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Research Article

ANTI-UROLITHIATIC ACTIVITY OF *NEERADAIPPU THELINEER* IN ETHYLENE GLYCOL INDUCED LITHIATIC RATS

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Abstract: Many Siddha medicines have been used in the treatment of urinary calculi. But a lacuna of inadequate scientific evaluation in understanding the mode of action exists till now. To fulfil this lacuna, in the present study an effort has been made to evaluate the Antiurolithiatic activity of *Neeradaippu Thelineer* in ethylene glycol induced lithiasis in rats. Experimentally induced urolithiatic rats were treated with standard drug cystone and trial drug *Neeradaippu Thelineer* in two different doses (1ml and 2ml/kg). The calcium, phosphate and oxalate content in urine and kidney homogenate were determined and serum estimation of creatinine, uric acid and urea nitrogen were performed on 28th day of experiment and were compared to the normal, control and standard drug group (cystone). The results suggested that *Neeradaippu Thelineer* at the dose of 2ml/kg provided lowering effect of urinary stone forming constituents which was closer to standard group on dose dependent manner. So the present work was confirmed the traditional use of *Neeradaippu Thelineer* for antiurolithiatic activity.

Key**words:** Anti-urolithiatic activity, cystone, ethylene glycol, *Neeradaippu Thelineer*, Siddha.

INTRODUCTION

Urinary stone disorder is a common disorder in global countries estimated to occur in approximately 12% of population, with a recurrence rate of 70-81% in males and 47-60% in females. Renal stone disease is 2-3 times more common in males than in females [1]. Kidney stones form as a result of physicochemical or genetic derangements leading to super saturation of the urine with stone forming salts or less commonly from recurrent urinary tract infection with urease produce bacteria[2 3].

Urolithiasis is a challenging problem nowadays, and many standard pharmaceutical drugs are available, they have a serious adverse effect that compromise long term use [4]. So in order to alleviate such critic medical condition, with a long term use, and unnecessary surgical therapies which are associated with renal damage [5]. Many traditional medicines have been used in the treatment of urinary stones. But a lacuna of inadequate scientific evaluation in understanding the mode of action exists till now. So in the present study an effort has been made to evaluate the Antiurolithiatic activity of *Neeradaippu Thelineer* prepared as per literature *'Agathiyar Vaithiya Pillai Tamil'* using ethylene glycol induced lithiasis in rats.

MATERIALS AND METHODS

Preparation of drug stock solution:

During the preparation of the drug *Neeradaippu Thelineer* the raw drugs were subjected to '*Suddhi'* (purification process) as per Classical Siddha text [6]. The drug is used for renal calculi (*Kalladaippu)*, instantly prepared everyday which is administered orally by gastric intubation method. The solution filtered finally was directly administered to animals to determine the antiurolithiatic activity and toxicity study maintaining the concentration 60mg/ml of active principle as a stock solution.

Animal selection:

Wistar albino mice of either sex weighing between 28 and 30 g were selected for acute toxicity studies and male Wistar weighing between 180-220 g were selected for antiurolithiatic study. The animals were acclimatized to standard laboratory conditions (temperature: 25±2°C) and maintained on 12-h light, 12-h dark cycle. These animals were provided with regular rat chow and drinking water ad *libitum*. This experiment was approved by the IAEC (Approval number: XIII/VELS/PCOL/20/2000/CPCSEA/IAEC/08.08.2012).

Acute toxicity studies:

As per the OECD guidelines 425 the acute oral toxicity study was carried out [7]. One-tenth of the median lethal dose was taken as an effective dose. The animals were kept under over night fasting providing only water, after which the *Neeradaippu Thelineer* at the dose level of 2 ml/kg body weight, was administered orally by gastric intubation and observed for 14 days. The dose administered was assigned as toxic dose if mortality was observed in two out of three animals. The same dose would be repeated again if mortality was observed in one animal to confirm the toxic dose. The doses were chosen for experiments according to the results of the acute toxicity test.

Ethylene glycol induced Urolithiasis model:

To determine the efficacy of *Neeradaippu Thelineer* urolithiatic model stimulated by ethylene glycol

was used in rat [8, 9, 10]. The study is intended to find out the effect of *Neeradaippu Thelineer* on therapeutic usage against ethylene glycol induced urolithiasis. All rats were kept in metabolic cages for entire duration of the experiment. Animals were divided into five groups. Each group contains six animals. 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 *Neeradaippu Thelineer* (*NT* - 1ml/kg body weight) and Group IV received *Neeradaippu Thelineer* (*NT* - 2ml/kg body weight) from 15th day till 28thday, Group V received standard antiurolithiatic drug, Cystone (750mg/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 *NT* (1ml/kg) + ethylene glycol
Group 4: Treated with *NT* (2ml/kg) + ethylene glycol
Group 5: Treated with Standard (ethylene glycol + Cystone)
All doses were administered once daily by oral route.

