 years[1] . The first evidence of urinary stone was found in Egyptian mummy E1amrah eygpt at 4800B.C[2].

Urinary stone constitute one of the commonest diseases in our country and pain due to kidney stones is known as worse than that of labour pain. In India, approximately 5 -7 million patients suffer from stone disease and at least 1/1000 of Indian population needs hospitalization due to kidney stone disease[2]

In India upper and lower urinary tract stones occur frequently but the incidence shows wide regional variation. The incidence of renal calculi is comparatively low in the southern part of country compared to other parts[3].

Treatment options and conservative measures, as well as 'surgical' interventions have also been known for a long time. In the recent few days new modern techniques are available to treat renal calculi which are not cost effective to low and middle socio-economic group. Even though our current preventive measures are definitively good the incidence and recurrence has not yet reduced markedly.

Assorted Siddha medicinal preparations are available to treat urolithiasis. Sarva Noi Linga Chenduram was one of the poly herbal formulation to treat Urolithiasis. Ethylene glycol induced urolithiatic model in rat on Sarva noi linga chenduram has not been evaluated so far.

Hence ethylene glycol induced urolithiatic model in rat was used to assess the effect of Sarva Noi Linga Chenduram[4].

Materials and Methods

Drugs, Chemicals and Reagents:

For the study Sarva Noi Linga Chenduram was prepared in Gunapadam lab ,NIS Chennai. All other reagents, assay kits and chemicals used in this work were purchased from Sigma Chemical Co. St Louis, MO, USA.

Collection and authentication of raw drug:

The raw drugs were procured from raw drug store in Chennai and authenticated by competent authority of Department of Gunapadam.

Preparation of the medicine:

Ingredients:

Purified Lingam (Cinnabar)	-	35g
Purified Venkaram (Borax)	-	140g

Purification methods:

Purification of Lingam(Cinnabar):[5] .

It was kept on a mud vessel and heated in low fire. The juices of Citrus lemon, Acalypha indica, cow's milk were mixed in equal proportions. The mixed liquid was poured drop by drop on lingam while heating**.**

Purification of Venkaram (borax):[6]

Venkaram ground by the Citrus lemon juice and then dried it .

Method of medicine preparation:

Lingam ground into tiny particles. Venkaram got placed in a mud vessel and heated in a low fire. When venkaram started melting purified lingam was sprinkled little by little. It had to be mixed well. Before melting of venkaram all quantity of lingam was sprinkled. After that the medicine was taken away from the heat. By the time, it got completely condensed. Then it was well ground in the kalvam and stored in an air tight container.

Animals:

Healthy adult male Wistar albino rats weighing between 150-200 g were selected for the antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions (temperature: 25±2°C) and maintained on 12-h light:12-h dark cycle. They were provided with regular rat chow and drinking water *ad libitum*. The animal care and experimental protocols were approved by Institutional Animal Ethical Committee (IAEC), Vels University (Approval number: (XIII/VELS/PCOL/37/2000/CPCSEA/IAEC/08.08.2012).All rats were housed in metabolic cages for entire duration of the experiment.

Ethylene Glycol Induced Urolithiasis Model [7]:

Animals were divided into five groups containing six animals in each. Group I served as control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was fed to Groups II-V for induction of renal calculi till 28th day. Group II received Ethylene glycol alone and served as urolithiatic control. Group III received standard antiurolithiatic drug, cystone (750mg/kg body weight) from 15th day till 28th day. Groups IV received Sarva Noi Linga Chenduram (50mg/kg body weight) from 15th day till 28th day, Group V received Sarva Noi Linga Chenduram (100mg/kg body weight) from 15th day till 28th day.

Group and Treatment

Group 1: Treated with Normal saline
Group 2: Treated with Control (ethylene glycol) + vehicle
Group 3: Treated with Standard (ethylene glycol + Cystone)
Group 4: Treated with Sarva Noi Linga Chenduram (50mg/kg) + ethylene glycol
Group 5: Treated with Sarva Noi Linga Chenduram (100mg/kg) + ethylene glycol
All doses were given once daily by oral route.

Assessment of antiurolithiatic activity

Collection and analysis of urine:

All animals were kept in individual metabolic cages and urine samples of 24h were collected on 28th day. Animals will be having free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine was analyzed for calcium, phosphate and oxalate content.

Serum Analysis:

After the experimental period, blood was collected from the retro-orbital vein under anesthetic conditions and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000x g for 10 min and analyzed for creatinine, uric acid and urea nitrogen.

Kidney homogenate analysis:

The abdomen was cut open to remove both kidneys form each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 80°C in a hot air oven. A sample of 100mg of the dried kidney were boiled in 10ml of 1N hydrochloric acid for 30min and homogenized. The homogenate was centrifuged at 2000x g for 10min and the supernatant was separated. The calcium, phosphate and oxalate content in kidney homogenate were determined.

