ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

PHARMACOLOGY

EFFECT OF A POLYHERBAL FORMULATION ON ETHYLENE GLYCOL INDUCED UROLITHIASIS.

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ABSTRACT

Antiurolithic effect of aqueous extract of Terminalia chebula, Glycyrrhiza glabra, Nelumbo nucifera, Zingiber officinale, Hemidesmus indicus, Myristica fragrans and Citrus aurantofolia. was tested. 24 albino wistar rats were divided into 4 groups of 6 each. Group-I is control. Groups-II, III and IV were fed 0.75% ethylene glycol by gastric lavage and 1 gm Ammonium chloride in drinking water . Groups-III and IV received aqueous extract of polyherbal formulation at a dose of 1mg/kg body weight and 2mg/kg body weight. . Statistical Analysis: One way of Analysis of Variance was used to test the statistical significance. Results.In urolithiatic animals,Calcium and Oxalate excretion was grossly increased. Groups III & IV showed weight gain, decreased calcium and oxalate excretion, increased levels of urinary volume , pH and stone inhibitors. Histopathological examination of renal tissues confirms this result. Conclusion: The polyherbal formulation used in this study has preventive effect in the formation of renal calculi.

KEY WORDS

Urolithiasis; Poly herbal formulation; Ethylene glycol; Ammonium chloride, urinary stone inhibitors.

INTRODUCTION

BACKGROUND INFORMATION :

Formation of stones in the urinary tract is called urolithiasis and in kidney it is termed nephrolithiasis. Urinary stone disease has affected mankind since antiquity. Urinary stones are a major cause of morbidity as it results in renal colic, urinary tract obstruction, urinary tract infection (UTI), and renal parenchymal damage. Ethylene glycol (EG) ingestion to male wistar rats has been widely used as an experimental model for the study of Urolithiasis. First evidence of urinary stones dates back to 4800 B.C., when a bladder stone was discovered in an Egyptian mummy at E1 Amrah Egypt. The practice of medical and surgical measures in the management of urological ailments prevailed in ancient India from the Vedic era around 3000 BC. Among many types of renal calculi calcium oxalate stones are the most common.The pathogenesis of formation of a calcium oxalate renal calculi is a dual process of nidus formation and development of nidus into a stone.[1]

Though there is a substantial progress in the understanding of the pathophysiology and management of urolithiasis, there is no satisfactory method available for complete cure of urolithiasis. Thus a drug with prophylactic effect [2,3] on urolithiasis would be of great interest. Several plants like Pergularia daemia,[4] catechin and epicatechin[5] , Wulingsan,[6] Trigonella foenum graecum, [7] have been used to treat kidney stones.

Traditional medical practitioners prescribe a combination of herbal products for synergistic action. One such Polyherbal formulation has folkloric claim of antilithiatic activity. (Table-1). The ingredients of this compound are claimed to have a cooling and ureterotonic effect.

Table-1
Composition of PHF

Sl. No	Common Name	Botanical Name	Part Used	Grams
1.	Indian Sarasaparilla	*Hemidesmus indicus*[8]	Root	1
2.	Dry ginger	*Zingiber officinale*[9]	Rhizome	0.05
3.	Licorice	*Glycyrrhiza glabra*[10]	Root	0.05
4.	Chebulic myrobalan	*Terminalia chebula*[11]	Fruit rind	0.025
5.	Lotus	*Nelumbo nucifera*[12]	Petals	0.050
6.	Nutmeg	*Myristicafragrans*[13]	Fruit rind	0.010

The present study is a preliminary investigation to demonstrate the prophylactic role of the compound when administered orally to male wistar rats in whom calcium oxalate crystals were induced .

MATERIALS AND METHODS

PLANT SOURCE

Various plants as mentioned in table-1 were identified and authenticated. The ingredients were dried, powdered and mixed as per the proportion mentioned in the table. The polyherbal formulation is abbreviated as PHF from now onwards.

After getting approval from Institutional animal ethical committee male albino Wistar rats[15] weighing around 150-250g were taken for the study. Rats were maintained in 24 hour light and dark cycle. Food and water were given ad libitum.

DRUGS AND CHEMICALS

All the chemicals,drugs and reagents used in this study were of analytical grade.

STUDY DESIGN:

EG induced urolithiasis has been considered clinically relevant method of stone induction in rats. EG is given either alone,[16] or in combination with AC, [17, 18] or α-Calcidol,[19] .

