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PHARMACOLOGICAL SCREENING OF *MUSA PARADISICA* LINN AGAINST ETHYLENE GLYCOL INDUCED RENAL CALCULI

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ABSTRACT
The effect of ethanol extract of dried roots of *Musa paradisica* Linn against ethylene glycol induced renal calculi in albino wistar rats are studied in this research. A renal calculus was induced in rats by ingesting 0.75% ethylene glycol in drinking water for 28 days and was manifested by high urinary calcium, oxalate, and low urinary magnesium contents. Simultaneous administration of 1ml (1 in 10) *Musa paradisica* Linn orally for 28 days along with ethylene glycol (0.75% v/v) reduced urinary calcium, oxalate and elevated urinary magnesium level. It also increased urinary volume thereby reducing the tendency for crystallization. The histopathological studies confirmed the induction as degenerated glomeruli, necrotic tubule and inflammatory cells was observed in section of kidney from animals treated with ethylene glycol. This was reduced; however after treatment with *Musa paradisica* Linn. These observations enable to conclude that *Musa paradisica* Linn is effective against ethylene glycol induced renal calculi
Key words: Renal calculi, *Musa paradisica* Linn, Ethylene Glycol, Calcium Oxalate Crystals.

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INTRODUCTION

A renal calculus is a recurrent disorder prominent in males than females. The present day medical management of renal calculi is either costly or not without side effects. Hence, the search for antilithiatic drugs from natural sources has assumed greater importance[1]. Many Indian plants have been quoted to be useful as antilithiatic agents[2]. They are effective with fewer side effects and are also inexpensive. Hence the Indian plants are constantly being evaluated for possible antilithiatic effects in a systemic manner[3]. One such plant is *Musa paradisica* Linn belonging to family Musaceae, which is used in some of the Intestinal Disorder[4], Constipation, Diarrhea, Arthritis, Gout, and Anemia[5]. The results of ethanol extracts detected a protein, amino acids, sugars, organic acids saponins and other substances, alcohol was detected in extracts. The present study was designed to investigate the antilithiatic activity of *Musa paradisica* Linn in ethylene glycol induced renal calculi.

MATERIALS AND METHODS

Male albino Wistar rats (200-220 gm) were obtained from National institute of bioscience, pune, MS, India). They were housed in well-ventilated cages, maintained at 25 ± 2^{0}C and 12 hour dark / light cycle. They were fed standard pellet diet and had free access to water. The animals were maintained, in these conditions for one week before the experimental session. Our institutional animal ethical committee (IAEC) approved this study. The *Musa paradisica* Linn was collected from wadagoan Maval, Pune, M.S, India.

Antilithiatic activity

The acclimatized animals were divided into three groups of six animals each designated as G-I, G-II and G-III. The animals of G-I served as the normal control. The G-II animals received 0.75% ethylene glycol in drinking water *ad libitum* for 28 days and served as the lithiatic control. The G-III group animals received 0.75% Ethylene glycol in drinking water *ad libitum*, along with *Musa paradisica* Linn 1 mL (1 in 10) by oral route for 28 days. The 24 hour urine samples were collected from rats housed in metabolic cages on 14th and 28th days and the volume noted. Urinary calcium, oxalate and magnesium concentration were estimated using standard methods. Also, the serum and urine creatinine levels were estimated. To confirm the incidence of Renal calculi, the animals were sacrificed and there kidney were subjected to histopathological studies[7].

Statistical Analysis

The results are expressed as mean ±SEM. Statistical analysis was carried out using one way ANOVA, followed by Newman-keuls multiple range test. Differences below P< 0.05 implied significance.

RESULTS

Urinary Parameter

The urinary excretion was increased significantly on the 14th day in ethylene glycol treated rats (G-II) compared with normal control rats (G-I). Maximum oxalate excretion was observed with G-II on 28th day (29.51±1.25mg/24 hr per rat). However the oxalate excretion was reduced significantly (22.45±1.46 mg/24 hr per rat) in the EEMP treated group (G-III), though normal values were not reached. The results are shown in table 1 & 2.Likewise, ethylene glycol treatment increased urinary calcium (9.95±0.24 mg/24 hr per rat) excretion significantly in lithiatic control group (G-II) on the 28th day. However after treatment with EEMP, these values were reduced to 6.89±0.34 mg/24 hr per rat in G-III. The magnesium excretion on the 28th day was reduced after treatment with ethylene glycol in G-II (0.921±0.076mg/24 hr per rat). Simultaneously, administration of extract to G-III, elevated the reduced magnesium level significantly (2.62± 0.35 mg/24 hr per rat), when compared with the lithiatic control group (G-II).

