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ANTIUROLITHIATIC ACTIVITY OF THE PLANT EXTRACTS OF *Peperomia tetraphylla* ON ETHYLENE GLYCOL INDUCED UROLITHIASIS IN RATS

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ABSTRACT

To study the antiurolithiatic activity of *Peperomia tetraphylla* (Piperaceae) in ethylene glycol induced urolithiasis in male Wister rats. The urolithiasis was induced in rats by oral feeding of ethylene glycolated water (0.75%v/v) for 28 days. Ethanolic extract of *Peperomia tetraphylla (PT)* (200 mg/kg, 400 mg/kg) was administered orally from 1st day for preventive regimen and from 15th day for curative regimen. It was observed that the inducing agent ethylene glycol elevated the ionic parameters, calcium and phosphate levels in urine, blood urea nitrogen (BUN), serum creatinine and serum uric acid levels. Treatment with ethanolic extract of *Peperomia tetraphylla* significantly ($P<0.001$) reduced the elevated levels of ions in urine as well as BUN, serum creatinine and serum uric acid levels. The elevated calcium and phosphate levels in urine, serum creatinine, blood urea nitrogen (BUN) and uric acid levels of urolithiasis induced rats were reduced with preventive and curative regimens of plant extract treatment. The histological findings also showed improvement in kidney architecture after treatment with the plant extract. These observations enable us to conclude that the *Peperomia tetraphylla* has curative and preventive properties for ethylene glycol induced urolithiasis in rats.

Keywords: Urolithiasis, Ethylene glycol, *Peperomia tetraphylla*, Hyperoxaluria, Micro crystals.

INTRODUCTION

Urinary stone disease is a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 70-81% in males, and 47-60% in females. Occurrence of urolithiasis requires formation of nidus, its reaction and growth in the urinary tract which cause obstruction of the ureter[1]. Urolithiasis is a complex process which is a consequence of an imbalance between promoters and inhibitors in the kidneys[2].

Peperomia tetraphylla belonging to Piperaceae family is commonly known as Ala ala wai nu Kani and Tamil name is vanabhrami.[3] *Peperomia tetraphylla* is a perennial shrub that is thought to have originated in Africa and is used as a medicinal plant to treat a wide range of disorders. The plant *Peperomia tetraphylla* (G.Forst) Hook & Arn**.** has been claimed to possess various medicinal properties. A juice of the whole plant is employed in treatment of convulsions, skin diseases, cough, asthma like symptoms and kidney disorders.[4,5]

From the ethanomedical information and folk claims it was observed that the plant *Peperomia tetraphylla* has medicinal properties related to urolithiasis and convulsant which has not been scientifically validated and only some of the phytochemical studies have been carried out and reported for the presence of Bio active compounds, Prenylated quinones, Piperogalins.[6-8]

EXPERIMENTAL

Collection and authentication of plant material

The plant specimen for the proposed study were collected from Trichy in the month of July 2010, the plant material was identified and authenticated by Dr. P. Jayaraman, Plant Anatomy Research Centre, Pharmacognosy Institute, Chennai.[9]

Extraction of Plant Material

500 g of the powdered material was mixed with absolute Methanol (2.5 ltr) and left for 72 h. The mixture was stirred at 6 h intervals using a sterile glass rod, the extract were passed through a filter paper. The filtrates were concentrated with a vacuum pump at 40°C, giving a yield of 3.78%, which was stored in universal bottles and refrigerated at 4°C prior to use. Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, saponins and glycosides were carried out on the plant powdered using standard procedures.[10-14]

Animal selection

Albino rats of Wistar strain, of either sex, aged around 2 to 3 months and weighing 150-200 g were used. They were housed in standard conditions of temperature (25 ± 2°C), relative humidity of 45-55%, and maintained on 12–hour light: 12–hour dark cycle in animal house. Experiments were conducted in accordance with internationally accepted standard guidelines for the use of animals. Animals were fed ad libitum on normal commercial chow and had free access to water.

