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EFFECTOF HYDRO ALCOHOLIC EXTRACT OF *PUNICA GRANATUM* ON ETHYLENE GLYCOL AND AMMONIUM CHLORIDE INDUCED NEPHROLITHIASIS

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ABSTRACT

Introduction:

Studying the chemistry of the urine with regard to the minerals that cause stones will give a solid indicator of the likelihood of stone formation. In ethylene glycol-induced nephrolithic rats, hypercalcinuria may be a factor that favours the formation of calcium phosphate crystals from urine precipitation and nucleation

Methods:

It is possible to predict the possibility of stone development by analysing the urine's chemistry in relation to the minerals that lead to stones. The development of calcium phosphate crystals from urine precipitation and nucleation may be aided by hypercalcinuria in ethylene glycol-induced nephrolithic rats.A renal calculus is1% and 0.75 percent of ethylene glycol, administered for 28 and 5 days, respectively, were used to generate the changes. In a preventive study, extract was administered at two different doses of 200 mg/kg and 400 mg/kg for 28 days. In a curative study, the same doses of extract were administered for 14 to 28 days. Numerous parameters have been evaluated, including creatinine, urea, and uric acid in the serum and oxalate, calcium, and phosphate in the urine.

Results:

When ethylene glycol and ammonium chloride intoxicated rats were compared to the control group, the

levels of biochemical parameters were elevated. When the extract was administered at doses of 200 and 400 mg/kg, biochemical parameters (urine:BUN, serum, urea, creatinine, calcium oxalate, and phosphate).

Conclusion:

Rats were prevented from developing nephrolithiasis caused by ethylene glycol by oral administration of a methanolic extract of *punica granatum* peel. The stimulated animals' oxalate synthesis is reduced by the protective action of MEPP. These findings imply that MEPP offers therapeutic protection against oxidative stress brought on by calcium oxalate accumulation.

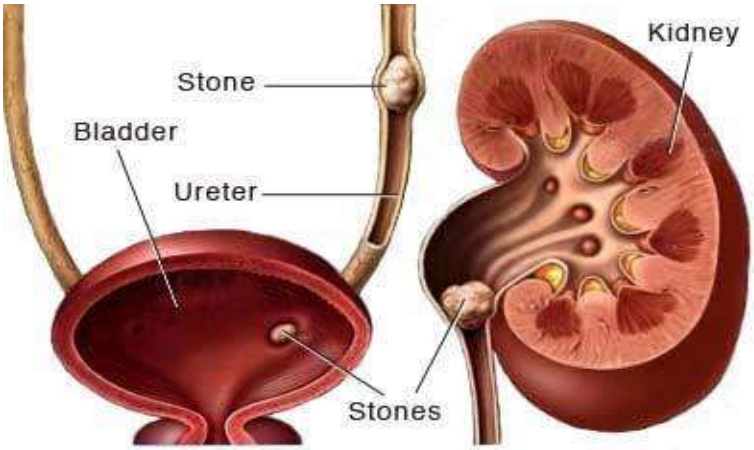
Keywords: *Punica granatum*, Nephrolithiasis, Calcium oxalate, Urolithiasis

INTRODUCTION

Since the dawn of time, herbal remedies have served as the cornerstone of therapy around the globe. They are still frequently employed today. Although it differs greatly between nations, the acceptance of their therapeutic uses is continually expanding **[1]**. A sizable population still relies on traditional doctors, medicinal herbs, and herbal remedies for their primary care in underdeveloped nations. Growing use of medicinal plants and their derivatives, which are widely utilised in traditional civilizations all over the world, has increased public interest in natural medicines significantly during the past few decades in developed nations. Nearly 80% of people worldwide use alternative and/or herbal medicine as their primary form of healthcare **[2]**. The public has developed an interest in herbs and herbal medications. Since ancient times, pomegranate extracts have been used to treat a variety of illnesses, such as parasite and microbial infections. In more recent times, uses have expanded to include oral hygiene, hormone replacement treatment, immune suppressions, and cardiovascular problems. Given that no treatment used in clinical therapy produces satisfactory results, research into drugs that can stop a disease from developing or from returning is very important. Urolithiasis can be prevented and treated with ordinary medications, but they are frequently ineffective, expensive, and recur frequently.

