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Research Article

EXPERIMENTAL EVALUATION OF KADALIKANDA PANEEYA KSHARA VIŚ - Á - VIŚ ASHMARIGHNA PROPERTY

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ABSTRACT

The present study was aimed to evaluate the *Ashmarighna* (antiurolithiatic) property of *Kadalikanda Kshara* (Test drug) against ethylene glycol (1%) induced urolithiasis in Wistar albino rats. Animals were divided into five groups as six animals in each group. First group served as normal control and received regular rat food and drinking water. Ethylene glycol (1%) was given in drinking water were fed to all other groups (Group 2 – 5). 2nd group served as only lithiatic control as ethylene glycol (1%) in drinking water. 3rd group Standard drug was given for 14 days (From 15th - 28th day). 4th & 5th group received *Kadalikanda Kshara* (Test drug) at doses of 22.5mg/kg (Therapeutic Equivalent dose - TED), 90mg/kg (Therapeutic Equivalent dose×4 - TED×4) respectively. After 28 days blood & urine samples collected and analyzed for blood and urine parameters. The kidneys were removed sectioned for histopathological examination. The experimental data were expressed as Mean ± SEM of mean and analyzed by one way analysis of variance followed by Dunnett's multiple "t" test as post hoc test was considered statistically significant. The rats treated with Test drug in both TED and TED ×4 groups has shown significant effect by increasing the volume of urine and elevating pH of urine & has shown very significant reduction in serum uric acid. Histopathology of kidney sections in Group 4 & 5 showed less tissue damage. *Kadalikanda Kshara* has protective effect in urolithiasis.

Keywords: *Ashmari,* Experimental study, Histopathology, *Kadalikanda Kshara*

INTRODUCTION

Ashmari (Renal Calculi) is one of the *Mahagadha* (Chronic disease) as mentioned in the classical treatises[1, 2]. It can be correlated to Urolithiasis that has become very common. At present it is estimated that 10-15% of global population suffers from urinary calculi[3, 4]. In *Samhita* (Classics) period, *Sushruta the* father of surgery has explained urinary calculus under the heading of *Ashmari* (Renal Calculi) in detail, including etiological factors, classifications, symptomatology, pathology, complications and its management in a most scientific manner. *Charaka* has advised medical management and *Sushruta* advised both conservative and surgical removal of stone.

The etiology of this disorder is multifactorial and is strongly related to dietary lifestyle habits or practices[5]. Modern science has emphasized on various factors like hereditary, age, sex, metabolic disease, sedentary life style, hydration status, mineral contents of water, nutritional deficiency along with different theories like supersaturated solution, hyperparathyroidism, vitamin A deficiency, etc. in relation to urinary stone formation[6]. Epidemiological studies revealed that nephrolithiasis is more common in men (12%) than women (6%) and is more prevalent between the ages of 20 to 40 in both sexes[7]. Urolithiasis is complex process that results from a succession of several physicochemical events including super saturation, nucleation, growth, aggregation and retention within the kidney. Calcium oxalate is the predominant component of most stones accounting for more than 80% of stones. The remaining 20% are composed of struvite, cysteine, uric acid and other stones[8].

Various medicines are suggested in Ayurveda for the treatment of Ashmari (Calculi). *Kshara Chikitsa* (Therapy with medicated caustic alkali) is considered as *pradhanatam* (Prior Importance) in *Shastra* (Surgical) and *Anushastra* (Para surgical) [9]. There are different opinions regarding the preparation of *Kshara* (Caustic alkali). *Paneeya Kshara* (Caustic alkali for internal administration)) is one of the types of *Kshara* (Caustic alkali) that is used for internal administration. One such *Kalpana* (Dosage form) utilized successfully by our *Acharyas,* to effectively deal the calculi due to its *Chedana* (Cuts), *Bhedana* (Splits), *Lekhana* (Scraps)[10-12], *Mutrala* (Diuretic)[13] and *Tridoshaghna* (Pacifies morbid *doshas*) properties is *Paneeya Kshara* (Caustic alkali for internal administration. Various theories of *Ashmari* (Renal Calculi) formation described in *Ayurveda*. *Acharya Sushruta* said *Asamshoditasheelata* (Uncorrected system) and *Mityahara Vihara (Incompatible food & activities)* [14] which causes *Kapha Prakopa* and by *Srotovaiguṇya* leads to *Ashmari*. It is mainly due to the *Kapha-Vata Sanghata (Obstruction of morbid doshas)* [15].