Assessment of antiurolithiatic activity:

Collection and analysis of urine:

All animals were kept in separate metabolic cages and their urine samples of 24h were collected on 28th day. Animals were allowed freely to drink water during the period of urine collection. A drop of concentrated hydrochloric acid was added to the urine before it is stored at 4°C. Urine was analyzed for calcium, phosphate and oxalate content.

Serum Analysis:

After the experimental period, animals were kept in the anesthetic condition and blood was collected by the retro-orbital puncture. After which they were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000 x g for 10 min and analyzed for creatinine, uric acid and urea nitrogen.

Kidney homogenate analysis:

The both kidneys from each animal were removed by the opening of abdomen. The extraneous tissue of both the isolated kidneys were cleaned and preserved in 10% neutral formalin. The kidneys were dried in a hot air oven at 80°C. 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 2000 x g for 10min and the supernatant was separated [11]. The calcium, phosphate and oxalate content in kidney homogenate were determined.

RESULTS AND DISCUSSION:

Various techniques were adopted for stimulation of urolithiasis, which cause acute and chronic types of hyperoxaluria. Acute hyperoxaluria is induced by the large single dose of lithogen is given to rats, while chronic hyperoxaluria is induced to the rat by continuous administration of small doses of lithogen for a period of time. In this study, chronic type of hyperoxaluria model was induced by ethylene glycol for the period of 28 days to assess the antiurolithiatic activity in albino rats.

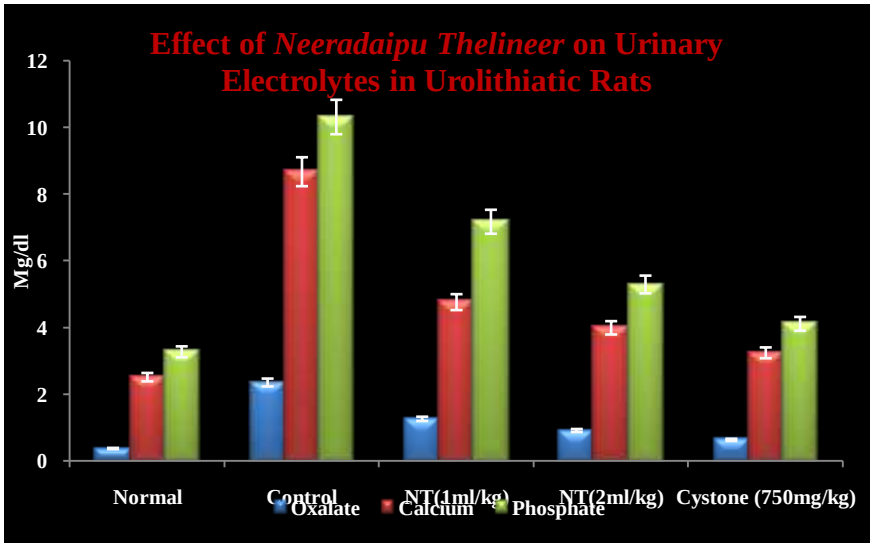
Urinary Electrolytes and Kidney Homogenate Electrolytes:

Urinary electrolyte estimation and estimation of electrolytes in kidney homogenate were summarized in Table No.1, Graph No.1 and Table No.2, Graph No.2 respectively. Chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to male albino rats presented with the formation of hyperoxaluria. In the calculi-induced (control) animals, urinary excretion of oxalate (2.36±0.09), calcium (8.68±0.34) and phosphate (10.32±0.14) were increased. The increase of urinary calcium concentration denotes the decrease of serum calcium and calcium oxalate stone formation. The level of urinary oxalate, calcium and phosphorus was decreased (1.27±0.21; 0.92±0.14, 4.77±0.19; 4.00±0.15 and 7.18±0.08; 5.30±0.10 respectively) in the trial drug treated rats (NT -1 and NT -2) which were highly significant when compared to the control group. Urinary excretion of oxalate, calcium and phosphorus were also significantly decreased in standard group as compared to the control (0.64±0.08, 3.25±0.24 and 4.12±0.12 respectively). Trial drug at the dose of 2 ml/kg showed marked response than the dose level of 1 ml/kg which represented the dose dependent activity of *Neeradaippu Thelineer*.