Rats were divided into four groups of six each. Group I served as the control and received only standard pellet and drinking water. Ethylene glycol (0.75%) and 1gm Ammonium Chloride were added in drinking water for a period of 28 days to groups II ,III and IV .

Group II : Urolithic Rats
2ml of 0.75% ethylene glycol (E.G) by oral gavage. 1g of Ammonium chloride (A.C) added to drinking water
Group III : Urolithic Rats + PHF by oral gavage (1g/kg body wt.
Group IV : Urolithic Rats + PHF by oral gavage(2g/kg body wt.)

At the end of 28 days animals were weighed,housed in metabolic cages for 24hours and urine was collected . Thymol crystal was added as preservative to urine sample. Blood was collected in sterile containers by sinuocular puncture in heparinised and non-heparinised tubes and sent for biochemical analysis . Animals were sacrificed under ether anesthesia by cervical dislocation. Kidneys were dissected, stored in 40% formalin and sent for histopathological examination. Cross sections of eosin haematoxylin stained kidney specimens were examined under polarized light microscope .

STATISTICS:

The results were expressed as mean ± standard error mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) . $P<0.05$ was considered significant.

Following comparisons were done.
Group II was compared with Group I.
Group III was compared with Group II.
Group IV was compared with Group II.

RESULTS

Table-2
Base line bodyweight of rats (gms)

		N	Mean	Std. Deviation	ANOVA	Significance level
Base line bodyweight of rats (gms)	Group I	6	192.6667	10.93008	17.939	0.000 ***
	Group II	6	233.3333	14.67878		
	Group III	6	200.6667	6.88961		
	Group IV	6	201.3333	7.11805		
	Total	24	207.0000	18.64077		

*_ P < 0.05 Significance, ** _ P < 0.01 Highly Significant, *** - P < 0.001 Very Highly Significant NS – Not Significant P > 0.05

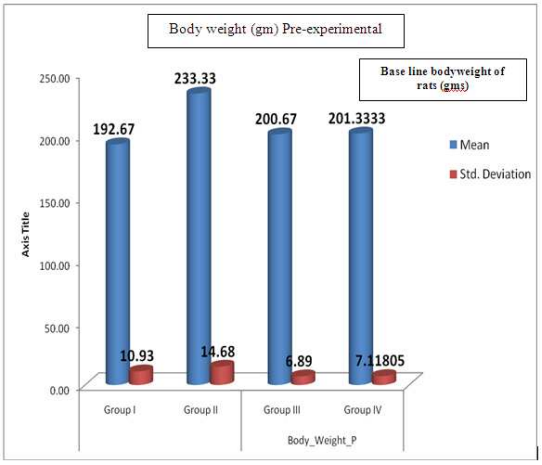


Table-3
Body weight at the end of 4 weeks

		N	Mean	Std. Deviation	ANOVA	Significance level
Body_weight At the end of 4 weeks	Group I	6	211.6667	13.70645	4.164	0.019 **
	Group II	6	211.0000	14.89966		
	Group III	6	214.6667	10.32796		
	Group IV	6	231.3333	3.50238		
	Total	24	217.1667	13.66578		

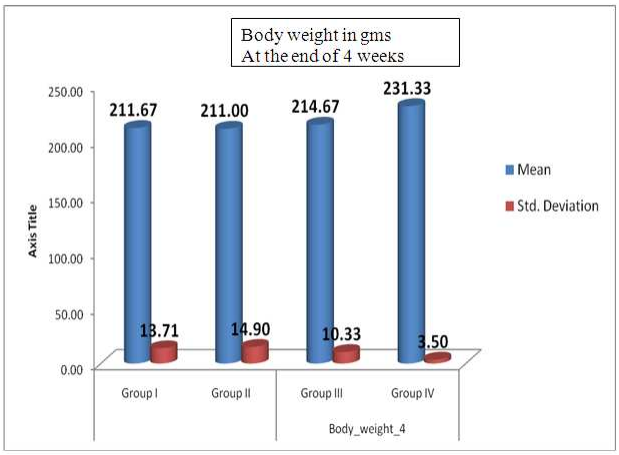


Table-4
Urinary volume in ml

		N	Mean	Std. Deviation	ANOVA	Significance level
Urinary Volume in ml	Group I	6	11.8333	.40825	184.148	0.000 ***
	Group II	6	10.5000	.44721		
	Group III	6	14.0833	.20412		
	Group IV	6	15.9167	.58452		
	Total	24	13.0833	2.16025		