Kadalikanda (Rhizome of *Musa paradisiaca* Linn.) possesses *Madhura* (Sweet), *Kashaya* rasa (Astringent taste), *Guru* (Heavy), *Ruksha* (Dry), *Sita* (Cold) *Guna* and *Kapha–Pittahara* (Pacifies *Kapha* & *Pitta dosha*) properties[16, 17]. Kadalikanda formulated into a Kshara dosage form, which possesses *Ushna* (Hot in nature)[18], *Tikshna* (Intense in nature)[19], *Pachana* (owing to digestive capability)[20], *Daarana* (Breaking)[21], *Mutrala* (Diuretic)[22], *Shodhana* (Cleansing)[23] and *Ropana* (Healing)[24] *properties*. Due to these properties breaks the Obstruction of Kapha – Vata (Morbid doshas) which is the main Doshadushya Sammurchana in the Samprapti of Ashmari formation and reducing the pain.

The present study was aimed to explore the *Ashmarighna* (antiurolithiatic) Property of *Kadalikanda paneeya kshara* (Test drug) in ethylene glycol (1%) induced calculi in Wistar albino rats.

MATERIALS AND METHODS

Test formulation

The raw drug *Kadalikanda* (Rhizome of *Musa paradisiaca* Linn.) required for the preparation of medicine was procured from Koratty, Thrissur (Dist.) Kerala, in the month of February 2016. The authentication of the raw drug was done at Department of Dravyaguna, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan (Reference no: - SDM/MD (Ayu)/01/2016). Other physical impurities were removed from stem, made into pieces and dried under sunlight for 7 days consecutively. After proper drying *Kadali Kanda* (Rhizome of *Musa paradisiaca* Linn.) was burnt to ash; to this ash 4 parts of water was added, stirred well and kept for 3 hours. After 3 hours it was macerated well with hand and filtered through a 3 folded cloth. The filtrate was heated in an iron pan to evaporate the water content. The *Kshara* (Caustic alkali) collected at the bottom of the vessel was obtained and preserved in air tight glass container, utilized for present study [25].

Experimental Animals

Wistar albino rats of either sex weighing between 150g-280g were used for the study. Animals were obtained from animal house a part of research lab, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. The experiment was conducted after obtaining the permission from the institutional ethics committee in accordance with the guide line formulated by CPCSEA (IEC No: SDM/IEC/25/2014-2015) and IAEC Approval No: (SDMCRA/IAEC/HSN/BK/01).

Thirty albino rats were selected and allotted to five groups of six rats each. Six animals were housed in each cage made up of poly - propylene with stainless steel top grill. The dry paddy husk was used as bedding material and was changed frequently to protect from infections. The rats were maintained under normal husbandry conditions and exposed to 12hr day and 12hr night and with ideal laboratory condition in terms of ambient temperature and humidity. Animals were fed with standard laboratory pellet feed and water ad libitum. Here two groups served as *Kadalikanda kshara* (Test drug) in minimum and maximum dosage levels. The Human dose of *Kshara* is 250mg – 1000mg [26] per day. So by these considerations made two groups with minimum (250mg) and maximum dosage (1000mg) levels and analyzed the study.

Considering this the animal dose of the experimental animals in both groups was calculated by extrapolating the human dose to rat dose as 22.5mg/kg & 90mg/kg respectively based on the body surface area ratio by referring to the standard table by Paget's and Barnes (1964) [27]. The human dose of standard drug was 900mg. Which was converted into animal dose as 550mg. Standard drug was finely powdered and made into suspension with adding of 55ml distilled water and administered according to 1ml/100g body weight. The test drug and standard drug was administered orally to rats with the help of rat feeding needle attached to syringe.

Lithiatic agent

Ethylene glycol (1%) was administered along with drinking water for 28 days. Daily 2ml of ethylene glycol was pipette out with 1 ml micropipette and mixed with 200 ml distilled water and kept for drinking in the cage of rats.

Experimental Methodology

Ashmari (Renal calculi) was induced by ethylene glycol (1%) in Wistar albino rats [28, 29]. Animals were divided into five groups containing six animals in each group. First group served as normal control and received regular rat food and drinking water ad libitum. Ethylene glycol (1%) was given in drinking water to second, third, fourth and fifth groups from 1st day to 28th day. Second group served as lithiatic control and given ethylene glycol (1%) from 1st day to 28th day in drinking water. To Third group Standard drug was given for 14 days (From 15th - 28th day) as standard control group. As curative regimen fourth group received test drug *Kadalikanda Kshara* (Test drug) at therapeutic equivalent dose in minimum (TED-250mg/kg – 22.5mg/kg) for 14 days (from 15th to 28th day) and fifth group received *Kadalikanda Kshara* (Test drug) in the maximum dose of TED×4 (1000mg/kg – 90mg/kg) for 28 days (From 1st to 28th day) as preventive regimen. All the drugs given once daily by oral route using gastric catheter of suitable size sleeved onto a disposable syringe. On the 28th day animals are kept in the separate metabolic cage for 24 hr for urine collection. On the 29th day urine samples were collected and kept for analysis. On the 29th day animals weighed again and anesthetized with diethyl ether and blood was collected from retro orbital plexus for estimation of serum biochemical parameters. Then animals were sacrificed by over dose of diethyl ether. The abdomen was opened by midline incision and kidney was dissected out carefully and cleaned off the extraneous tissue. Kidney was weighed and one kidney of each animal was transferred to 10% formalin solution and sent for histopathological studies.