Histopathological Studies

Kidney Sections were treated with ethylene glycol showed marked dilation of tubules, tubular damage and infiltration of inflammatory cells into the interstitial space. However kidney sections of animals treated with EEMP showed improvement of the above symptoms and reduced crystal deposition as shown in fig 1, 2 and 3.

DISCUSSION

Renal calculi is the formation of stones in the urinary tract, causing pain and bleeding, and may lead to secondary infection. It is the third most common affliction of the urinary tract. Of many types of stones that are formed, the most common are calcium oxalate. Calcium oxalate stone disease is the most common human urinary stone disease in the Western Hemisphere. To understand different aspects of the disease, calcium oxalate renal calculi in the rat is used as a model. Spontaneous calcium oxalate renal calculi are very rare in rats. Thus the disease is experimentally induced and the rats are generally made hyperoxaluric either by administration of excess oxalate, exposure to the toxin ethylene glycol, or various nutritional manipulations. All the experimental models show renal injury associated with crystal deposition. One of the important phenomena that characterize renal calculi is its high recurrence. Thus, a protective system is required including extracorporeal shock wave lithotripsy and medicament treatment. Unfortunately, these means remain costly and in most cases are invasive and with side effects. Therefore, it is worthwhile to look for an alternative to these conventional methods by using medicinal plants or phytotherapy. Therefore, it is highly recommended to explore new drugs coming from medicinal plants to treat and prevent the formation of kidney stones. Ideally, conventional and phytotherapy should supplement one another and have all the need available for renal calculi patients [8-11].The present study showed ethylene glycol can induce stone formation. In accord with this experiment, urinary calcium and urinary oxalate excretion were significantly higher in group fed with EG (G-II), than those in the control group (G-I) and other treated group (G-III). Changes in ionic pattern of urine are the major determinant of stone formation. In this study, the ionic pattern was found disturbed by treatment with EG. It has been reported that daily oral administration of EG for more than four weeks resulted in a significant increases in oxalate excretion and that the kidneys are the targets for the EG toxicities which gets oxidized to oxalic acid leading to hyperoxaluria[12-13]. Hyperoxaluria is reported to be a more significant risk factor in the pathogenesis of stone formation. Likewise ethylene glycol administration increased the urinary calcium level. It has been stated that hyperoxaluria favors precipitation of calcium oxalate from urine. Thus the high oxalate and calcium ion concentration in urine tend to form calcium oxalate crystals. The growth of calcium oxalate crystals is further favored by disturbances in the urinary levels of other ions like magnesium and phosphate. The available literature states that, high urinary calcium which induces further deposition of calcium oxalate on it [16]. Magnesium is considered as a potent inhibitor of calcium oxalate crystallization in-vitro, and binds to oxalate to form a soluble complex, consequently reducing the concentration available for calcium oxalate precipitation [17-18]. Our study also revealed a similar observation. Thus, ethylene glycol administration induces stone formation by raising urinary calcium, and oxalate, and by lowering magnesium as noted in G-II[19]. The increase in urine volume may also minimize the tendency for crystallization. It was found that kidney function was impaired in the group of animals treated with ethylene glycol alone: however in the group treated with ethylene glycol and ethanol extract of dried roots of *Musa paradisica* Linn, the kidney function was found to improve. Thus it has been concluded that ethanol extract

of dried roots of *Musa paradisica* Linn has inhibitory potential on ethylene glycol induced renal calculi.

CONCLUSION

Biochemical analysis showed that the rats treated with EG alone had higher amounts of calcium in the kidneys compared to negative control rats. This EG induced increase in kidney calcium levels was inhibited by the administration of ethanol extract of dried roots of *Musa paradisica* Linn. Histology showed that rats treated with EG alone had large deposits of calcium oxalate crystals in all parts of the kidney, and that such deposits were not present in rats also treated with *Musa paradisica* Linn. These data suggest that ethanol extract of dried roots of *Musa paradisica* Linn has a protective activity against renal calculi.

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Table 1: Effect of ethanolic extract of dried roots of *Musa paradisica* Linn on urinary biochemical parameters on the 14th day.