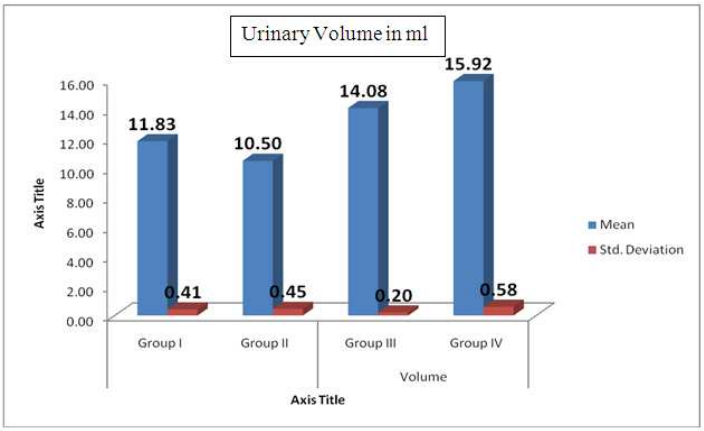


Table-5
Urinary pH

		N	Mean	Std. Deviation	ANOVA	Significance level
Urinary pH	Group I	6	7.4050	.00837	6176.675	0.000 ***
	Group II	6	5.6300	.03521		
	Group III	6	6.8967	.03445		
	Group IV	6	7.3083	.00983		
	Total	24	6.8100	.72312		

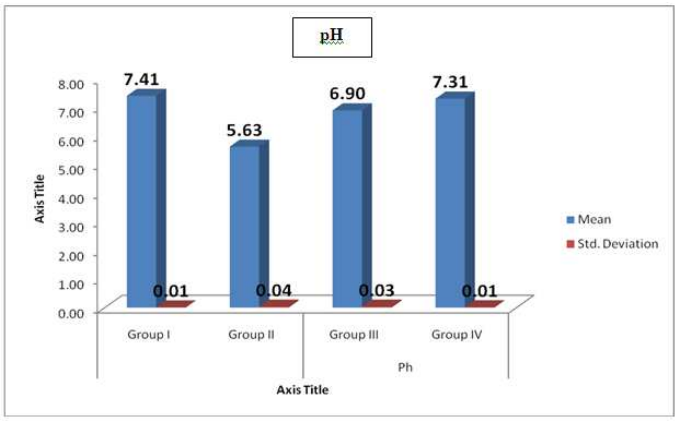


Table-6
Urinary calcium

		N	Mean	Std. Deviation	ANOVA	Significance level
Urinary Calcium mg/24hr urine	Group I	6	1.1783	.04215	9360.104	0.000 ***
	Group II	6	3.9667	.02066		
	Group III	6	1.5600	.04195		
	Group IV	6	1.3200	.02191		
	Total	24	2.0063	1.16497		

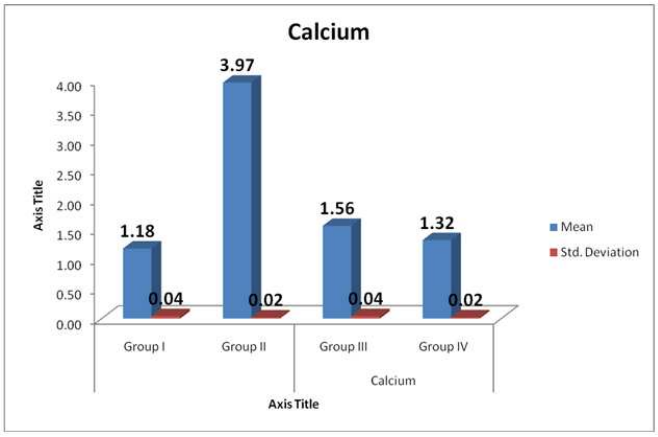


Table-7
Urinary oxalate

		N	Mean	Std. Deviation	ANOVA	Significance level
Urinary Oxalate mg/24hr urine	Group I	6	.3133	.01211	6023.142	0.000 ***
	Group II	6	1.9100	.02098		
	Group III	6	.9167	.01966		
	Group IV	6	.4400	.03347		
	Total	24	.8950	.64156		