Statistical analysis

The experimental data were expressed as Mean ± SEM (Standard Error of Mean).
The data obtained was analyzed using Graph pad In sat version 3.05 by "t" test for comparison between groups and rest of the data were analyzed by one way analysis of variance (ANNOVA) followed by Dunnett's multiple "t" test as post hoc test for determining the level of significance of the observed effects. A 'p' value of less than 0.05 was considered statistically significant.

RESULTS

Effect of *Kadalikanda Kshara* on Body weight and Relative organ weight

Ethylene glycol control group has shown non-significant decrease in percentage change in body weight gain compared to normal control group. Whereas the standard drug group and both test drug groups (*Kadalikanda Kshara* (TED)) & (TED×4)) shown non-significant increase in percentage change in body weight when compared to ethylene glycol control group. The ethylene glycol control group has shown non-significant increase in the related kidney weight as compared to normal control group. In Standard drug, both test drug groups has shown non-significant decrease in kidney weight when compared to ethylene glycol control group. (Table 1)

Effect of *Kadalikanda Kshara* on urine parameters

Ethylene glycol control group non – significantly decreased the urine volume & pH and non-significantly increased in specific gravity and calcium oxalate crystals in urine as compared to normal control group. Whereas urine volume and pH is significantly increased in both test drug groups and non – significantly decreased in specific gravity as compared to ethylene glycol control group. In case of calcium oxalate crystals in urine has significantly decreased in test drug (*Kadalikanda Kshara* (TED)) & non- significantly decreased in Test drug (*Kadalikanda Kshara* (TED×4)) group as compared to ethylene glycol control group. In standard drug group non – significant increase has shown pH and non - significantly decreased in urine volume and specific gravity. Whereas significantly decreased in Calcium oxalate crystals in urine as compared to ethylene glycol control group. (Table 2)

Effect of *Kadalikanda Kshara* on serum biochemical Parameters

Ethylene glycol control group non – significantly increased the urea & calcium level and significantly increased the creatinine and uric acid as compared to normal control group. Both test drug (*Kadalikanda Kshara* TED & TED×4) and standard drug groups has non – significantly decreased the urea, creatinine and calcium. Whereas the test drug (*Kadalikanda Kshara* TED) has non significantly decreased in uric acid, and significantly increased in test drug (*Kadalikanda Kshara* TED×4) and standard drug groups. (Table 3)

Histopathology

In positive control group administration of ethylene glycol through drinking water was found to be efficacious in inducing stone formation. Marked stone formation was observed one rat; in other rats crystal formation was mild to moderate. However, degenerative changes in the form of cell infiltration and fatty changes were observed in the majority of the rats.
Note: - Dilatation of the convoluted tubules was observed in majority of the sections. (Photomicrograph of the representative sections from this group has been provided in Figure-2 A1 & A2)

In reference standard administered group the stone formation was minimum or not seen in many of the sections.
Note: - The cell infiltration, tubular dilatation and cell degeneration were found to be only mild. (Photomicrograph of the representative sections from this group has been provided in Figure -2 B1 & B2)

In test drug TED and TED x 4 dose administered groups the stone formation was minimum or not seen in many of the sections.
Note: - The cell infiltration, tubular dilatation and cell degeneration were found to be only mild to moderate. (Photomicrograph of the representative sections from this group has been provided in Figure -2 C1, C2 and No. D1, D2)

Table 1: Effect of Kadalikanda Kshara on Body weight and Relative organ weight

Group	Body weight % change	Relative organ weight
		Kidney weight (g)
Normal control	9.64 ± 0.24	1.46 ± 0.08
Ethylene glycol control	6.04 ± 2.37	1.60 ± 0.16
Standard drug	14.06 ± 4.20	1.26 ±0.01
K.K Kshara (22.5mg/kg)	16.65 ± 5.76	1.36 ± 0.08
K.K Kshara (90mg/kg)	33.63 ± 6.27	1.52 ± 0.05

Data: MEAN ± SEM, * P<0.05, ** P<0.01 in comparison to ethylene glycol control,
@ - compared with normal control, #-compared with EG control

Table 2: Effect of Kadalikanda Kshara on Urine parameters

Group	Urine volume (ml)	Urine pH	Urine Specific gravity	Calcium oxalate crystal (Cumm)
Normal control	9 ± 1.36	9 ± 0.00	1± 0.00	3396.66 ±569.01
Ethylene glycol control	8.16 ± 1.74	7.83 ± 0.10	1.00 ± 0.00	6496.66 ± 1170.4
Standard drug	7.5 ± 1.70	8 ± 0.00	1± 0.00	1200 ± 468.73$^{\#\#}$
K.K Kshara (22.5mg/kg)	24.83 ± 7.54$^{\#}$	9 ±0.00$^{\#\#}$	1.00 ± 0.00	398.33 ± 101.57$^{\#\#}$
K.K Kshara (90mg/kg)	24.16 ± 4.90$^{\#}$	9 ± 0.00$^{\#\#}$	1.00 ± 0.00	4223.66 ± 1751.4

