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EVALUATION OF ANTI-UROLITHIASIS POTENTIAL OF SIDDHA FORMULATION MITHRAN AMBU CHOORANAM IN ETHYLENE GLYCOL-FED RATS

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ABSTRACT

Urolithiasis is a comprehensive disease condition wherein various endogenous or exogenous etiological factors, as well as multivariate pathogenesis, are involved. The current treatment strategies for urolithiasis include shockwave lithotripsy, ureteroscopy and percutaneous stone extractions. However, these treatments are wrought with various side effects. When coupled with the high recurrence rate of stone formation (over 50% in 10 years), it strongly calls for new treatment options. Traditional therapy becomes an innumerable part of the human health since several centuries. Siddha system of medicine reveals obvious opportunity in treating metabolic issues like urolithiasis. Mithran ambu chooranam (MAC) is one such potential traditional formulation with unique blend of herbal components have a significant ability in rejuvenation. The main aim of the present investigation is to evaluate the anti-urolithiasis screening of siddha formulation MAC in ethylene glycol (EG) induced urolithiasis model in wistar rats. Results of the present investigation clarifies that there is a significant increase in serological parameters such as blood urea nitrogen (BUN), creatinine and uric acid in EG treated rats. Oral administration of MAC at the dose of 200 and 400 mg/kg shown marginal decrease in serum biochemistry profile of all the rats subjected to MAC treatment. Reports on urine biochemistry like magnesium, calcium, phosphate and uric acid reveals that there was a profound increase in mentioned cations in the EG rats when compared to that of the control rats. Treatment with MAC at the dose of 200 and 400 mg/kg signifies declination in cations including uric acid profile of the treated rats. It was concluded from the evidence based data of the present study is that siddha formulations like mithran ambu chooranam possess magnificent anti-urolithiasis potential against the EG induced nephrolithiasis and further screening must be extrapolated with proper clinical validation.

KEY WORDS: ***Urolithiasis, Traditional therapy, Siddha system, Mithran ambu chooranam, Ethylene glycol, Urine biochemistry, Serological parameters***

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1. Introduction

Urolithiasis is a typical medical condition in which there is a formation of urinary calculi (stone) anywhere in the urinary tract is one of the most painful and third prevalent ailments, has beset humans from centuries [1]. It is reported as complaint with an increasing incidence and prevalence worldwide. It is estimated that it affect about 12% of world population and expected to rise further with the advancement in the industrialization [2-4]. This increased incidence of urinary stones over the last few years, are associated with decrease in age of onset has an important effect on the healthcare system [5]. Recurrence is another major factor that makes it more serious issue to address. On recurrence, the subsequent relapse risk is raised and the interval between recurrences is shortened [6]. Common features associated with recurrence include a young age of onset, family history, frequent infections and underlying medical conditions [7].

Calcium oxalate stones are most common neproliths, accounting for more than 80% of stones, whereas 5–10% of uric acid stones are present. The other types are cystine, struvite, and urate stones, have very less percentage. Urolithiasis is the outcome of various physicochemical changes such as supersaturation of urine, nucleation, growth of crystal and aggregation. Urine is invariably saturated with the stone-forming components such as calcium, oxalate, urate, cystine, xanthenes, and phosphate. However, the natural tendency to inhibit crystallization prevents stone formation, whereas this natural inhibition capacity varies person to person and which is poor in stone formers [8]. An imbalance between urinary stone promoting and inhibiting factors is predominantly responsible for the formation of renal stones [9], which is a multistep process involving nucleation, crystal growth, aggregation and finally retention of crystals [10].

Retention of calcium oxalate monohydrate (COM) crystals on cellular surface is a key event for the development of kidney stones owing to the interaction between cell membranes and oxalate crystals, mediated by surface of crystal and surface of renal cell membrane [11]. High rate of recurrence in stone formation were been documented which is around 50% at a 5 years follow-up [12], makes it a chronic condition which underscores the importance of preventive therapy [13]. In spite of substantial progress in the study of the biological and physical manifestation of urolithiasis, its mechanism is still not clearly understood [14] and there is no satisfactory drug available for the treatment of urolithiasis, especially for the prevention of recurrence of the stones [15]. The agents used clinically for prophylactic therapy are primarily aimed to correct the underlying metabolic disorders but the evidence for their effectiveness is still not convincing in addition to their side effects and tolerability [16, 17]. One reason for a limited success of chemical drugs in urolithiasis is that multiple factors are involved in its pathogenesis [18] and thus treatment demands multiple targets, such as antispasmodic, antioxidant, anti-inflammatory activities [19].