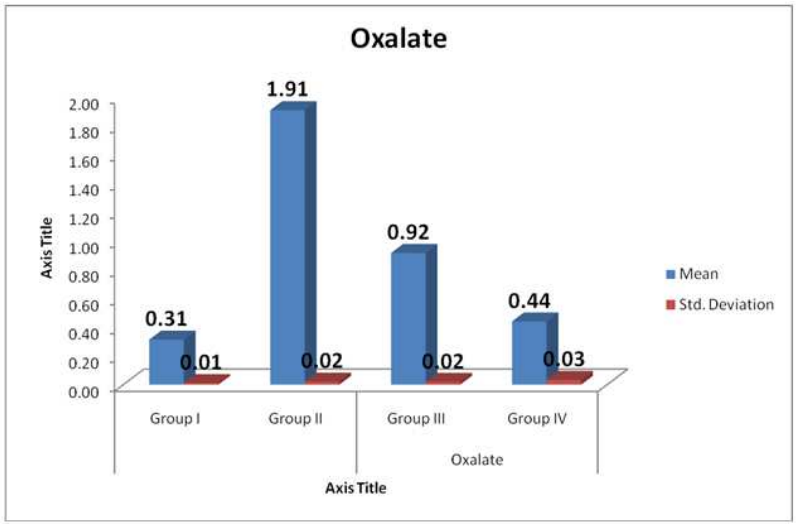


Table-8
Urinary phosphate

		N	Mean	Std. Deviation	ANOVA	Significance level
Urinary Phosphate (mg/24hr urine)	Group I	6	2.9950	.02811	1875.156	0.000
	Group II	6	7.0567	.17131		
	Group III	6	3.5933	.07659		
	Group IV	6	4.5767	.07090		
	Total	24	4.5554	1.58674		

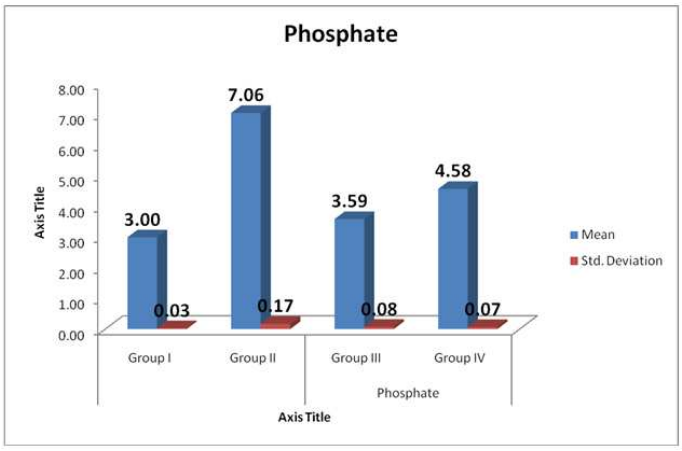


Table-9
Urine magnesium

		N	Mean	Std. Deviation	ANOVA	Significance level
Urinary Magnesium (mg/24hr urine)	Group I	6	6.2467	.04131	1888.207	0.000 ***
	Group II	6	4.3300	.10392		
	Group III	6	6.2933	.01033		
	Group IV	6	6.7233	.04320		
	Total	24	5.8983	.94589		

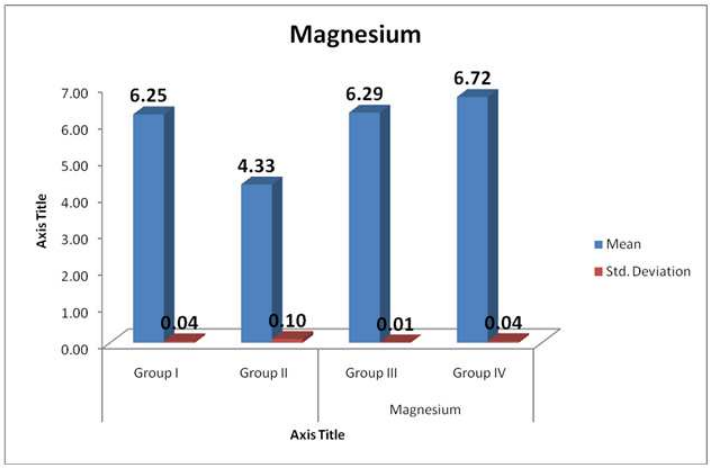
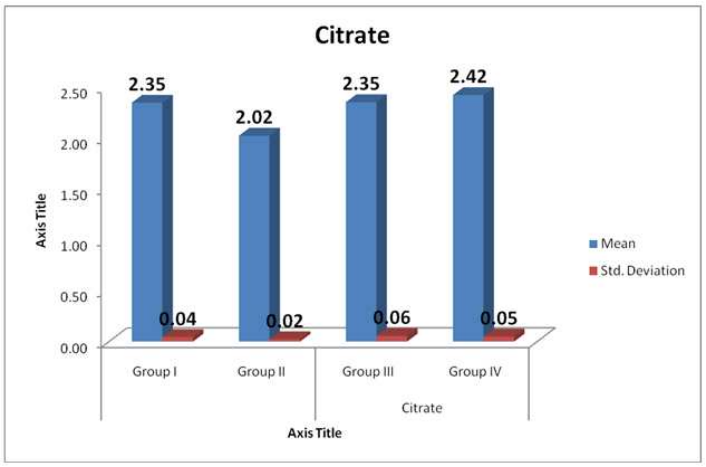