Table1. 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 (Saline)	0.38±0.06	2.52±0.16	3.28±0.06
2	Positive control (0.75% EG)	2.36±0.09$^{©,x}$	8.68±0.34$^{©,x}$	10.32±0.14$^{©,x}$
3	*Neeradaippu Thelineer* 1ml/kg	1.27±0.21^{c,***}	4.77±0.19^{a,***}	7.18±0.08^{a,***}
4	*Neeradaippu Thelineer* 2ml/kg	0.92±0.14***	4.00±0.15***	5.30±0.10^{a,***}
5	Standard (Cystone 750 mg/kg)	0.64±0.08$^{©,***}$	3.25±0.24$^{©,***}$	4.12±0.12$^{©,***}$

All values are expressed as mean ±S.E.M for six rats in each group.



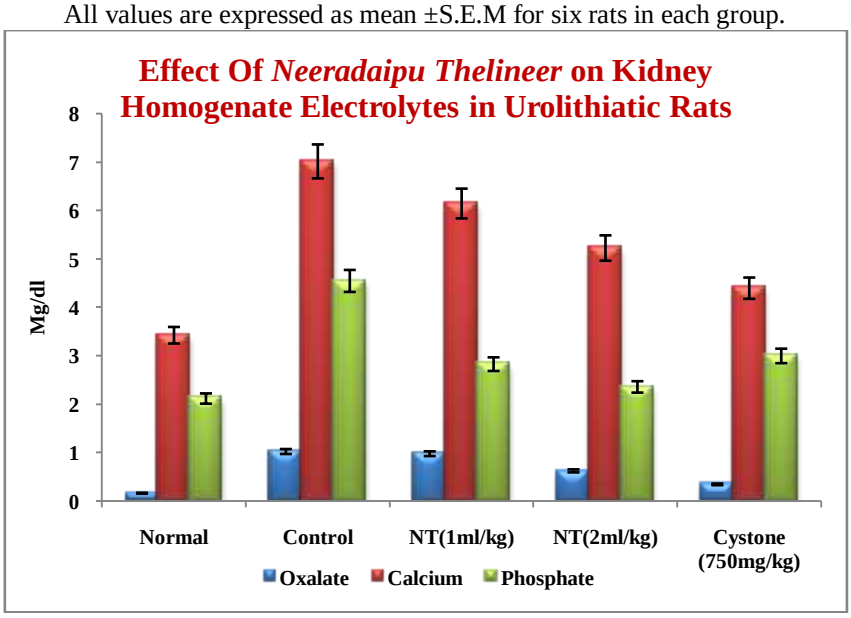
Graph No.1.Showing the effect of NT on urinary electrolytes in urolithiaitic rats.

Estimation of Kidney Homogenate Electrolytes of Normal and Urolithiatic rats also revealed the same result as those in urinary electrolyte excretion analysis. NT – 1 and NT – 2 showed significant decrease in the level of oxalate, calcium and phosphorus (0.98±0.10; 0.625±0.11, 6.15±0.20; 5.23±0.13 and 2.83±0.14; 2.36±0.09) when compared to the control group. This result also confirmed the dose dependent activity of *Neeradaippu Thelineer*.

Table2. 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)	0.165±0.06	3.428±0.34	2.12±0.08
2	Positive control (0.75% EG)	1.025±0.08$^{©,x}$	7.02±0.26$^{©,x}$	4.55±0.18$^{©,x}$
3	*Neeradaippu Thelineer* 1ml/kg	0.98±0.10^{a}	6.15±0.20^{a}	2.83±0.14***
4	*Neeradaippu Thelineer* 2ml/kg	0.625±0.11*	5.23±0.13***	2.36±0.09^{c,***}
5	Standard (Cystone 750 mg/kg)	0.346±0.08$^{©,***}$	4.40±0.18$^{©,***}$	3.00±0.12^{α,***}

All values are expressed as mean ±S.E.M for six rats in each group.



Graph No.2. Showing the effect of NT – 1 and NT – 2 on kidney homogenate electrolytes in urolithiatic rats.