Acute toxicity studies

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (No.265/CPCSEA). One- tenth of the median lethal dose (LD50) was taken as an effective dose.

Ethylene glycol induced urolithiasis model

Ethylene glycol induced hyperoxaluria model[13,15] was used to assess the antilithiatic activity in albino rats. Animals divided into seven groups containing six animals in each group. Group I serves as control and received regular rat food and drinking water ad libitum. Group II receive as a standard drug cystone, Ethylene glycol (0.75%) in drinking water was fed to Group III for induction of renal calculi till 28th day. Group III and IV received PT methanolic and chloroform extracts of (200mg/kg body weight), PT of (400mg/kg body weight), and Group V received as a positive control. All extracts were given once daily by oral route.

Assessment of Antiurolithiatic activity

Collection of urine analysis all animals were kept in individual metabolic cages and urine samples of 24 hr were collected on 15th day and 28th day. Animals had free access to drink water during the urine collection period. A drop of concentrated hydrochloride acid is added to the urine before being stored at 4ºC. Urine was analysed for calcium, phosphate and oxalate content.[16-18]

Serum analysis

After the experimental period, blood was collected from the retro-orbital under anaesthetic conditions and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000×g for 10 min and analysed for creatinine, uric acid and urea nitrogen.[19,20]

Kidney homogenate analysis

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 80ºC in a hot air oven.

A sample of 100mg of the dried kidney was boiled in 10ml of 1N hydrochloric acid for 30min and homogenized.[21-24,26]

Statistical Analysis

All the values are expressed as mean ± SEM. The data were statistically analysed by One-way ANOVA followed Tukey multiple comparison test. P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Urinary excretion of calcium and phosphorous

In the present study, chronic induction of EG (0.75% v/v) to male Sprague dawley rats resulted in significant (P<0.001) increase in urinary excretion of calcium and phosphate. Whereas the cystone-treated group II animals were shown significant reduction in calcium (P<0.0001) and phosphate (P<0.001) levels. Similarly treatment with *Peperomia tetraphylla* significantly lowered the elevated levels of calcium (P<0.0001) and phosphate (P<0.0001) in urine curative regimens and preventive regimen as compared to EG induced group I animals (Table-1).

Serum analysis

The blood urea nitrogen (BUN), serum creatinine and serum uric acid levels were significantly (P<0.0001) increased in calculi-induced animals. While the BUN, serum creatinine and serum uric acid levels were significantly (P<0.001) decreased in cystone-treated group II animals. However, the BUN and serum creatinine levels were significantly (P<0.0001) decreased in both preventive and curative regimen groups. It is also found that there is no significant reduction in serum uric acid levels in curative regimens (Group IV and Group V), but the preventive regimens shows a significant (P<0.0001) reduction in elevated serum uric acid levels (Table-1).

Kidney Weights and Urinary Volume

The kidney weight was significantly (P<0.001) increased in ethylene glycol induced group –I animals when compare to the control group animals. Whereas the standard treated group animals was shown significant (P<0.001) reduction in the kidney weight when compared to the EG induced group I animals. Similarly a significant (P<0.0001) decrease in the kidney weight is also identified in *Peperomia tetraphylla* treated preventive and curative regimens (Table-2). The urinary volume was significantly (P<0.001) decreased in ethylene glycol induced group-I animals when compared to the control group animals. Whereas the standard treated group animals was shown significant (P<0.01) increase in urine output when compared to the EG induced group I animals. Similarly a significantly increase the urinary volume is also identified in *Peperomia tetraphylla* treated preventive regimen (P<0.0001) and curative regimen (P<0.05) animals (Table-2).

Histopathological studies

Histopathological studies of kidneys clearly revealed that the tissue section of Group I rats showing normal size tubules with single epithelial lining along the margin. Whereas the Group I rats showed dilated tubules and degeneration of epithelial lining with presence of crystals. But the kidney specimen from standard and extract treated groups showed characters similar normal control group (Figure-1).