Table-10
Urinary citrate

		N	Mean	Std. Deviation	ANOVA	Significance level
Urinary Citrate (mg/24hr urine)	Group I	6	2.3483	.04491	95.592	0.000 ***
	Group II	6	2.0233	.02338		
	Group III	6	2.3533	.05502		
	Group IV	6	2.4233	.04967		
	Total	24	2.2871	.16391		



Urolithic rats showed gross weight reduction at the end of study period (Group II in Table – 3, (P< 0.01) But PHF supplemented rats showed significant weight gain.(Groups- III & IV table – 3) (P<0.001).

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
_ Baseline bodyweight(gm)	Group I	Group II	-40.66667*	6.00740	.000	-57.4810	-23.8523
		Group III	-8.00000	6.00740	.554	-24.8143	8.8143
		Group IV	-8.66667	6.00740	.489	-25.4810	8.1477
	Group II	Group I	40.66667*	6.00740	.000	23.8523	57.4810
		Group III	32.66667*	6.00740	.000	15.8523	49.4810
		Group IV	32.00000*	6.00740	.000	15.1857	48.8143
	Group III	Group I	8.00000	6.00740	.554	-8.8143	24.8143
		Group II	-32.66667*	6.00740	.000	-49.4810	-15.8523
		Group IV	-.66667	6.00740	.999	-17.4810	16.1477
	Group IV	Group I	8.66667	6.00740	.489	-8.1477	25.4810
		Group II	-32.00000*	6.00740	.000	-48.8143	-15.1857
		Group III	.66667	6.00740	.999	-16.1477	17.4810
Bodyweight at the end of 28 days	Group I	Group II	.66667	6.63827	1.000	-17.9134	19.2468
		Group III	-3.00000	6.63827	.968	-21.5801	15.5801
		Group IV	-19.66667*	6.63827	.036	-38.2468	-1.0866

* The mean difference is significant at the 0.05 level.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Volume	Group I	Group II	1.33333*	.25000	.000	.6336	2.0331
		Group III	-2.25000*	.25000	.000	-2.9497	-1.5503

		Group IV	-4.08333*	.25000	.000	-4.7831	-3.3836
	Group II	Group I	-1.33333*	.25000	.000	-2.0331	-.6336
		Group III	-3.58333*	.25000	.000	-4.2831	-2.8836
		Group IV	-5.41667*	.25000	.000	-6.1164	-4.7169
	Group III	Group I	2.25000*	.25000	.000	1.5503	2.9497
		Group II	3.58333*	.25000	.000	2.8836	4.2831
		Group IV	-1.83333*	.25000	.000	-2.5331	-1.1336
	Group IV	Group I	4.08333*	.25000	.000	3.3836	4.7831
		Group II	5.41667*	.25000	.000	4.7169	6.1164
		Group III	1.83333*	.25000	.000	1.1336	2.5331
pH	Group I	Group II	1.77500*	.01470	.000	1.7339	1.8161
		Group III	.50833*	.01470	.000	.4672	.5495
		Group IV	.09667*	.01470	.000	.0555	.1378
	Group II	Group I	-1.77500*	.01470	.000	-1.8161	-1.7339
		Group III	-1.26667*	.01470	.000	-1.3078	-1.2255
		Group IV	-1.67833*	.01470	.000	-1.7195	-1.6372
	Group III	Group I	-.50833*	.01470	.000	-.5495	-.4672
		Group II	1.26667*	.01470	.000	1.2255	1.3078
		Group IV	-.41167*	.01470	.000	-.4528	-.3705
	Group IV	Group I	-.09667*	.01470	.000	-.1378	-.0555
		Group II	1.67833*	.01470	.000	1.6372	1.7195
		Group III	.41167*	.01470	.000	.3705	.4528
Urine Calcium	Group I	Group II	-2.78833*	.01924	.000	-2.8422	-2.7345
		Group III	-.38167*	.01924	.000	-.4355	-.3278
		Group IV	-.14167*	.01924	.000	-.1955	-.0878
	Group II	Group I	2.78833*	.01924	.000	2.7345	2.8422
		Group III	2.40667*	.01924	.000	2.3528	2.4605
		Group IV	2.64667*	.01924	.000	2.5928	2.7005
	Group III	Group I	.38167*	.01924	.000	.3278	.4355
		Group II	-2.40667*	.01924	.000	-2.4605	-2.3528
		Group IV	.24000*	.01924	.000	.1861	.2939
	Group IV	Group I	.14167*	.01924	.000	.0878	.1955
		Group II	-2.64667*	.01924	.000	-2.7005	-2.5928
		Group III	-.24000*	.01924	.000	-.2939	-.1861