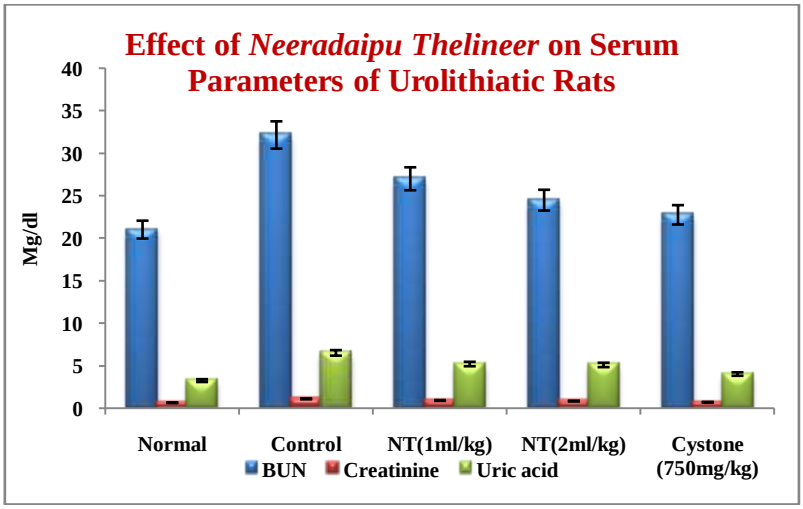
Serum Parameters

Result of serum parameters of normal and urolithiatic rats were summarized in Table No.3 and Graph No.3. When compared to the control rats the BUN level of drug treated groups (NT -1, NT -2 and standard group) were significantly decreased. Creatinine clearance of lithiatic control rats were altered (1.120±0.08), but it improved significantly (P<0.001) in standard (0.720±0.08) and *NT – 2* (0.855±0.10) treated groups (P<0.001). The serum creatinine levels were restored to normal limits after treated with *Neeradaippu Thelineer* at the dose of 2ml/kg. Uric acid level of NT – 1 and NT – 2 were slightly decreased when compared to the standard and normal group, but within the normal range.

Table3. 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)	21.02±0.24	0.646±0.05	3.26±0.08
2	Positive control (0.75% EG)	32.15±0.36$^{©,x}$	1.120±0.08^{α,z}	6.52±0.09$^{©,x}$
3	*Neeradaippu Thelineer* 1ml/kg	27.00±0.64^{a,***}	0.928±0.11	5.21±0.06^{a,***}
4	*Neeradaippu Thelineer* 2ml/kg	24.48±0.42***	0.855±0.10	5.10±0.08^{a,***}
5	Standard (Cystone 750 mg/kg)	22.76±0.38***	0.720±0.08$^{@,***}$	4.03±0.07^{α,***}

All values are expressed as mean ±S.E.M for six rats in each group



Graph No.3. Showing the effect of NT - 1 and NT – 2 on serum parameters of experimental rats.

HISTOPATHOLOGICAL STUDY OF RAT KIDNEY

Histopathological study:

Tissue samples from the kidneys of the normal group clearly revealed that tubules with single epithelial lining on histopathological studies (Fig No.1) and they were of normal in size. In lithiasis induced animals, all the tubules showed the presence of crystals, there were marked dilation of the tubules and total degeneration of epithelial lining with infiltration of inflammatory cells in to the interstitial space (Fig No.2). But examination of the kidney in standard and *Neeradaippu Thelineer* (NT – 1 and NT – 2) treated groups (Fig No.3, Fig No.4 and Fig No.5 respectively) showed characters just as the normal control group. The biochemical mechanism involved in this process is coupled with a raise in the oxalate concentration of urine. Stone formation in ethylene glycol-fed animals is caused by hyperoxaluria, which provides reason for increased renal retention and excretion of oxalate[12]. One aspect of its function is to get rid of the body's waste products that accumulate as a result of cellular metabolism, thus proving the excretory function of urinary system. The primary functions of kidneys are to maintain a stable internal environment for optimal cell and tissue metabolism. They segregate urea, mineral salts, toxins and other waste products from the blood. Urolithiasis refers to deposits forming calcifications in the urinary system, primarily effects over kidney or ureter, and may also migrate into bladder or urethra, the lower urinary system. Hyperoxaluria much prone to stone formation, which leads to increased renal retention and excretion of oxalate. Magnesium one of the inhibitors of stone formation, reduces the super saturation of calcium oxalate by reducing the saturation of calcium oxalate and the growth of calcium oxalate crystals.