Groups	Calcium (mg/dl)	Oxalate (mg/dl)	Protein (mg/dl)	Magnesium (mg/dl)	Creatinine (mg/dl)
Control (G-I)	5.74±0.33	16.29±1.90	3.21±1.005	1.75±0.175	55.19±4.95
Lithiatic control (G-II)	7.90±0.35▪▪▪	22.59±1.09▪▪▪	5.55±0.88▪	1.11±0.070▪▪▪	32.39±0.58▪▪
Treatment with EEMP (G-III)	6.78±0.48*	13.48±0.66***	1.90±0.27**	1.80±0.129**	38.89±2.32NS

Values are expressed as mg/dl/24 hr urine sample. Values are expressed as mean ± SEM for six animals in each group. Newman-Keuls multiple range test ($p < 0.05$) was used.
▪▪▪ Values are significantly different from normal control (G-I), $p<0.001$.
▪▪ Values are significantly different from normal control (G-I), $p<0.01$.
▪ Values are significantly different from normal control (G-I), $p<0.05$.
*** Values are significantly different from lithiatic control (G-II), $p<0.001$.
** Values are significantly different from lithiatic control (G-II), $p<0.01$.
* Values are significantly different from lithiatic control (G-I), $p<0.05$.

Table 2: Effect of ethanolic extract of dried roots of Musa paradisica Linn on urinary biochemical parameters on the 28th day.

Groups	Calcium (mg/dl)	Oxalate (mg/dl)	Protein (mg/dl)	Magnesium (mg/dl)	Creatinine (mg/dl)
Control (G-I)	7.12±0.63	16.82±0.81	4.25±0.79	3.26±0.23	53.69±5.29
Lithiatic control (G-II)	9.95±0.24▪▪▪	29.51±1.25▪▪▪	11±1.16▪▪▪	0.921±0.076▪▪▪	4.56±0.30▪▪▪
Treatment with EEMP (G-III)	6.89±0.34***	22.45±1.46**	13±1.88	2.5±0.14***	12.98±3.52NS

Values are expressed as mg/24 hr urine sample. Values are expressed as mean ± SEM for six animals in each group. Newman-Keuls multiple range test ($P < 0.05$) was used.
▪▪▪ Values are significantly different from normal control (G-I), $p<0.001$
*** Values are significantly different from lithiatic control (G-II), $p<0.001$
** Values are significantly different from lithiatic control (G-II), $p<0.01$

Histopathology of kidney

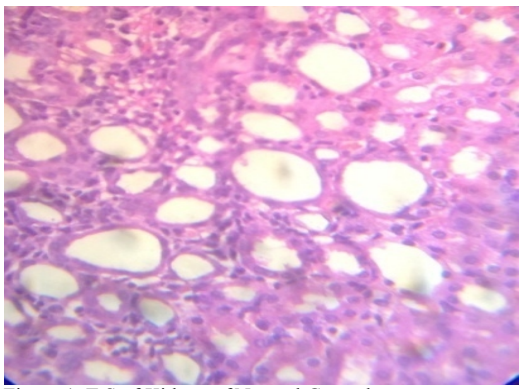

Figure 1. T.S of Kidney of Normal Control group

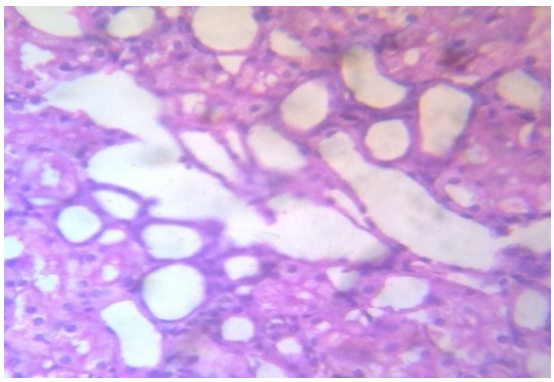

Figure 2.T.S of Kidney of Lithiatic Control group

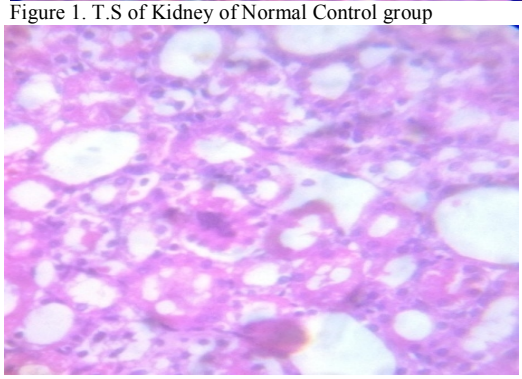

Figure 3. T.S of Kidney of EEG treated group

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