Urinary Oxalate	Group I	Group II	-1.59667*	.01321	.000	-1.6336	-1.5597
		Group III	-.60333*	.01321	.000	-.6403	-.5664
		Group IV	-.12667*	.01321	.000	-.1636	-.0897
	Group II	Group I	1.59667*	.01321	.000	1.5597	1.6336
		Group III	.99333*	.01321	.000	.9564	1.0303
		Group IV	1.47000*	.01321	.000	1.4330	1.5070
	Group III	Group I	.60333*	.01321	.000	.5664	.6403
		Group II	-.99333*	.01321	.000	-1.0303	-.9564
		Group IV	.47667*	.01321	.000	.4397	.5136
	Group IV	Group I	.12667*	.01321	.000	.0897	.1636
		Group II	-1.47000*	.01321	.000	-1.5070	-1.4330
		Group III	-.47667*	.01321	.000	-.5136	-.4397
Urinary Phosphate	Group I	Group II	-4.06167*	.05847	.000	-4.2253	-3.8980
		Group III	-.59833*	.05847	.000	-.7620	-.4347
		Group IV	-1.58167*	.05847	.000	-1.7453	-1.4180
	Group II	Group I	4.06167*	.05847	.000	3.8980	4.2253
		Group III	3.46333*	.05847	.000	3.2997	3.6270
		Group IV	2.48000*	.05847	.000	2.3163	2.6437
	Group III	Group I	.59833*	.05847	.000	.4347	.7620
		Group II	-3.46333*	.05847	.000	-3.6270	-3.2997
		Group IV	-.98333*	.05847	.000	-1.1470	-.8197
	Group IV	Group I	1.58167*	.05847	.000	1.4180	1.7453
		Group II	-2.48000*	.05847	.000	-2.6437	-2.3163
		Group III	.98333*	.05847	.000	.8197	1.1470
Urinary Magnesium	Group I	Group II	1.91667*	.03474	.000	1.8194	2.0139
		Group III	-.04667	.03474	.547	-.1439	.0506
		Group IV	-.47667*	.03474	.000	-.5739	-.3794
	Group II	Group I	-1.91667*	.03474	.000	-2.0139	-1.8194
		Group III	-1.96333*	.03474	.000	-2.0606	-1.8661
		Group IV	-2.39333*	.03474	.000	-2.4906	-2.2961
	Group III	Group I	.04667	.03474	.547	-.0506	.1439
		Group II	1.96333*	.03474	.000	1.8661	2.0606
		Group IV	-.43000*	.03474	.000	-.5272	-.3328
	Group IV	Group I	.47667*	.03474	.000	.3794	.5739
		Group II	2.39333*	.03474	.000	2.2961	2.4906

		Group III	.43000*	.03474	.000	.3328	.5272
Urinary citrate	Group I	Group II	.32500*	.02591	.000	.2525	.3975
		Group III	-.00500	.02591	.997	-.0775	.0675
		Group IV	-.07500*	.02591	.041	-.1475	-.0025
	Group II	Group I	-.32500*	.02591	.000	-.3975	-.2525
		Group III	-.33000*	.02591	.000	-.4025	-.2575
		Group IV	-.40000*	.02591	.000	-.4725	-.3275
	Group III	Group I	.00500	.02591	.997	-.0675	.0775
		Group II	.33000*	.02591	.000	.2575	.4025
		Group IV	-.07000	.02591	.061	-.1425	.0025
	Group IV	Group I	.07500*	.02591	.041	.0025	.1475
		Group II	.40000*	.02591	.000	.3275	.4725
		Group III	.07000	.02591	.061	-.0025	.1425

*. The mean difference is significant at the 0.05 level.