NEPHAROLITHASIS – INTRODUCTION

Renal calculus, or lithiasis, is one of the urinary tract's most prevalent disorders. Urolithiasis, which is the presence of stones anywhere in the urinary tract, is separated from nephrolithiasis, which is the presence of renal calculi **[3, 4]**. A body that resembles a stone formed of urinary salts and bound together by a colloid matrix of organic substances is known as urine calculus. Urine salts are arranged in concentric layers around a central point.



MOLECULAR BASIS OF THE PATHOGENSIS OF NEPHROLITHIASIS

Oxidative stress, which is caused by an increase in reactive oxygen (nitrogen) intermediate generation, is the most frequent cause of cell damage **[5]**. High oxalate concentrations can lead to oxidative stress, as demonstrated by the fact that exposure to these species of RO (N) can lead to a variety of cellular effects,like the activation of crucial transcription factors by means of gene induction, cell growth, or necrotic/apoptotic cell death **[6]**.

1. Increased lipid peroxidation (thiobarbituric acid reactive substance (TBARS))
2. Reduced glutathione levels
3. An increase in the number of free radical generations
4. Increased arachindonic acid release via phospholipase-A2 **[7, 8]**

OXIDATIVE STRESS AND CALCIUM OXLATE NEOHAROLITHASIS

High Ox and CaOX crystal concentrations have also shown that renal cells produce more extracellular matrix, inflammatory mediators, and crystallisation regulators. Reactive oxygen species play a role in the activation of signalling molecules including protein kinase C (PKC), c-jun N-terminal kinase (JNK), and p38 mitogen -activated protein kinase (MAPK)N, with effect over transcription factors including NF-B) and activated protein protein-1 (AP -1). We have demonstrated that experimentally induced hyperoxaluria in rats **[9, 10]**. in results in renal tubular cell damage and CaOx crystal deposition. Both apoptotic and necrotic injuries have been detect In vitro studies have shown that membrane and cellular degradation products are excellent nucleator of CaOx crystals at super saturation normally found in the renal tubular fluids .

this attachment mediated by Ox – induced exposure of phosphatidylserine (ps) on cell surfaces. Interestingly, apoptosis involves the exposure of PS on cell surfaces **[11]**.

PUNICA GRANATUM

Pomegranate (*Punica granatum*) an ancient fruit –bearing delicious shrub, is the predominant member of two species comparing the punicaceae family. it is native of the Himalayas in northern India. The fruit can be divided into three parts: the seeds and the juice, **[12]** which represent about and 30% of the fruit weight, respectively, and the peels, which include the mentioned inner network of membrane with distinct chemical compositions and potential medical benefits.

Figure 1: Whole plant of *punica granatum*

Figure 2: Peel of *punica granatum*

PREPRATION OF PLANT EXTRACT

Manually separated, shade-dried, and powdered pomegranate skins were used. The powder (25g) was extracted by stirring 100 ml of methanol with a magnetic stirrer at 30°C for one hour. **[13]**. To get rid of the peel particles, the extract was filtered. The same solvent was used to extract the residue once more. The extracts were combined and vacuum-concentrated at 40°C.

EXPERIMENTAL PROTOCOL

STUDIES ON Acute Oral Toxicity

The acute toxic class method is a step-by-step process with 3 animals of a single sex per step that was carried out in accordance with OECD guidelines (organisation of economic corporation and development**) [14]**. Depending on the animals' mortality and/or morbidity status. A substance can be graded and classified using the globally harmonised system (GHS) **[15, 16]** for the categorization of chemicals that induce acute toxicity thanks to the method's defined dosages (200mg/kg body weight). *Punica granatum* Linn's methanolic extract was chosen to be 2000 mg/kg/bw/p.o. Three male rats weighing between 250 and 300 g were employed for the study. Rats that had been fasted over night and given unlimited access to water before the medication was given to them received it orally. Prior to and following treatment, the rats' body weights were record **[17, 18]**.

ANIMAL GROUPINGS:

In both preventive and therapeutic studies, the male Wistar rats were randomly assigned to 5 separate groups (n = 6)

CURATIVE STUDY

GROUP A:

Received regular rat food and water and libitum and served as normal

GROUP B:

In Group B, drinking water was supplemented with 1% ammonium chloride for 5 days and 0.75% ethylene glycol for 28 day

GROUP C:

Group C was given 1% ammonium chloride for five days, 0.75 percent ethylene glycol for 28 days, drinking water, and 750 mg/kg cystone from day 14 to day 28.