Data: MEAN ± SEM, * P<0.05, ** P<0.01 in comparison to ethylene glycol control,
@ - compared with normal control, #-compared with EG control

Table 3: Effect of Kadalikanda Kshara on Serum biochemical Parameters

Group	Blood urea (mg/dL)	Serum creatinine (mg/dL)	Uric acid (mg/dL)	Serum calcium (mg/dL)
Normal control	36.66 ± 1.08	0.26 ± 0.03	1.08 ± 0.07	9.06 ± 0.32
Ethylene glycol control	43 ± 9.36	0.51 ± 0.06$^{@@}$	1.59 ± 0.08$^{@@}$	10.25 ±0.36
Standard drug	32.5 ± 0.88	0.41 ± 0.04	1.53 ± 0.08	9.53 ± 0.31
K.K Kshara (22.5mg/kg)	32.83 ± 4.86	0.46 ± 0.04	1.35 ± 0.09	10.01 ± 0.40
K.K Kshara (90mg/kg)	37.83 ± 3.95	0.35 ± 0.04	1.13 ± 0.08$^{\#\#}$	10.06 ± 0.49

Data: MEAN ± SEM, * P<0.05, **P<0.01 in comparison to ethylene glycol control, @-compared with normal control, #-compared with EG control

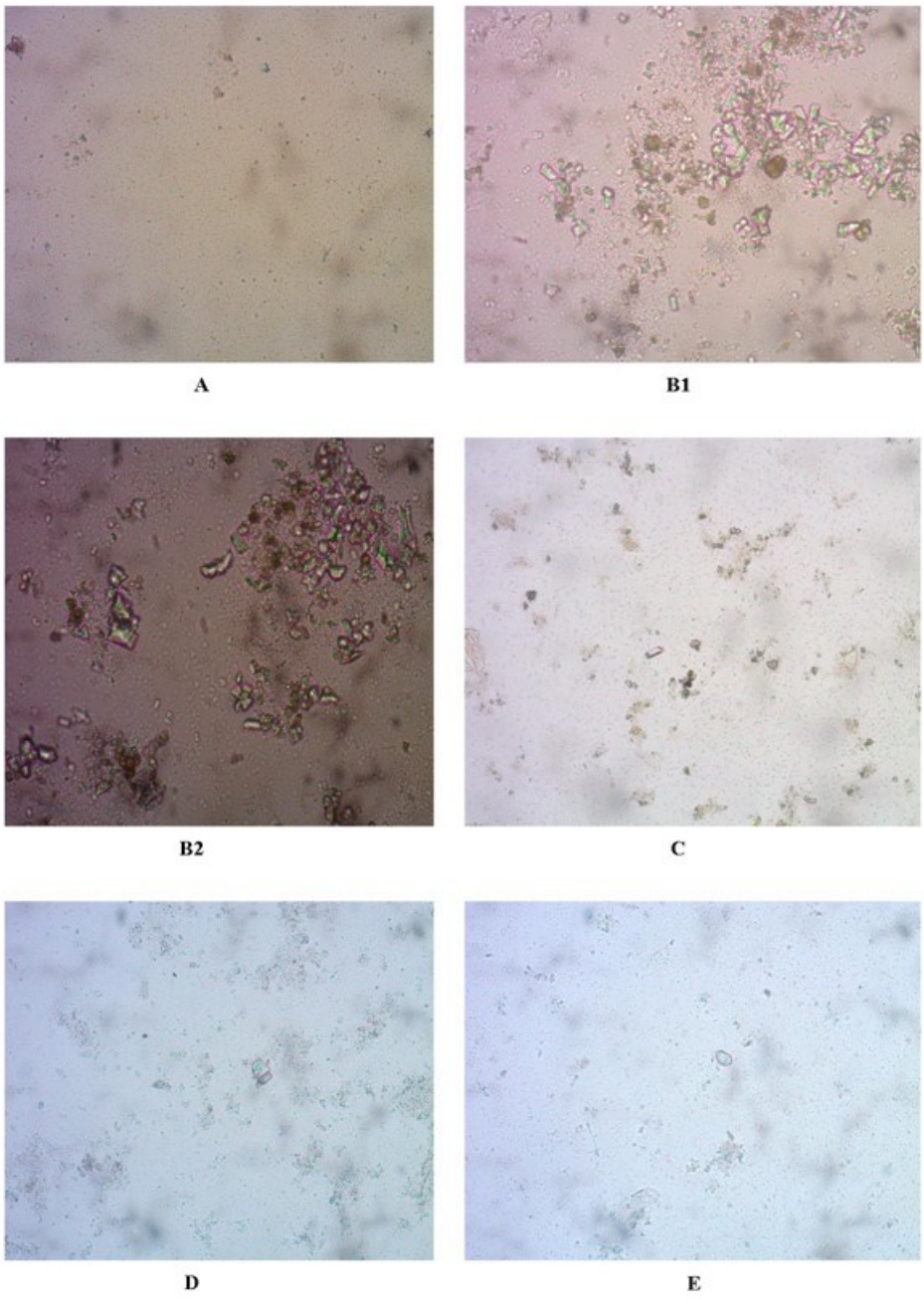