Diuretic activity:

Standardization Of Furosemide

Seven groups of six male wistar albino rats were employed four doses of 10,15,20,25-mg/kg b.w of furosemide were administered intraperitoneaaly to each group of rats separately. The control animals received normal saline alone. The animals were placed in separate cages and the urine output over 24hr period was collected. This procedure was repeated. The most consistent dose (15mg/kg b.w) was adapted for dosing.

Evaluation of diuretic activity

Five groups of six male Wistar albino rats were used. First group received normal saline. Second group received Sarva noi linga chendooram 50mg/kg. The third group received Sarva noi linga chendooram 100mg/kg. The fourth group was administered furosemide 20mg/kg. Immediately after administration of the drug, the rats were placed in metabolic cages, specially designed to separate urine and feacal matter and was observed at room temperature. The animals were denied for food and water during the experiment. The urine volume (ml/day) was measured and then assayed for Na^+ and K^+ and Cl^- concentrations in mMol/l, Cl was measured using routine method.

Statistical analysis:

Results were expressed as mean ± S.E.M. Differences among data was determined using one-way ANOVA followed by Dunnet 't' test.

Results and Discussion

The results of acute toxicity study revealed that the SNLC is tolerable upto 1000mg/kg and the therapeutic dose was fixed as 100 and 50mg/kg for further pharmacological investigation. Ethylene glycol induced urolithiasis resulted in significant elevation of urine volume, kidney calcium, oxalate, inorganic phosphate, serum blood urea nitrogen, creatinine and uric acid compared to normal control group. Treatment with cystone (750 mg/kg) and Sarva noi linga chenduram reduced the bio-chemical changes induced by ethylene glycol. In order to probe the possible mechanism by which Sarva Noi Linga Chenduram cures renal damage caused by ethylene glycol, investigation on levels of various stone inhibitors like total protein, magnesium and citrate was studied. There was significant rise on total protein, magnesium and citrate after treatment with Cystone and Sarva Noi Linga Chenduram. .

Administration of ethylene glycol significantly reduced the body weight, urine volume and pH of urine as compared to normal group. Rats treated with cystone and Sarva Noi Linga Chenduram also showed significant increase in body weight, urine volume and

pH of urine as compared to control group. The results were shown in the table (1-14.). The histopathological study of the kidney sections also supported the above results. In all the stone forming rats there was damage to the last part of the nephron, collecting system and peritubular interstitium as compared to the normal rat kidney architecture. The tubules appeared focally ecstatic and surrounded by inflammatory infiltration.

Table 1: Diuretic activity of Sarva noi linga Chenduram in rats

Group	Treatment and Dose	Volume of Urine (ml/4hrs)	Sodium (mMol/l)	Potassium (mMol/l)	Chloride (mMol/l)
I	Saline (10ml/ kg)	0.86±0.17	79.8±7.5	61.0±5.5	95.6±9.0
II	SNLC (50 mg/ kg)	0.94±0.15[a]	105.2±9.5	83.4±7.2	116.1±12.4
III	SNLC (100 mg/ kg)	1.35±0.14[a]	110.4±8.1	92.1±6.0[*]	128.6±7.8
IV	Frusemide (20 mg/ kg)	4.12±0.24	128.6±5.2	107.5±8.1	144.2±10.3

All values are expressed as mean ±S.E.M for six rats in each group.
Comparisons made between [*]p<0.05; T_1,T_2 V_s normal control.; [a]p<0.001 T_1,T_2 V_s Standard.

Table 2: Estimation of Urinary Electrolytes of Normal and Urolithiatic Rats.

S.No	Group & Drug Treatment	Estimation of Urinary Electrolytes		
		Oxalate(mg/dl)	Calcium(mg/dl)	Phosphate(mg/dl)
1	Normal control (Saline)	0.38±0.04	2.74±0.15	3.45±0.05
2	Calculi induced(0.75% EG)	2.13±0.06[©]	9.21±0.43[©]	8.16±0.10[©]
3	Standard (Cystone 750 mg/kg)	1.22±0.07[x]	3.38±0.23[x]	3.92±0.07[x]
4	T_1 (SNLC 50 mg/kg)	0.751±0.23[***]	5.79±0.10[***]	5.11±0.09[a,***]
5	T_2 (SNLC 100 mg/kg)	0.448±0.12[b,***]	4.36±0.14[a,***]	4.32±0.08[c,***]

All values are expressed as mean ±S.E.M for six rats in each group.
Comparisons made between
[a]p<0.001,[b]p<0.01,[c]p,<0.05; T_1,T_2 V_s Standard.
[***]p<0.001,[**]p<0.01,[*]p<0.05 ; T_1,T_2 V_s Calculi induced.
[©]p<0.001 ,[α]p<0.01,@p<0.05; Calculi induced V_s normal control.
[x]p<0.001,[y]p<0.01,[z]p,<0.05; Calculi induced V_s Standard., One-way ANOVA followed by Tukey test.