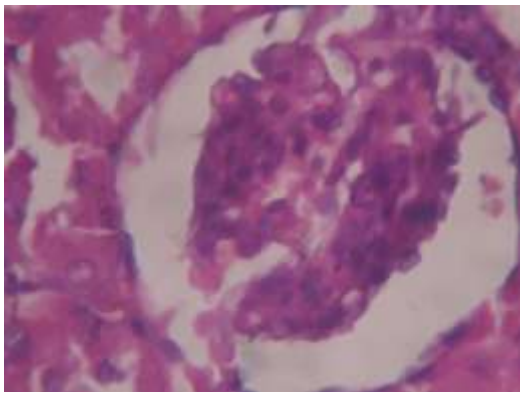


Fig No.1. Showing the normal architecture Crystals and dilatation

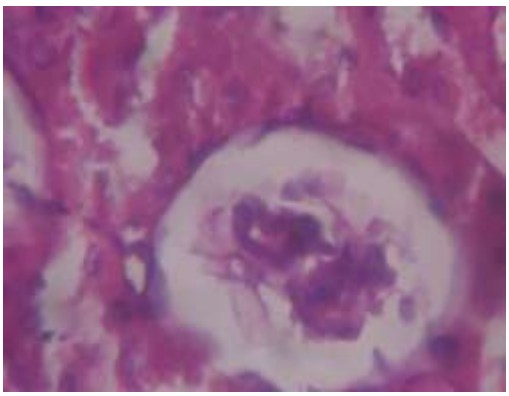


Fig No.2. Control group showing the

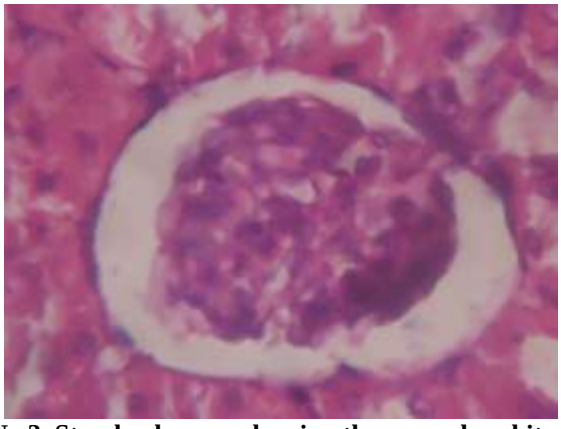


Fig No.3. Standard group showing the normal architecture

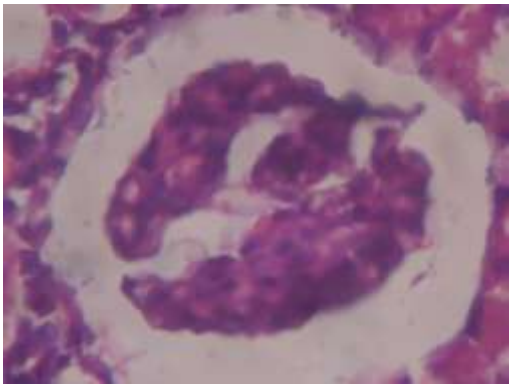


Fig No.4. NT- 1 showing the normal architecture

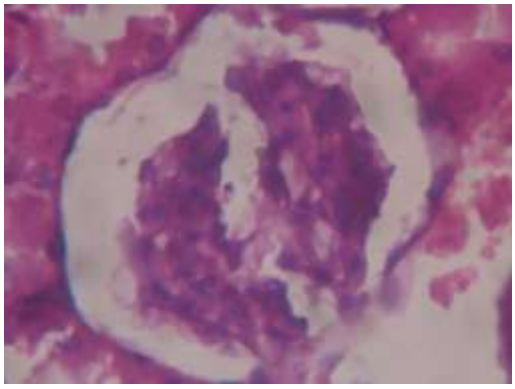


Fig No. 5. NT – 2 showing normal architecture

Conclusion:

In conclusion, the present findings confirm the effectiveness of the *Neeradaippu Thelineer* against urinary pathologies. It provides much effect which was closer to standard drug cystone for urinary calculi with dose dependent manner. *Neeradaippu Thelineer* 2ml/kg showed marked effect than *Neeradaippu Thelineer* 1ml/kg. Further researches can be done towards implementing this as one of the standard drug for urinary pathology. The mechanism underlying may be apparently related to its diuretic properties and lowering of urinary concentrations of stone-forming constituents, which may be attributed to the presence of natural active principles.

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