Figure 1: - Urine microscopy of presence of calcium oxalate crystals in urine
A - Normal control group; B1 & B2 - Positive control group (Ethylene glycol induced); C - Standard drug group; D - Test drugs – Kadalikanda Kshara (TED) group; E - Kadalikanda Kshara (TED×4) group.

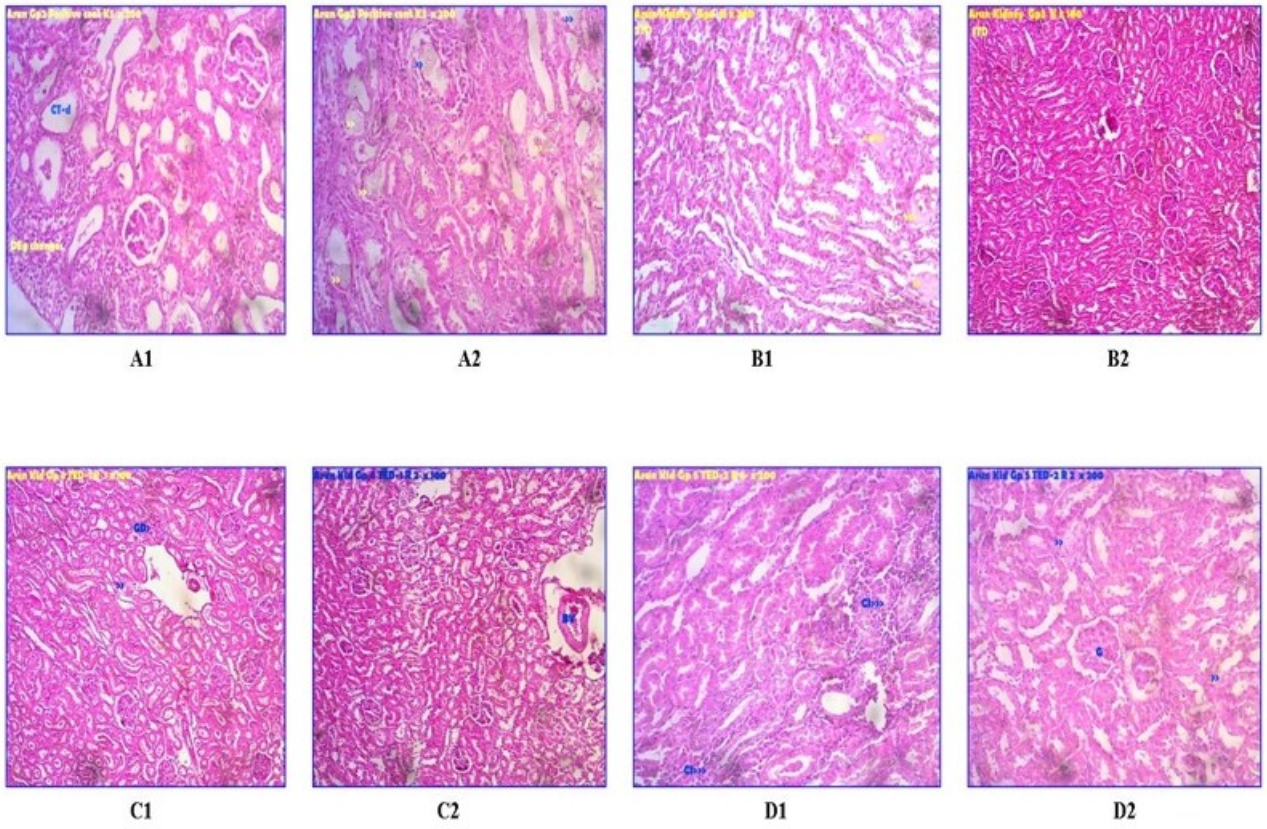

Figure 2 :- Photomicrograph of the representative sections of kidney tissue
A1 & A2 – Positive control group (Ethylene glycol induced) has shown dilatation of the convoluted tubules was observed in majority of the sections. B1 & B2 – Reference standard drug group has shown the cell infiltration, tubular dilatation and cell degeneration were found to be only mild. C1, C2 & D1, D2 – Test drugs – Kadalikanda Kshara (TED) & (TED×4) group has shown the cell infiltration, tubular dilatation and cell degeneration were found to be only mild to moderate.