Traditional and herbal supplements have been used as traditional healthcare system from the centuries. The WHO has listed 20 000 medicinal plants globally in which contribution of India is 15–20% [20]. The WHO reported that 80% of global countries depend on the medicinal plants [21]. The main objective of the present investigation is to evaluate the anti-urolithiasis potential of siddha formulation MAC in ethylene glycol (EG) induced urolithiasis model in wistar rats.

2. Materials and Methods

2.1. Experimental Animals

Healthy adult Wistar albino rats of either sex weighing between 200-220 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit. A 12 light / dark cycle were maintained. Room temperature was maintained between 22 ±2ºC and relative humidity 50–65%. They were provided with food and water ad libitum. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of science and technology, Chennai, Tamil Nadu, India. The IAEC approval number: SU/CLATR/IAEC/XIII/124/2019

2.2. Animal grouping and Methodology [22,23]

The animals were grouped into four groups of 6 animals each. Group I (Control group) -received normal saline, Group II – Urolithiatic control received EG (0.75% w/v, p.o.) in drinking water for 28 days ad

libitum (Day1 and Day 28). Group III - Received EG (0.75% w/v, p.o.) in drinking water and treated with 200mg/kg of MAC for the period of 28 days. Group IV Received EG (0.75% w/v, p.o.) in drinking water and treated with 400mg/kg of MAC for the period of 28 days.

2.3. Sample Collection [24,25]

At the end of the study, before sacrifice, the animals were fasted for overnight with free access to water. Animals were sacrificed with excess anesthesia. Blood samples were collected from retro orbital sinus puncture and stored in EDTA (ethylenediamine –tetra acetate) test tubes for Hematological analysis and in clot activator coated test tubes for serum biochemical analysis. Kidney sample were harvested and carefully investigated for gross lesions. The organ (kidney) were preserved in 10% formalin for histopathological assessment.

2.4. Urine Sample Analysis

Urine samples (24 h) will be collected on the 28th day by keeping the animals in an individual metabolic cage. The animal had free access to drinking water during urine collection period.

2.5.Parameters

The parameters such as serum magnesium, calcium, Phosphate, uric acid and urine biochemistry such as BUN, pH, uric acid and Creatinine was estimated.

2.6. Statistical Method

The statistical analysis was carried by one-way analysis of variance ANOVA (GRAPH PAD PRISM 5 computer program). Results are expressed as ±SEM. The data were statistically analyzed by ONE WAY ANOVA followed by Dunnett's multiple comparison test. Probability P values < 0.05 were considered as significant.

3.Results

3.1. Effect of MAC on Urine output, crystal size and pH of EG Induced urolithiatic rats

Urine output with respect to volume and pH is one of the measurable index of the routine kidney function. Profound increase or decrease in the volume reflects the hindering efficiency of the kidney function. From the data's of the present study it was evident that there was a potential variation in urine volume evidenced with presence of large sized crystal and pH in the EG alone treated rats, whereas treatment with MAC at both the dose level of 200 and 400mg/kg reveals marginal increase in urine volume with severe decrease in the size of urinary crystals and also maintains average urine pH. As shown in table 1 and figure 1.