GROUP D:

Group D received, 0.75% ethylene glycol for 28 days and 1% ammonium chloride for 5 days adding in drinking water and 400mg/kg of MEPP was administrated orally from 14^{th} day to 28^{th} day.

GROUP E:

Group E received 200 mg/kg of MEPP orally from the 14th day through the 28th day, along with 0.75% ethylene glycol for 28 days, 1% ammonium chloride for 5 days, and drinking water additives.

PREVENTIVE STUDY:

GROUP A:

Received regular rat food for water ad libitum and served as normal

GROUP B:

Group B received 1% ammonium chloride added to drinking water for 5 days and 0.75% ethylene glycol for 28 days.

GROUP C:

Group C received stone induction treatment, 0.75% ethylene glycol for 28 days and 1% ammonium chloride for 5 days along with 750 mg /kg of cystone for 28 days

GROUP D:

Group D received stone induction treatment, 0.75% ethylene glycol for 28 days and 1% ammonium chloride for 5 days along with 400 mg /kg of MEPP for 28 days

GROUP E:

Group E received stone induction treatment, 0.75% ethylene glycol for 28 days and 1% ammonium chloride for 5 days along with 200 mg /kg of MEPP for 28 days

RESULTS

Punica granatum peel extract significant improved the protective effect in nephrolithiasis ethylene glycol-induced in rats and results were discussed as follows.

Table 1: Effect of MEPP On Urinary Calcium Level (Curative Study)

		Identity Number						
	Groups	R1	R2	R3	R4	R5	R6	Mean ±SEM
Calcium (mg/dl)	A	1.27	1.2	2.91	1.32	1.66	1.76	1.686±0.23
	B	4.51	6.27	8.15	4.64	5.32	4.21	5.5163±0.55###
	C	1.64	1.58	1.32	1.96	1.89	1.26	1.608±0.131***
	D	2.13	2.32	1.86	1.94	1.89	1.56	1.95±0.234***
	E	1.7	1.7	2.83	2.53	1.96	2.35	2.34±0.162***

Significant: * P <0.05 , **<0.001,*P<0.001,NS non significant**
Values are expressed as mean ±SEM of 6 animals

Comparison were made between **# Group B vs Group A and *group C, D & E vs group B**

Symbol represents the statistical significances done by ANOVA one way test, followed by Tukey 's multiple comparison test.

Table 2: Effect of MEPP on Urinary Oxalate Level (Curative Study)

		Identity Number						
	Groups	R1	R2	R3	R4	R5	R6	Mean ±SEM
Oxalate (mg /dl)	A	0.37	0.35	0.34	0.58	0.41	0.49	0.423 ±0.044
	B	3.64	3.39	2.1	3.47	3.15	2.98	3.121±0.205###
	C	0.58	0.61	0.96	0.82	0.59	0.98	0.756±0.069***
	D	1.35	1.65	1.23	1.12	1.08	0.98	1.235±0.089***
	E	1.89	1.91	1.35	1.42	1.56	1.16	1.54±0.112***

SIGNIFICANT : * P <0.05 , **<0.001,*P<0.001,NS non significant**
Values are expressed as mean ±SEM of 6 animals

Comparison were made between **# Group B vs Group A and *group C, D & E vs group B**

Symbol represents the statistical significances done by ANOVA one way test, followed by Tukey 's multiple comparison test.

Table 3: Effect of MEPP on Urinary Phosphate Level (Curative Study)

		Identity Number						
	Groups	R1	R2	R3	R4	R5	R6	Mean ±SEM
Phosphate (mg/dl)	A	3.44	3.64	3.27	3.35	3.14	3.92	3.46±0.113
	B	7.29	6.27	7.29	8.18	7.25	6.98	7.21±0.221###
	C	3.62	3.81	3.51	3.91	3.45	3.54	3.64±0.006***
	D	4.01	3.89	4.12	4.6	5.3	4.89	4.463±0.207***
	E	5.32	4.98	4.82	5.02	5.16	4.7	5.0±0.090***

SIGNIFICANT: * P <0.05, **<0.001, ***P<0.001, NS non significant
Values are expressed as mean ±SEM of 6 animals

Comparison were made between **# Group B vs Group A and *group C, D & E vs group B**

Symbol represents the statistical significances done by ANOVA one way test, followed by Tukey 's multiple comparison test.