DISCUSSION

Ashmarighna property against ethylene glycol induced urolithiasis in rats

An analysis show that in both minimum (TED) and maximum dose (TED ×4) of *Kadalikanda Kshara* (Test drug) possesses *Ashmarighna* activity (anti-urolithiatic activity) against ethylene glycol induced calculi. *Ashmarighna* (anti-urolithiatic) activity was found to be 93.86 % in minimum dose (Therapeutic effective dose (TED)) and 34.98 % in maximum dose (TED×4) of *Kadalikanda Kshara* (Test drug). This shows that minimum dose (TED) level showed remarkable changes compared to Maximum dose (TED×4) which possess moderate change.

Ethylene glycol produced stone by causing hyperoxaluria as well as increased activities of oxalate synthesizing enzymes of the liver i.e., glycolate oxidase (GAO), glycolate dehydrogenase (GAD), etc. and decreases colloids like mucins, GAGS, etc. which are the inhibitors of stone formation. Ethylene glycol produces oxalate load which increase the oxidative stress due to the production of reactive oxygen species. Oxidative stress causes renal damage followed by inflammation. Due to renal cell damage retention of oxalate and calcium occurs which form calcium oxalate stone. Stone formed by super saturation, nucleation growth of stone promoter substances. The effect of *Kadalikanda Kshara* (Test drug) against ethylene glycol induced urolithiasis was evaluated by employing several parameters like effect on ponderal changes like body weight, kidney weight, urinary parameters like urine volume, pH, calcium oxalate presence, serum biochemical parameters like urea, creatinine, uric acid, calcium and Histopathology of kidney.

Ethylene glycol feeding resulted in non-significant increase in weight of kidney, as well as increased renal excretion of calcium and reduction of weight was shown in the drug treated groups (*Kadalikanda Kshara*) and reference standard drug group. Ethylene glycol moderately enhanced the level of blood urea, significant increase in serum creatinine, uric acid and moderate increase in serum calcium. Significant reduction was shown in the drug treated groups and reference standard group. The calcium oxalate contents were increased in ethylene glycol group and there is attenuation in the drug treated groups as well as reference standard.

Effect on body weight

Body weight indicates health status of living beings. In the animals gain in body weight is observed which indicates normal progressive health status of the animals and when decrease is observed it is considered to indicate interference with body functions and possibility of degenerative changes. In the ethylene glycol model body weight of animals decreased which indicates toxicant induced degenerative changes in the body.

In the Standard drug group and *Kadalikanda Kshara* (Test drug) treated groups, it possessed increased body weight that indicates normal progressive health status of animals, which shows

protective effect of test formulation. This effect can be considered as moderate since it was statistically non-significant.

Effect on kidney weight

In the present study there was increase in the kidney weight in the urolithiasis control rats as compared to normal control. These observed changes in weight might be due to increased deposition of urate crystals and renal injury which results in inflammation and degenerative changes. This increase supported the results of stone deposition in kidney and also supported by histopathological study in which increased calcium oxalate stones were present in sections of kidney of this group. Further, extensive degenerative changes are also observed in kidneys of this group. These changes were significantly attenuated by both the doses of *Kadalikanda Kshara* (Test drug) which was also evidenced by cytoarchitecture of kidney from treated groups.

Effect on urine volume

Many factors affect the urine formation due to the formation of stone in the kidney. Among them, are the altered glomerular filtration, tubular re-absorption and excretion. In the present study there was significant increase in the urine volume in *Kadalikanda Kshara* (TED) (204.28 %) and *Kadalikanda Kshara* (TED×4) (196.07 %) groups when compared to Ethylene glycol induced group. Non- significant decrease was found in standard group (8.08 %). *Kadalikanda Kshara* (Test drug) possess diuretic property due to the presence of sodium, potassium salts which increases the formation and volume of urine which helps in flushing the urinary tract and results in the reduction of super saturation of crystals forming salts and also help in the expulsion of already formed crystals. Thus, indicating that the test drug is having diuretic activity and reduction in stone formation.

Effect on urine pH

Urinary pH influences the formation and persistence of several types of crystals. Therefore, it is often useful to consider pH when interpreting crystalluria. Crystalluria is pH dependent, thus by changing urinary pH, dissolution of calculi can be attained. In general magnesium ammonium phosphate, calcium phosphate is associated with alkaline urine, whereas calcium oxalate, uric acid, ammonium urate, cysteine uroliths tends to be associated with acidic urine. In the present study, there was significant increase in urinary pH in *Kadalikanda Kshara* (Test drug) in both TED and TED×4 (14.94 %) dose level groups compared to ethylene glycol induced group. Standard drug shows non - significant increase in urinary pH (2.17 %). *Kadalikanda kshara* (Test drug) is a strong alkaliser as it changes the pH of urine this, helps in lowering the saturation of urine and helps in dissolution of the calculi.