Table 3: Estimation of Kidney Homogenate Electrolytes of Normal And Urolithiatic Rats.

S.No	Group &Drug Treatment	Estimation of Kidney Homogenate Parameters		
		Oxalate(mg/dl)	Calcium(mg/dl)	Phosphate(mg/dl)
1	Normal (Saline)	0.186±0.03	3.431±0.28	2.65±0.05
2	Positive control (0.75% EG)	1.742±0.09[©]	6.024±0.20[©]	4.10±0.14[©]
3	Standard (Cystone 750 mg/kg)	0.446±0.05[x]	4.326±0.19[x]	3.20±0.08[x]
4	T_1 (SNLC 50 mg/kg)	0.689±0.05[***]	5.452±0.26[c]	3.88±0.12[a]
5	T_2 (SNLC 100 mg/kg)	0.575±0.06[***]	4.234±0.18[***]	3.01±0.09[***]

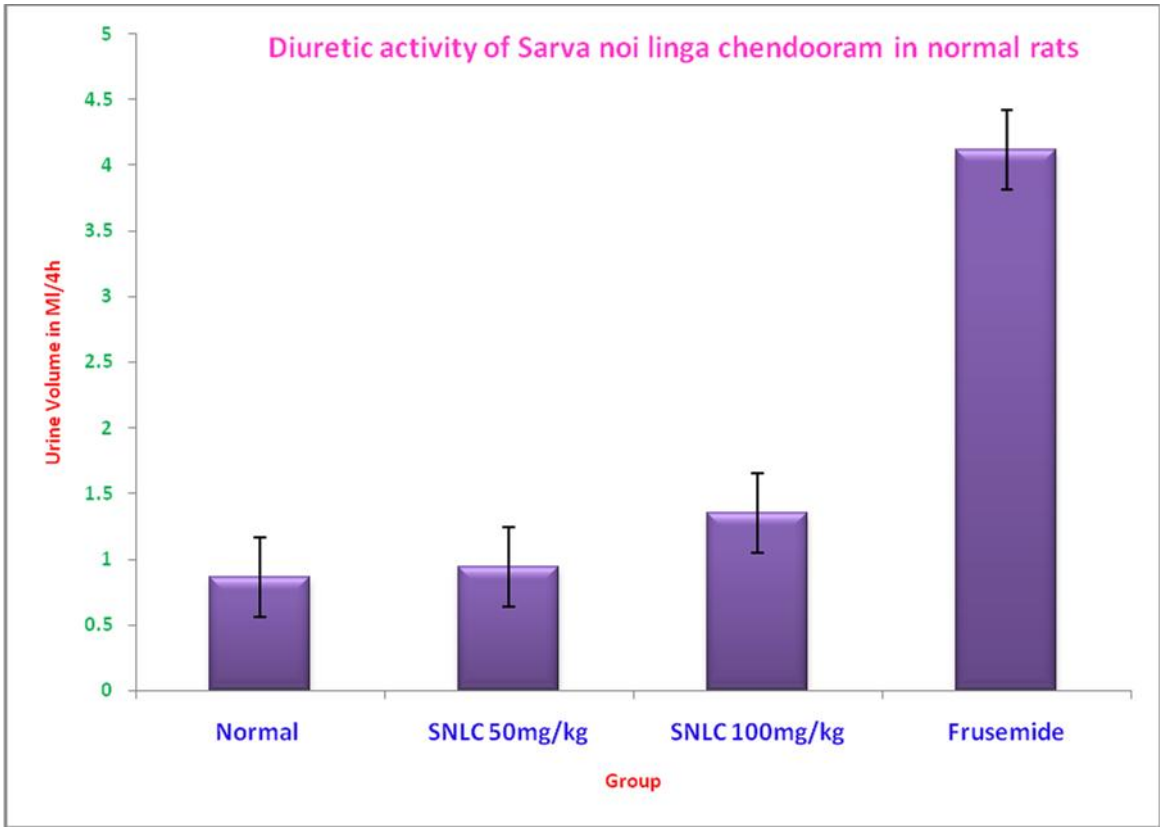
All values are expressed as mean ±S.E.M for six rats in each group.
Comparisons made between
[a]p<0.001,[b]p<0.01,[c]p,<0.05; T_1,T_2 V_s Standard.
[***]p<0.001,[**]p<0.01,[*]p<0.05 ; T_1,T_2 V_s Calculi induced.
[©]p<0.001 ,[α]p<0.01,@p<0.05; Calculi induced V_s normal control.
[x]p<0.001,[y]p<0.01,[z]p,<0.05; Calculi induced V_s Standard., One-way ANOVA followed by Tukey test.