Table 4: Effect of MEPP on Serum Bun Level (Curative Study)

		Identity Number						
	Groups	R1	R2	R3	R4	R5	R6	Mean ±SEM
serum Bun (mg/dl)	A	37.6	36.09	36.5	36.4	35.4	36.2	36.41±0.0275
	B	49.9	51.12	52.6	51.2	51.9	50.5	51.215±0.355###
	C	39.3	37.5	38.4	39.7	38.2	39.1	38.7±0.302***
	D	41.6	40.9	40.98	41.54	40.62	41.32	41.16±0.416***
	E	41.6	42.3	43.9	44.54	44.32	42.6	43.21±0.44***

SIGNIFICANT: * P <0.05 , **<0.001,*P<0.001, NS non significant**
Values are expressed as mean ±SEM of 6 animals

Comparison were made between **# Group B vs Group A and *group C, D & E vs group B**

Symbol represents the statistical significances done by ANOVA one way test, followed by Tukey 's multiple comparison test.

Table 5: Effect of MEPP on Serum Creatinine Level (Curative Study)

	Groups	Identity Number						Mean ±SEM
		R1	R2	R3	R4	R5	R6	
CREATININE (mg/dl)	A	0.75	0.89	0.72	0.68	0.74	0.69	0.745±0.029
	B	0.94	1.56	1.468	1.39	1.25	1.46	1.346±0.082###
	C	0.81	0.79	0.88	0.86	0.85	0.93	0.0853±0.0187***
	D	0.89	0.91	0.99	0.86	1.06	0.96	0.945±0.027***
	E	1.12	1.02	1.35	1.45	1.06	1.46	1.243±0.074***

SIGNIFICANT: * P <0.05, **<0.001, ***P<0.001, NS non significant
Values are expressed as mean ±SEM of 6 animals

Comparison were made between

 # Group B vs Group A and *group C, D & E vs group B

Symbol represents the statistical significances done by ANOVA one way test, followed by Tukey 's multiple comparison test.

Table 6: Effect of MEPP on Serum Uric Acid Level (Curative Study)

	Groups	Identity Number						Mean ±SEM
		R1	R2	R3	R4	R5	R6	
Uric acid (mg /dl)	A	1.49	1.45	1.5	1.48	1.51	1.42	1.475±0.012
	B	3.64	3.68	3.63	3.65	3.12	3.25	3.54±0.079###
	C	1.71	1.68	1.61	1.66	1.54	1.98	1.696±0.056***
	D	2.06	1.84	1.69	2.32	2.15	1.86	1.986±0.085***
	E	1.94	3.08	2.94	2.89	2.63	2.54	2.67±0.152***

SIGNIFICANT: * P <0.05, **<0.001, ***P<0.001, NS non significant
Values are expressed as mean ±SEM of 6 animals

Comparison were made between

 # Group B vs Group A and *group C, D & E vs group B

Symbol represents the statistical significances done by ANOVA one way test, followed by Tukey 's multiple comparison test.

PREVENTIVE STUDY:

Table 7: Effect of MEPP on Urinary Calcium Level (Preventive Study)

	Groups	Identity Number						Mean ±SEM
		R1	R2	R3	R4	R5	R6	
Calcium (mg/dl)	A	1.27	1.2	2.91	1.32	1.66	1.76	1.686±0.63
	B	4.51	6.27	8.15	4.64	5.32	4.21	5.516±1.48###
	C	1.64	1.58	1.32	1.96	1.89	1.26	1.608±0.286***
	D	2.08	2.11	1.78	1.56	1.89	1.64	1.843±0.225***
	E	1.18	2.29	2.63	2.34	1.97	1.94	2.163±0.370***

SIGNIFICANT: * P <0.05, **<0.001, ***P<0.001, NS non significant
Values are expressed as mean ±SEM of 6 animals

Comparison were made between

 # Group B vs Group A and *group C, D & E vs group B

Symbol represents the statistical significances done by ANOVA one way test, followed by Tukey 's multiple comparison test.

Table 8: Effect of MEPP on Urinary Oxlate Level (Preventive Study)

	Group	Identity Number						Mean ±SEM
		R1	R2	R3	R4	R5	R6	
oxalate (mg/dl)	A	0.37	0.35	0.34	0.58	0.41	0.49	0.423±0.63
	B	3.64	3.39	2.1	3.47	3.15	2.98	3.121±1.216###
	C	0.58	0.61	0.96	2.1	0.59	0.98	0.756±0.93***
	D	0.72	0.91	0.93	0.96	0.86	0.79	0.816±0.099***
	E	1.35	1.65	1.23	0.93	1.08	0.98	1.235±0.288***

SIGNIFICANT: * P <0.05, **<0.001, ***P<0.001, NS non significant;
Values are expressed as mean ±SEM of 6 animals

Comparison were made between

 # Group B vs Group A and *group C, D & E vs group B

Symbol represents the statistical significances done by ANOVA one way test, followed by Tukey 's multiple comparison test.