Table 1: Effect of MAC on Urine output and pH of EG Induced urolithiatic rats

Group	Urine Output Volume in ml	pH
Control Group	7.61 ±0.33	6.63 ± 0.23
EG – Treated Rats	12 ± 0.61	7.53 ± 0.16
EG+ 200 mg/kg MAC	17.32 ± 0.73	6.9 ± 0.18
EG+ 400 mg/kg MAC	21.28 ± 1.18	6.08 ± 0.06

Values represent mean ± SEM of 6 experimental animals

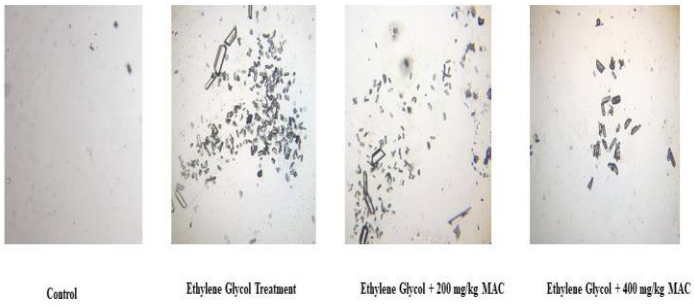

Figure 1: Morphology of Urine Crystals of rats belongs to control , EG treatment and MAC treated groups

3.2.Effect of MAC on Serum biochemistry of EG Induced urolithiatic rats

Results of the present investigation clarifies that there is a significant increase in serological parameters such as blood urea nitrogen (BUN), creatinine and uric acid in EG treated rats. Oral administration of MAC at the dose of 200 and 400 mg/kg shown marginal decrease in serum biochemistry profile of all the rats subjected to MAC treatment. As shown in table 2.

Table 2: Effect of MAC on Serum biochemistry of EG Induced urolithiatic rats

Group	Blood urea nitrogen (BUN)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control Group	13.67 ±1.229	0.466 ± 0.076	3.933 ± 0.33
EG – Treated Rats	37.5 ± 2.975**	1.867 ± 0.033*	9.25 ± 0.11*
EG+ 200 mg/kg MAC	24.33 ± 1.308*	1.167 ± 0.088*	7.35 ± 0.281*
EG+ 400 mg/kg MAC	21± 0.9309 *	0.6667 ± 0.058*	5.483 ± 0.183*

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001.

3.3.Effect of MAC on Urine Biochemistry of EG Induced urolithiatic rats

Reports on urine biochemistry like magnesium, calcium, phosphate and uric acid reveals that there was a profound increase in mentioned cations in the EG rats when compared to that of the control rats. Treatment with MAC at the dose of 200 and 400 mg/kg signifies declination in cations including uric acid profile of the treated rats. As shown in table 3.

Table 3: Effect of MAC on Urine Biochemistry of EG Induced urolithiatic rats

Group	Magnesium (mg/24hr)	Calcium (mg/24hr)	Phosphate (mg/24hr)	Uric acid (mg/dl)
Control Group	4.86 ± 0.15	5.74 ± 0.26	36.53 ± 1.47	9.1 ± 0.16
EG – Treated Rats	1.10 ± 0.02*	24.45 ± 1.43*	80.67 ± 1.20*	17.35 ± 0.51*
EG+ 200 mg/kg MAC	2.96 ± 0.20*	17.85 ± 0.76*	53.5 ± 1.31*	13.7 ± 0.47*
EG+ 400 mg/kg MAC	3.36 ± 0.12*	14.53 ± 0.94*	46.83 ± 1.8*	11.2 ± 0.43*

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001.

3.4.Effect of MAC on weight and histology of urolithiatic rats

Mean weight (2.61±0.02 gms) of kidney belongs to control rats was significantly higher than the control rats (1.29 ±0.05 gms). Treatment with MAC at the dose of 200mg/kg (2.25±0.09 gms) and 400g/kg (1.83 ±0.06 gms) significantly restores the weight almost to the normal index. Histological finding reveals normal glomerulus surrounded by a narrow capsular space and the parietal layer of Bowman's capsule. Epithelial lining on proximal convoluted tubule appears normal. Lumen of distal convolutes tubule and collecting duct was normal in sample belongs to control group rats. Ethyl glycol treated group reveals hypertrophic tubules, others are dilated and evident with glomerular degeneration and increased crystal deposition. Significantly reduced level of crystal deposition was observed in sample belongs to MAC 200mg/kg and 400 mg/kg treated rats. Accumulation of calcium oxalate deposits inside the tubules was much controlled in treatment group when compare to EG alone treated group. As shown in table 4 and figure 2.