Table 4: Estimation of Serum Parameters of Normal and Urolithiatic Rats.

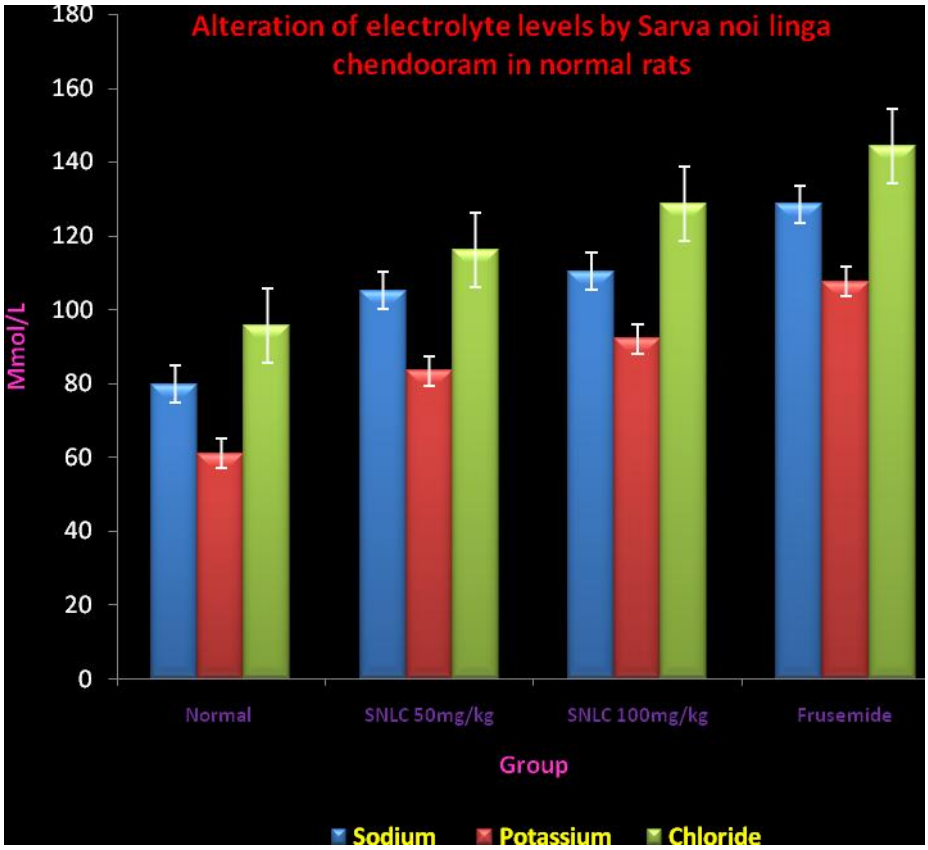
S.No	Group & Drug Treatment	Estimation of Serum Parameters		
		BUN (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
1	Normal (Saline)	18.256±0.32	0.682±0.05	4.99±0.05
2	Positive control (0.75% EG)	$27.109\pm0.36^{©}$	$0.902\pm0.05^{@}$	$6.72\pm0.09^{©}$
3	Standard (Cystone 750 mg/kg)	22.754 ± 0.44^{x}	0.918±0.04	5.56 ± 0.07^{x}
4	T_1 (SNLC 50 mg/kg)	$34.022\pm0.65^{a,***}$	$1.226\pm0.05^{b,**}$	$6.04\pm0.09^{b,***}$
5	T_2 (SNLC 100 mg/kg)	$30.239\pm0.48^{a,***}$	1.101±0.07	$6.12\pm0.08^{a,***}$

All values are expressed as mean ±S.E.M for six rats in each group.
Comparisons made between
$^{a}p<0.001, ^{b}p<0.01, ^{c}p,<0.05$; T_1, T_2 Vs Standard.
$^{***}p<0.001, ^{**}p<0.01, ^{*}p<0.05$; T_1, T_2 Vs Calculi induced.
$^{©}p<0.001$, $^{\alpha}p<0.01$, $@p<0.05$; Calculi induced V_s normal control.
$^{x}p<0.001, ^{y}p<0.01, ^{z}p,<0.05$; Calculi induced V_s Standard., One-way ANOVA followed by Tukey test.