Table 9: Effect of MEPP on Urinary Phosphate Level (Preventive Study)

		Identity Number						
	Groups	R1	R2	R3	R4	R5	R6	Mean ±SEM
Phosphate (mg/dl)	A	3.44	3.64	3.27	3.35	3.14	3.92	3.46±0.281
	B	7.29	6.27	7.29	8.18	7.25	6.98	7.21±0.62###
	C	3.62	3.81	3.51	3.91	3.45	3.54	3.64±1.36***
	D	4.25	3.96	3.63	3.39	4.01	4.23	4.005±0.226***
	E	4.97	5.23	4.82	4.29	5.12	5.27	5.1±0.358***

SIGNIFICANT: * P <0.05, **<0.001, ***P<0.001, NS non significant
Values are expressed as mean ±SEM of 6 animals Comparison were made between

 # Group B vs Group A and *group C, D & E vs group B

Symbol represents the statistical significances done by ANOVA one way test, followed by Tukey 's multiple comparison test.

Table 10: Effect of MEEP on Serum Bun Level (Preventive Study)

		Identity Number						
	Groups	R1	R2	R3	R4	R5	R6	Mean ±SEM
serum Bun (mg/dl)	A	37.6	36.09	36.5	36.7	35.4	36.2	36.415±0.73
	B	49.97	51.12	52.6	51.2	51.9	50.5	51.215±0.94###
	C	39.3	37.5	38.4	39.7	38.2	39.1	38.7±0.803***
	D	39.2	39.1	40.02	40.23	38.9	39.6	39.508±0.533***
	E	45.2	41.3	49.34	46.23	43.6	46.8	45.411±2.76***

SIGNIFICANT: * P <0.05, **<0.001, ***P<0.001, NS non significant
Values are expressed as mean ±SEM of 6 animals

Comparison were made between

 # Group B vs Group A and *group C, D & E vs group B

Symbol represents the statistical significances done by ANOVA one way test, followed by Tukey 's multiple comparison test.

Table 11: Effect of MEPP on Serum Creatinine Level (Preventive Study)

		Identity Number						
	Groups	R1	R2	R3	R4	R5	R6	Mean ±SEM
CREATININE (mg/dl)	A	0.75	0.89	0.72	0.68	0.74	0.69	0.745±0.07
	B	0.94	1.56	1.48	1.39	1.25	1.46	1.346±0.208###
	C	0.81	0.79	0.88	0.86	0.85	0.93	0.0853±0.05***
	D	0.86	0.85	1.14	0.98	0.93	0.9	0.938±0.106***
	E	1.23	1.07	1.07	0.97	0.8	0.89	0.9993±0.139***

SIGNIFICANT: * P <0.05, **<0.001, ***P<0.001, NS non significant
Values are expressed as mean ±SEM of 6 animals

Comparison were made between

 # Group B vs Group A and *group C, D & E vs group B

Symbol represents the statistical significances done by ANOVA one way test, followed by Tukey 's multiple comparison test.

Table 12: Effect of MEPP on Serum Uric Acid Level (Preventive Study)

	Group	Identity Number						Mean ±SEM
		R1	R2	R3	R4	R5	R6	
Uric acid (mg/dl)	A	1.49	1.45	1.5	1.48	1.51	1.42	1.475±0.033
	B	3.64	3.68	3.63	3.65	3.12	3.52	3.54±0.212###
	C	1.71	1.68	1.61	1.66	1.54	1.98	1.696±0.157***
	D	1.94	1.89	2.08	1.91	1.86	1.99	1.945±0.079***
	E	2.59	1.89	2.56	2.32	2.46	2.01	2.305±0.293***

SIGNIFICANT: * P <0.05, **<0.001, ***P<0.001, NS non significant
Values are expressed as mean ±SEM of 6 animals

Comparison were made between

 # Group B vs Group A and *group C, D & E vs group B

Symbol represents the statistical significances done by ANOVA one way test, followed by Tukey 's multiple comparison test.

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