Table-1: Effect of *Peperomia tetraphylla* extracts on urinary and Serum biochemical data and experimental urolithiasis

S.No.	Groups	Calcium (mg/dl)	Phosphorous (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
1	Normal (Vehicle)	6.45±0.06	8.21±0.04	0.35±0.01	3.15±0.05
2	Standard (cystone)	7.07±	7.31±0.06	0.32±0.02	27.68±0.03
3	Methanolic extract of PT 200mg	7.95±0.08	8.35±0.09	0.45±0.01	24.25±0.07

4	Methanolic extract of PT 400mg	7.02±0.076	8.65±0.07	0.44±0.01	20.90±0.05
5	Positive control	9.52±0.10	8.65±0.10	0.60±0.03	37.65±0.10

Statistical significant test for comparisons was done by ANOVA, followed by Dunnet'- 't'test p-values ***p<0.001, **p<0.01 and *p<0.05. Ns – Non significant

Table-2: Effect of *peperomia tetraphylla* extracts of Kidney calcium homogenate

S.No.	Groups	Kidney homogenate (calcium)(µg/gm)
1	Normal (Vehicle)	7.65±0.04
2	Standard (cystone)	7.58±0.07
3	Methanolic extract of PT 200mg	4.66±0.09
4	Methanolic extract of PT 400mg	4.85±0.07
5	Positive control	25.62±0.10

The values are expressed as Mean ± SEM (n=6). Comparisons were made between: Control vs Group-II & Group-II vs Group-III,IV,V,VI,V. Statistical significant test for comparisons was done by ANOVA, followed by Dunnet'-'t'test p-values ***p<0.001, **p<0.01 and *p<0.05. Ns – Non significant.