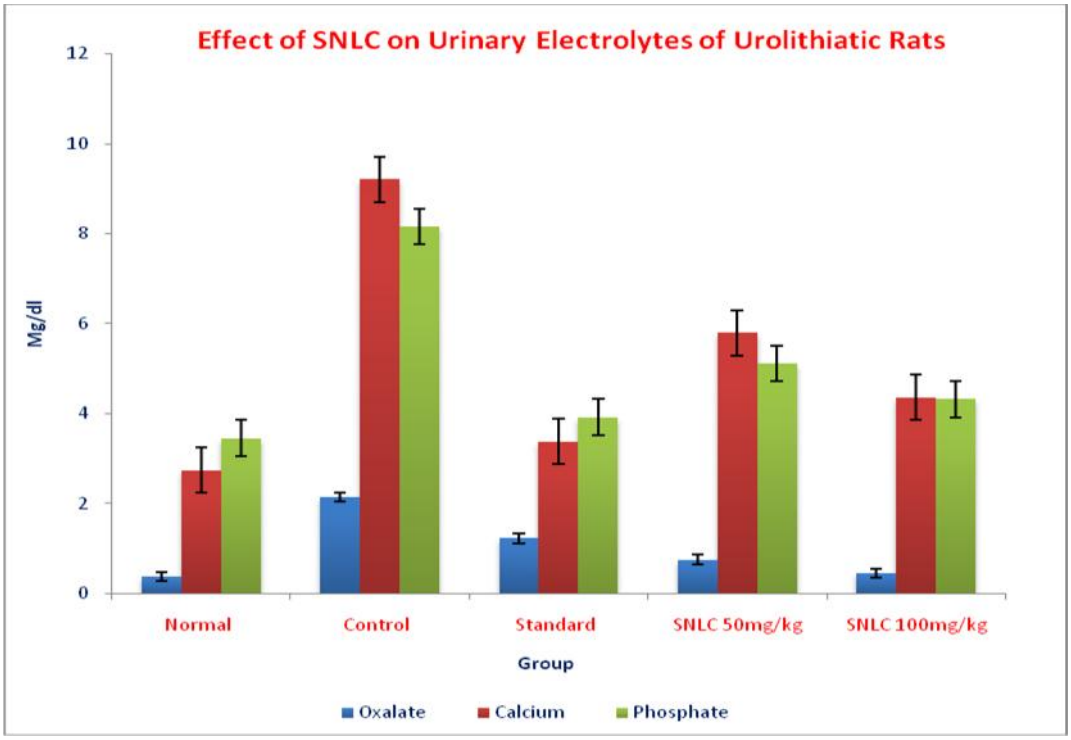
Bar diagram: 1



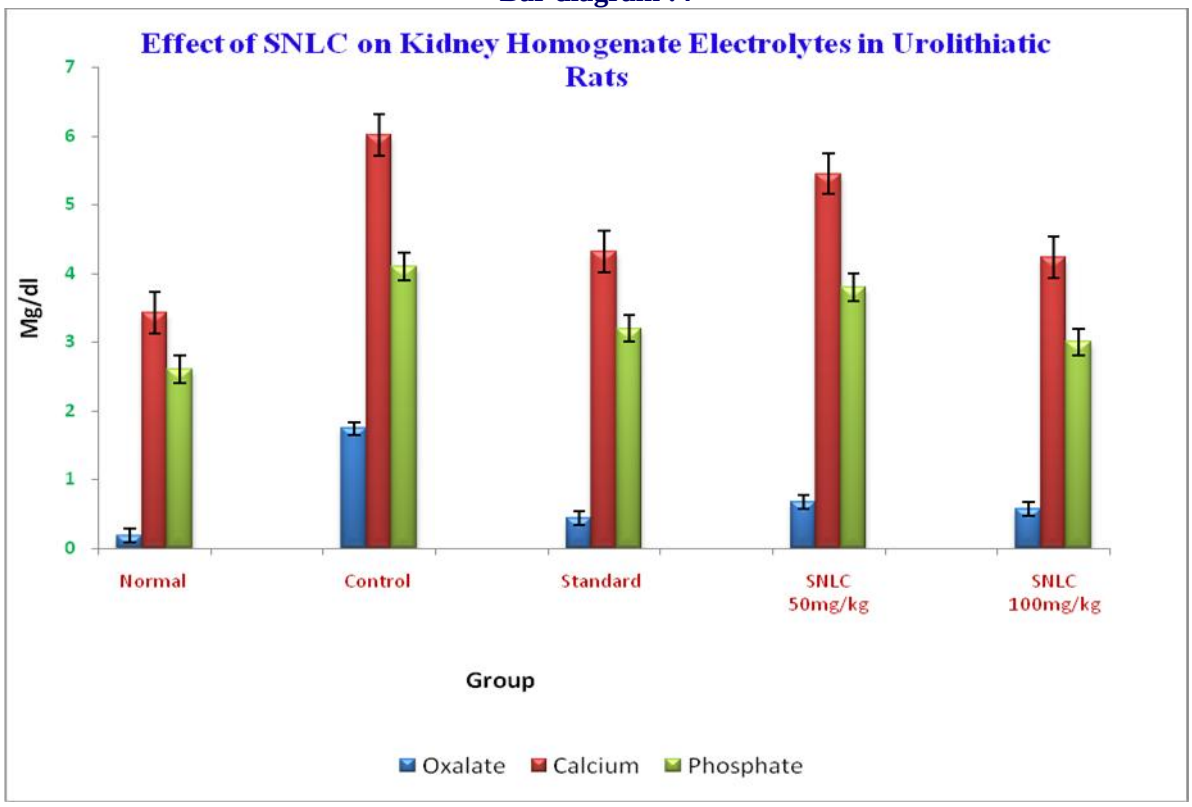
Bar diagram: 2



Bar diagram :3



Bar diagram :4



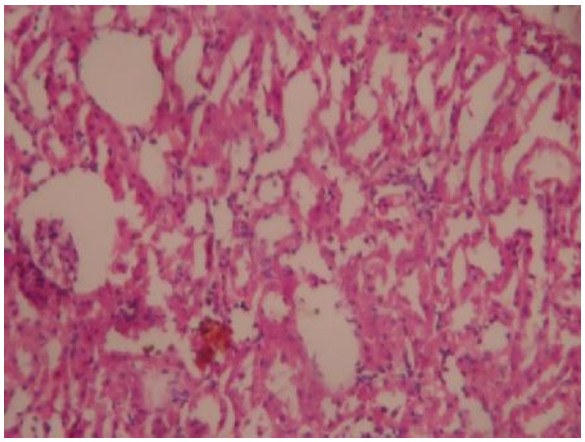


Fig 1: Control

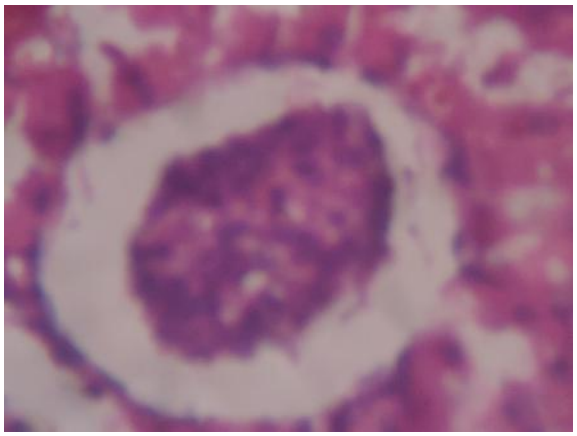


Fig 2: Control 400x

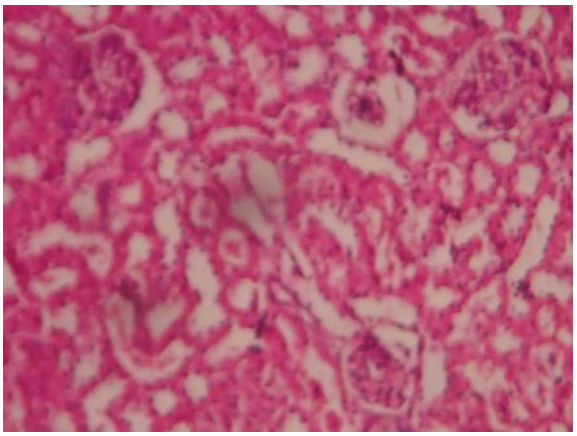


Fig 3: Normal

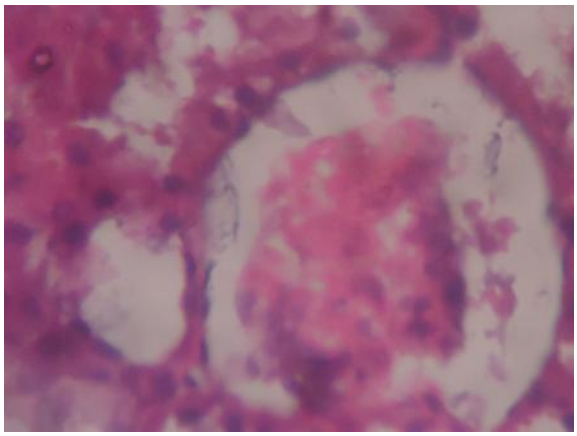


Fig 4: Normal 400x

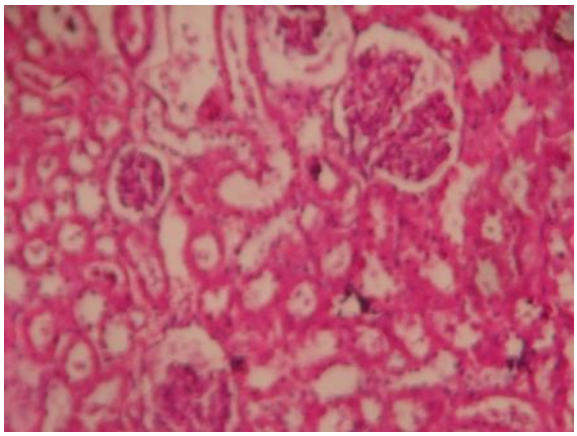


Fig 5: SNLC 50mg

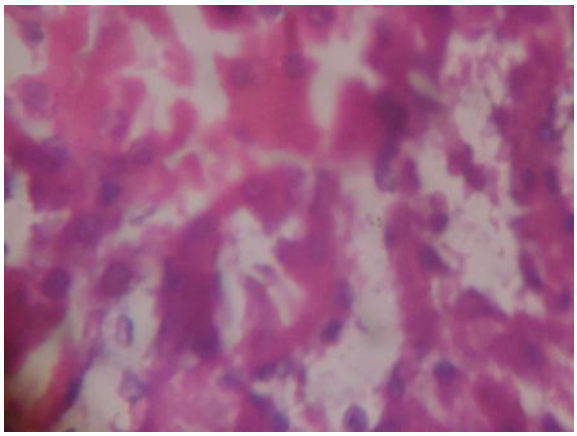


Fig 6:SNLC 50mg 400x