Urinary data 24 hour urine volume was significantly increased in PHF treated groups as compared to urolithic rats(Groups III & IV, Table – 4) (P< 0.001) Acidic pH was noted in urolithic rats (Group II, Table- 5) PHF treated groups showed a raise in urine pH in a dose dependent manner (Groups III & IV), Table – 5) (P< 0.001) .Chronic administration of 0.75 % (v/v) EG with 1 g AC resulted in hyperoxaluria. Excretion of calcium, oxalate and phosphate was grossly increased in calculi induced rats. (Tables – 6, 7 & 8) (Group – II) wheras the same were lowered significantly in PHF treated rats (Groups III & IV, Tables – 5, 6 & 7) (P<0 .001). In urolithic rats, the levels of Citrate and Magnesium in urine were reduced. (Group II, Tables 9 & 10) However PHF supplemented rats showed signficant (P< 0.01) raise in urinary citrate & magnesium. (Groups III & IV, Tables 9 & 10).

HISTO PATHOLOGICAL STUDIES :

Kidney sections treated with EG & AC showed multiple calcium oxalate crystals under poralised light.(Fig – 2).Urolithic rats who were supplemented with PHF simultaneously showed gross reduction in calcium (Fig.3) in kidney. The kidney section was almost near normal in rats who received a higher dose (Fig.4) and it was comparable with that of control rats.

ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

PHOTOMICROGRAPHS OF RENAL HISTOPATHOLOGY

Fig.1

Kidneys of Control rats which received distilled water and observed by polarized light (H&E, ×100)

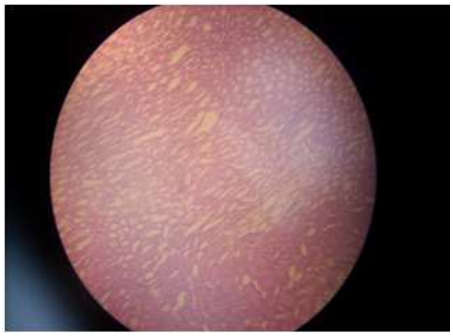


Fig.2

Intratubular crystals in kidneys of ethylene glycol & Ammonium chloride-treated rats, crystals observed by polarized light (H&E, ×100)

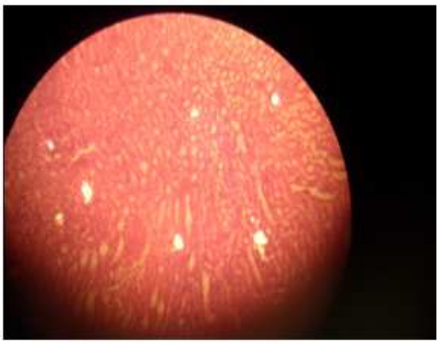


Fig.3

Reduction in crystals in kidneys of ethylene glycol ,Ammonium chloride-treated rats & PHF-1gm/kg b.wt. crystals observed by polarized light (H&E, ×100)

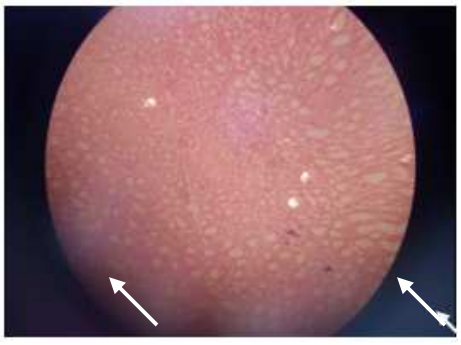
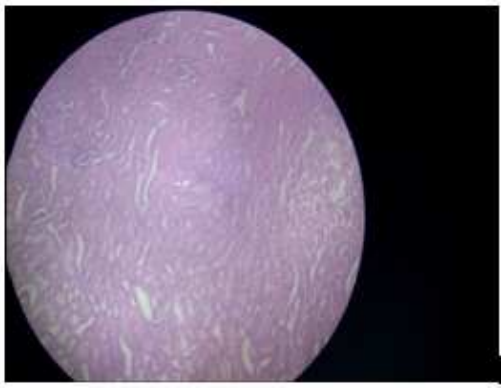


Fig.4

Marked reduction in crystals in kidneys of ethylene glycol ,Ammonium chloride-treated rats & PHF-2 gm/kg b.wt showing near normal renal structure. crystals observed by polarized light (H&E, ×100)