Histological Section of Kidney

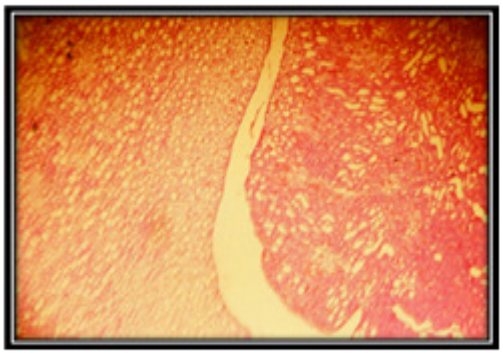

Fig.-1: Crystalline formation in Normal

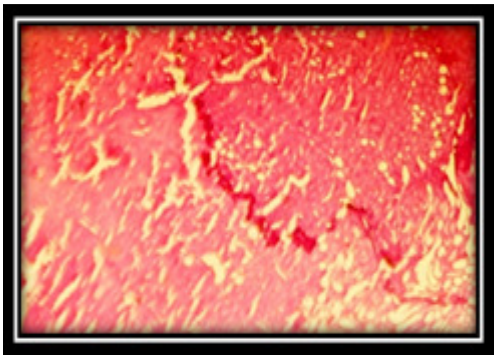

Fig.-2: Standard cystone2mg/kg bw

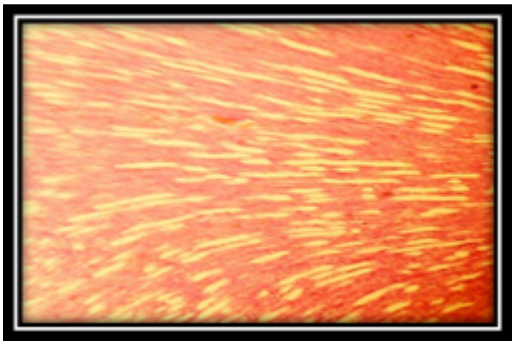

Fig.-3: Methanolic Extract of *Peperomia tetraphylla*200mg/kg bw

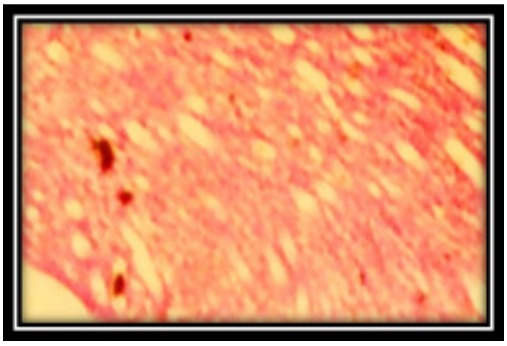

Fig.-4: Methanolic Extract of *Peperomia tetraphylla* 400mg/kg bw

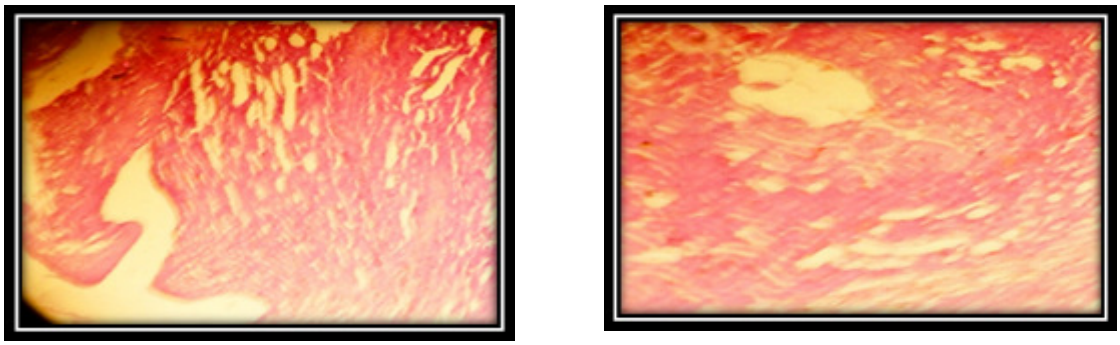

Fig.-5: Formation in Kidney of Positive control (A)(B)

RESULTS AND DISCUSSION

Urinary lithiasis is generally the result of an imbalance between inhibitors and promoters in the kidneys. Human kidney stones are usually composed of CaOx1, and several studies have examined the effect of the citrus juices on calcium salt crystallization. However, the conclusions from those studies were not consistent. Many in vivo models have been developed to investigate the mechanisms involved in the formation of urinary stones, and to ascertain the effect of various therapeutic agents on the development and progression of the disease.[22-24] Rats are the most frequently used animals in models of CaOx deposition in the kidneys, a process that mimics the etiology of kidney stone formation in humans. Rat models of CaOx urolithiasis induced by either EG alone or in combination with other drugs such as AC, are often used to study the pathogenesis of kidney crystal deposition24. Using the accelerated model, in the present study rats were treated with 0.75% EG and 2% AC for 10 days. All positive control rats (Group 5) developed CaOx depositions during that time. The present study examined the effect of extracts of *Peperomia tetraphylla* on the deposition of CaOx crystals within the rat kidney. The current study analysed body weight, kidney calcium level, serum concentrations of calcium, phosphorus, urea and creatinine and the histopathology of the kidney. We found that Group 1 rats (normal group) remained active and gained weight, while Group 2, 3, 4 and 5 rats lost weight over the 10 days of treatment. Microscopic examination using polarized light of kidney sections derived from nephrolithiasis rats showed intra-tubular and interstitial crystal deposits. These crystal deposits were observed in the kidneys of all Group 5 rats. Rats treated with Methanolic extracts had far less kidney calcification and lower renal tissue calcium levels than the positive control rats (Group 5) (Table-1 and 2).

CONCLUSION

The findings of the histopathological studies suggested that no microcrystalline deposition and deposition and kidney damage in the *Peperomia tetraphylla* extract treated groups all these observations enabled us to confirm the preventive curative potential of *Peperomia tetraphylla* on ethylene glycol induced lithiasis. In conclusion, the ethanolic extract of *Peperomia tetraphylla* has both preventive as well as curative property in urolithiasis of rats. These finding, thus prompt the necessity for further study to carry out the antilithiatic effect of *Peperomia tetraphylla* by isolation of constituents and find out the actual constituent that active against stone formation.

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