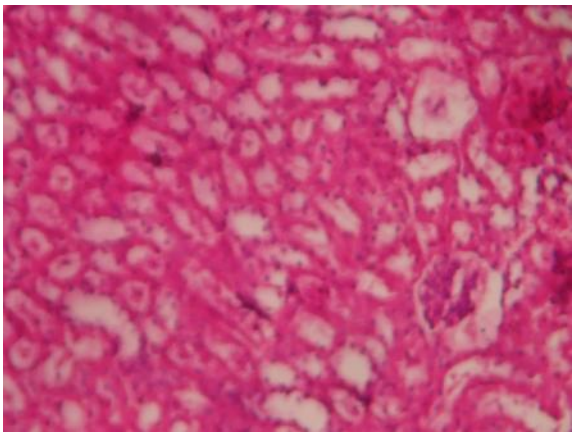


Fig 7:SNLC 100mg

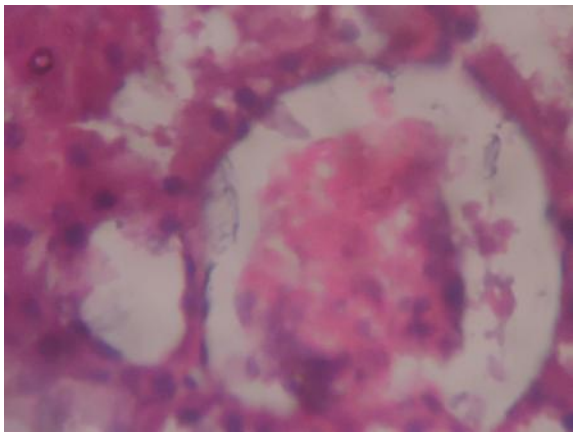


Fig 8:SNLC100mg 400x

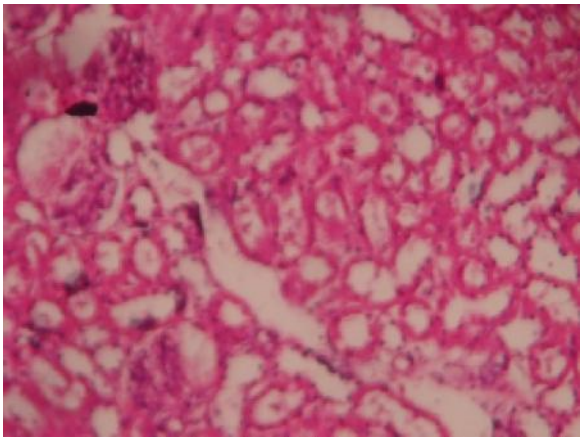


Fig 9:Standard Cystone

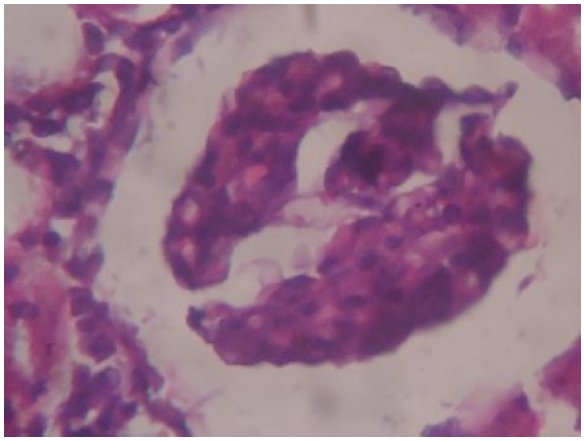


Fig 10:Standard Cystone 400mg

Flattened epithelium with focal vacuolar degeneration and single cell necrosis bordered the tubules, which focally contained hyaline casts. Inflammatory infiltration was mainly composed of mature lymphocytes infiltrating tubular epithelium. Irregular crystals were present inside the tubules and in the peritubular interstitium, along the nephron and at papillary level. The Sarva Noi Linga Chenduram treated groups showed normal histology of the kidney, and showed normal glomeruli, slight oedema of the tubular cells compared to standard drug treated animals. The kidneys excised from ethylene glycol treated group were larger and heavier than from the control animals. When observed under light microscope, many crystalline deposits in the histological preparations were seen in tubules of all regions of kidney.