Effect on Specific gravity of urine

Specific gravity of urine is an indication of the kidney's ability to reabsorb water and chemicals from glomerular filtrate. Specific gravity of urine depends upon the person's state of hydration, the integrity of the posterior pituitary and the renal tubules. One of the main roles of the kidney in humans and other mammals is to aid in the clearance of various water-soluble molecules, including toxins, toxicants, and metabolic waste. The body excretes some of these waste molecules via urination, and the role of the kidney is to concentrate the urine, such that waste molecules can be excreted with minimal loss of water and nutrients. The concentration of the excreted molecules determines the urine's specific gravity. This related to hypersthenuria and hyposthesuria determines the increase and decrease of solute concentration in urine. In the present study there was decrease in urine specific gravity level in standard drug Standard drug group (0.29%), *Kadalikanda Kshara* (TED) (0.19%) and *Kadalikanda Kshara* (TED×4) (0.29%) when compared to the ethylene glycol control group, the observed decrease was found to be statistically non significant.

Effect on Calcium oxalate crystal in urine

In the present study, calcium oxalate excretion progressively increased in calculi- induced animals. Since it is accepted that hyperoxaluria, is a far more risk factor in the pathogenesis of renal stones than hypercalciuria. Increased urinary calcium is a factor favoring the nucleation and precipitation of calcium oxalate from urine and subsequent crystal growth. Kidney stones are partially or entirely of the calcium oxalate type. They form when urine has been persistently acidic. Calcium oxalate exists in monohydrate and dihydrate forms, which can be distinguished by the shape of the respective crystals. Here the presence of calcium oxalate crystals was analyzed through urine microscopy in different fields. In this study shows that *Kadalikanda Kshara* (Test drug) (TED) (93.86 %) and Standard drug group (81.52 %) possess significant decrease in calcium oxalate crystals compared to ethylene glycol induced group. The *Kadalikanda Kshara* (Test drug) (TED×4) group (34.98%) also shows decrease in crystals compared to ethylene g**l**ycol compared group. But it is statistically non - significant. This may be due to the physiological changes in the body of rats. (Figure 1)

Effect on blood urea, serum creatinine, uric acid

Blood urea nitrogen is derived in the liver from proteins/amino acids from diet or tissue sources and is normally excreted in the urine. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance. Creatinine, on the other hand is mostly derived from endogenous sources by tissue creatinine breakdown. The plasma creatinine concentrations in normal individuals are usually affected by a number of factors such as the muscle mass, high protein diet, and catabolic state. Urea, uric acid and creatinine are the waste products which are excreted through urine but due to the presence of stones, there is an obstruction to the out flow of urine in urinary system and because of this reason the glomerular filtration rate (GFR) also decreases. Reduction in the GFR leads to accumulation of the waste products, particularly nitrogenous substances such as urea, creatinine, and uric acid in blood.

In the present study, it is observed that there was decrease in levels of urea, creatinine compared to ethylene glycol induced group. But statistically the observed effect is non- significant.

There was decrease in urea levels in *Kadalikanda Kshara* (Test drug) (TED) (23.65%), *Kadalikanda Kshara* (Test drug) (TED×4) (12.02%) and standard groups (24.41%) when compared to ethylene glycol induced group. In case of creatinine levels non -significant decrease was found in *Kadalikanda Kshara* (Test drug) (TED) (9.80%), *Kadalikanda Kshara* (Test drug) (TED×4) (31.37%) groups and Standard drug group (19.60%) when compared to ethylene glycol induced group.

Uric acid levels show significant decrease in *Kadalikanda Kshara* (Test drug) (TED×4) group (28.93%) when compared to ethylene glycol induced group. Non - significant decrease was

found in *Kadalikanda Kshara* (Test drug) (TED) group (15.09%) and Standard drug group (3.77%).

The above effect on urea, creatinine and uric acid parameters are indicative of presence of protective effect of test drug against toxicant.

Effect on serum calcium

Calcium is present in the extracellular and intracellular compartments. In the kidney 90% of the calcium is reabsorbed from the glomerular filtrate. However, the regulation of calcium balance is achieved at the distal convoluted tubules. Due to alteration of renal function, calcium absorbed by tubules increase the serum calcium level which indicates the state of hypercalcemia and renal calculi. In the present study, it is observed that there is decrease in calcium levels of both drug treated groups with *Kadalikanda Kshara* (Test drug) (TED) (2.34%) and *Kadalikanda Kshara* (Test drug) (TED×4) (1.85%) and Standard drug administered group (7.02%) when compared to ethylene glycol induced group. But the observed decrease was statistically non- significant.

Histopathology

Histopathological examination of the kidney sections showed marked reversal of the ethylene glycol induced crystal formation and degenerative changes in the reference standard and test drug administered groups indicating that the *Kadalikanda Kshara* (Test drug) has significant potential for the treatment of urolithiasis.