DISCUSSION

Urolithiasis is a complex,multi-factorial disorder which result from the combined influence of dietary, goegraphical, biochemical and genetic risk factors. Ureteric colic is a severe painful condition which is comparable to that of labor pain. Urolithiasis is a highly recurrent condition. Currently, in the management of urinary stones drugs like alpha blockers, 5 alpha reductase inhibitors, analgesics, antispasmodics, surgical procedures and extra corporeal shock wave lithotripsy are commonly employed.[20] The drugs temporarily relieve the symptoms but they result in autonomic, endocrinal and gastrointestinal side effects whereas the procedure is expensive. The major drawback of these procedures is recurrence of stones. Reverse pharmacology is use of plants in the treatment of diseases. Phytotherapy can be either used alone or as an adjuvant with conventional therapy.

Ethylene glycol ingestion has been used widely as an experimental model for the study of urolithiasis. To achieve a uniformly high rate of kidney crystal deposition, Ammonium chloride was added in conjunction with EG [21]. In current study, the rats treated with 1% AC & 0.75% EG demonstrated Calcium oxalate crystals in urine within a week. Hyperoxaluria could be an outcome of one among the following.

- Increased consumption of oxalate rich diet.
- Increased denovo synthesis from EG
- Altered renal excretion of oxalic acid

In the current study since all animals receive normal pellet feed, hyper oxaluria could not be of dietary origin. EG per se is non toxic as it is eliminated by the kidney[22]. Ethylene glycol is a metabolic precursor of oxalate. EG is metabolized to glycaldehyde, glycolic acid, glycoxalic acid and oxalic acid. Oxalic acid was largely excreted in the urine as Calcium oxalate and got deposited in the renal tubules. Rats supplemented with PHF showed a decline in oxalate excretion. In PHF treated groups crystalluria and oxalate deposition were significantly reduced in a dose dependent manner. This may be due to decrease synthesis of oxalate from UG as a result of PHF administration.

Ingestion of AC resulted in higher urinary acidification, which may be responsible

for increased deposition of Calcium oxalate crystals in the kidneys. In general urolithiasis occurs as a result of super saturation of urine. When there is a gross imbalance between the stone promoters and inhibitors calculi are formed. Some of the promoters of stone formation include Calcium, Oxalate, low urine pH and low urine volume whereas Magnesium, Citrate, high urine pH and high urine volume inhibit stone formation. PHF has increased urine pH and urinary volume as well in groups III & IV. As the alkaline pH is one of the inhibitory factors, the formation of stones is grossly reduced . The diuretic effect can be accounted to the actions of Terminalia chebula, Nelumbo nucifera, Glycyrrhiza glabra and Hemidesmus indicus.

The urinary levels of Magnesium and citrate were also increased in the test groups III & IV. It is there by understood that the PHF elevates the level of these inhibitors in urine and along with diuresis and alkaline pH it has brought about a marked reduction in the stone formation.

The levels of urinary Calcium & Oxalate were greatly increased in urolithic rats leading to excessive stone formation. On the other hand, their levels in the test groups III & IV were reduced which was comparable to that of control group . Decreased urine levels of Calcium & Oxalate in PHF treated groups rendered them unavailable for stone formation since they are the principal stone promoters. Thus PHF not only decreased the level of stone promoters in urine but also increased the level of stone inhibitors The effectiveness of PHF as a prophylactic agent in urolithiasis was also confirmed histopathologically. When examined in polarized light oxalate crystals were higher in urolithic rats while in PHF treated rats number of crystals were reduced.

CONCLUSION

The PHF resulted in an increase in urinary volume, decrease in Calcium, Oxalate and Phosphate excretion along with increased excretion of Citrate and Magnesium. Further the Histopathological examination of renal tissues showed drastic reduction in stone formation. So, it is concluded that the Polyherbal formulation used in the present study is an effective drug in the management of urolithiasis and could be tried in the treatment of urolithiasis. However further studies are required for authentication in human beings.

ACKNOWLEDGEMENT

1) Dr.S.Vembar, Advisor to Vice chancellor, Annamalai University for his support in carrying out this study.
2) The TamilNadu State Council of Science and Technology(TANSCST) gave us a cash award of Rs.5,000 from their student project scheme.
3) Dr.S.Ramasamy Ph D for his guidance in doing this study
4) Rumi Herbals Private limited ,Chennai.
5) Mr.S.Arunai Nambiraj , VIT for giving us information regarding methodology and histopathological examination.

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