In Sarva noi linga chendooram along with EG treated rats, such deposits were small and less abundant. Microscopic examination of kidney sections derived from EG induced urolithiatic rats showed calcification inside the tubules which causes dilation of the proximal tubules. Co-treatment with Sarva Noi Linga Chenduram decreased the calcification in different parts of the renal tubules and also prevented damages to the tubules and calyxes. Organ-body weight ratio is a marker of cell constriction and inflammation. The non-significant effect on the rat kidney-body weight ratio following the administration of various doses of the Sarva Noi Linga Chenduram suggests that the drug did not induce inflammation or constriction of the kidney cells.

Pathologic studies have shown that the renal failure from EG is associated with proximal tubule cell necrosis leading to production of several metabolites (glycol aldehyde, glycolate, glyoxylate and oxalate, in that order) and accumulation of large calcium oxalate monohydrate crystals in tubular lumen.

An Ayurvedic compound preparation (Cystone) was found to contain water soluble substances, which inhibited the initial precipitation of calcium and phosphate ions in the form of a mineral phase bound to the organic matrix and the subsequent growth of the preformed mineral phase.

In the present study, concurrent administration of Ethylene glycol with cystone/ Sarva Noi Linga Chenduram caused significant curative effect in Ethylene glycol induced changes. The effect was dose dependent. The effectiveness of Sarva Noi Linga Chenduram was comparable to Cystone.

It was mentioned in the Siddha text Cinnabar cures the disease related with fluids. Borax had lithontriptic and diuretic activity.

The architectural appearance of the kidneys from the rats in the control group, presented a normal histological appearance with no calcium oxalate depositions with normal glomeruli, tubules surrounded by the Bowmanís capsule, proximal and distal convoluted tubules without any inflammatory changes and normal blood vessels. On the other hand, disrupted renal parenchyma showing loss of structural arrangement of renal tubules, early degenerative changes in glomeruli and focal calcification in glomerulo-tubular structures and congested blood vessels were observed in the renal tissue of urolithiatic rats.

The renal tissue of Ethylene glycol along with Sarva noi linga chenduram showed only few stray areas of calcification in glomeruli and normal tubular structures with no congestion in blood vessels. The renal tissue of standard drug treatment still showed moderate calcification in many tubules and few glomeruli. It has been reported that the kidneys are the principle target organ for ethylene glycol toxicity and administration of ethylene glycol for 3 weeks resulted in significant urinary oxalate excretion and deposition of crystals in kidney, hence in our study ethylene glycol was chosen to induce lithiasis. Following the induction of lithiasis the urinary volume and composition were found to be altered.

In our study also the urinary output was markedly decreased in lithiatic control rats on day 28, however in Sarva Noi Linga Chenduram and standard treated rats the urinary volumes were increased when compared to that of lithiatic Group. This suggested that Sarva Noi Linga Chenduram might have moderate diuretic effect. Following ethylene glycol administration the excretion of calcium, oxalate, phosphate and protein were found to be increased in lithiatic group while in standard, test groups these levels were significantly decreased ($P<0.01$).The results were shown in the bar diagram(1-4)

On contrary to this the magnesium level was decreased in lithiatic group while in standard and Sarva noi linga chendooram treated groups those levels were increased significantly ($P<0.01$). The serum creatinine levels of Sarva noi linga chendooram treated rats restored to normal limits and the creatinine clearance was also found to be improved. The findings of the histopathological studies suggested that no microcrystalline deposition and kidney damage in the Sarva noi linga chendooram treated groups. All these observations enabled us to confirm the inhibitory potential of Sarva noi linga chendooram on ethylene glycol induced lithiasis.

Conclusion

The presented data indicated that administration of the Sarva Noi Linga Chenduram to rats with ethylene glycol induced lithiasis reduced the formation of urinary stones, regarding antiurolithiatic activity of the Sarva noi linga chenduram. The mechanism underlying this effect is still unknown, but is apparently related to diuresis and lowering of urinary concentrations of stone forming constituents. These effects could conclude the antiurolithiatic property of Sarva Noi Linga Chendooram.

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