Probable Mode of Action of *Kadalikanda Kshara*

Various theories of *Ashmari* (Renal Calculi) formation are described in *Ayurveda*. *Acharya Sushruta* said *Asamshoditasheelata* (uncorrected system) and *Mityahara Vihara* (incompatible food & activities) which causes increase of *Kapha dosha* and by *Srotovaiguṇya* (derangement of channels) lead to *Ashmari* (Calculi). It is mainly due to the obstruction of *Kapha - Vata dosha. Kadalikanda* (Rhizome of *Musa paradisiaca* Linn.) possess *Madhura* (Sweet), *Kashaya* rasa (Astringent taste), *Guru* (heavy), *Ruksha* (Dry), *Sita* (Cold) *Guna* and *Kapha–Pittahara* (Pacifies *Kapha* & *Pitta dosha*) properties. In *Ashmari*, the drug should possess *Ushna* (hot nature), *Tikshna* (Intense) *gunas* which lead to break up the obstruction of *Kapha - Vata. So Kadalikanda* (Rhizome of *Musa paradisiaca* Linn.) was formulated into *Kshara* (Caustic alkali) dosage form. *Kshara* (caustic alkali) is considered as the *pradhanatam* (Superior) and *Sreshta* (Best) in *Shastra* (surgical procedures) and *Anushastra* (Para surgical procedures) due to its *Chedana* (Cuts), *Bhedana* (Splits), *Lekhana* (Scraps), *Mutrala* (Diuretic) and *Tridoshaghna* (Pacifies morbid *doshas*) properties. *Kadalikanda Kshara* (Test drug) has *Ushna* (Hot in nature), *Tikshna* (Intense in nature), *Pachana* (owing to digestive capability), *Daarana* (Breaking), *Mutrala* (Diuretic), *Shodhana* (Cleansing) and *Ropana* (Healing) *properties*. *Kadalikanda Kshara* (Test drug) breaks the *Kaphavata dosha* obstruction which is the main *Doshadushya Sammurchana* (localization of morbid doshas) in the *Samprapti*(Pathogenesis) of *Ashmari* (Renal Calculi) formation and reducing the pain. The *Pachana* (Owing to digestive capability) and *Daarana gunas* (Breaking) of the drugs helps in breaking the *sanghata* (Obstruction) of *Ashmari* (Renal Calculi) and helps in dissolution and disintegration of stone, i.e., urolithiatic property. The purificatory and diuretic properties help to expel out the stones from urinary tract and reduce the burning micturition, i.e., diuretic property. The *Ropana* (Healing) property of drug helps in reducing the hematuria by healing property.

According to modern science various risk factors have been identified for stone formations and these includes hot climate, vitamin A deficiency, excessive administration of vitamin D, metabolic disorders, hyperthyroidism, gout, idiopathic hypercalciuria, acid urea, family history of stone, geographic area, dietary factors rich with calcium like red meat, fish, cereals and pulses, fluoride rich water and recurrent urinary tract infections also plays an important role as a risk factors. *Kadalikanda Kshara* (Test drug) is alkaline in nature. So it is strong alkali as it changes the pH of urine this helps in lowering the saturation of urine and helps in dissolution of the calculi. Due to the diuretic property it increases the formation and volume of urine which helps in flushing the urinary tract and reduces the chances of deposition and increase in size of stone. *Kadalikanda Kshara* (Test drug) contains potassium which lowers the level of phosphate and carbonate in urine which are the causative factors in the formation of oxalate and phosphate stones. The high concentration of uric acid in urine favors the formation of calcium oxalate and uric acid stones. As an alkaliser the drug reduces the level of uric acid and formation of stone.

Kadalikanda Kshara (Test drug) is prepared from single herbal plant of *Kadali* which is available easily and preparation of *Kadalikanda Kshara* (Test drug) is a simple procedure.

CONCLUSION

Administration of ethylene glycol through drinking water was found to be efficacious in inducing stone formation. Both doses (TED & TED*4) produced significant diuretic effect by increasing the urine volume and elevating pH. The higher dose (TED*4) of *Kadalikanda Kshara* (Test drug) has shown significant uric acid lowering effect. Presence of Calcium oxalate crystals was analyzed and resulted that in TED shows 93.86 % expulsion than TED*4 (34 %) - indicating that in TED gives significant result for Calcium oxalate expulsion. Histopathological examination also reveals that the kidney sections showed marked reversal of the ethylene glycol induced crystal formation and degenerative changes in the test drug administered groups indicating that the *Kadalikanda Kshara* (Test drug) has significant potential for the treatment of urolithiasis. These can be considered the beneficial attributes in the test formulation that can be taken up for treatment. *Kadalikanda* (Rhizome of *Musa paradisiaca* Linn.) based preparation can be considered for preventive purpose in people vulnerable to renal stone formation. Thus the study provides evidence to the fact that there is sound basis for the use of this formulation for the treatment of renal stones.

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