Table 4: Effect of MAC on kidney weight of EG Induced urolithiatic rats

Group	Kidney weight (gms)
Control Group	1.29 ± 0.05
EG – Treated Rats	2.61 ± 0.02
EG+ 200 mg/kg MAC	2.25 ± 0.09
EG+ 400 mg/kg MAC	1.83± 0.06

Values represent mean ± SEM of 6 experimental animals

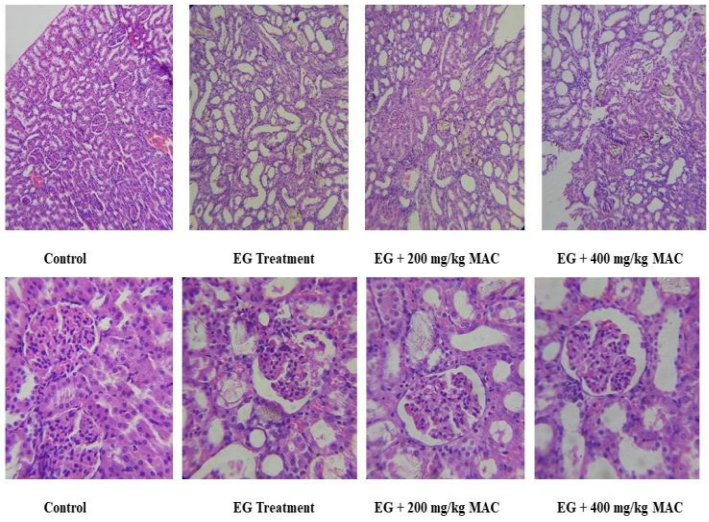

Figure 2: Histopathology of rat Kidney belongs to control ,EG treatment and MAC treatment groups under low and high power magnification

4.Discussion

Urolithiasis refers to the solid non-metallic minerals in the urinary tract. This is the third most common condition of the urinary tract after urinary tract infection and pathologic condition of prostate [26]. Urolithiasis is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidneys. The formation of kidney stones involves several physicochemical events beginning with crystal nucleation, aggregation, and end with retention within the urinary tract [27]. Among the several types of kidney stones, the most common are calcium oxalate stones representing up to 80% of the analyzed stones [28].

The surgical operation, lithotripsy, and local calculus disruption using high-power laser are commonly used techniques to remove the calculi. However, these procedures are associated with the risk of acute renal injury leading to decrease in renal function. Moreover,

an increase in stone recurrence is also observed [29]. Hence exploration of traditional supplement of herbal origin grabs the attention of the researcher around the globe. Siddha medicine pioneers the concept of rejuvenation and healing with its composite medicine with unique blend of herbal and minerals. From the data's of the present study it was evident that there was a potential variation in urine volume evidenced with presence of large sized crystal and pH in the EG alone treated rats, whereas treatment with MAC at both the dose level of 200 and 400mg/kg reveals marginal increase in urine volume with severe decrease in the size of urinary crystals and also maintains average urine pH.

A kidney stone is a hard mass developed from crystals that separate from the urine within the urinary tract. Normally, urine contains chemicals that prevent or inhibit the crystals from urinary tract. These crystals remain tiny enough; they will travel through the urinary tract and pass out of the body in the urine without being noticed. A less common type of stone is caused by infection in the urinary tract. This stone is called struvite or infection stone. Another type of stone, uric acid stones, are a bit less common, and cystine stones rare [30]. Kidney stones are composed of inorganic and organic crystals amalgamated with proteins. Crystallisation and subsequent lithogenesis can happen with many solutes in the urine. Calcareous stones are still by far the most common nephroliths, 17 % accounting for more than 80% of stones [31]. In the present study reports on urine biochemistry like magnesium, calcium, phosphate and uric acid reveals that there was a profound increase in mentioned cations in the EG rats when compared to that of the control rats. Treatment with MAC at the dose of 200 and 400 mg/kg signifies the declination in cations including uric acid profile of the treated rats.

Blood urea nitrogen and creatinine is the marginal index of the normal kidney function, filtration efficiency of the kidney is calibrated with the sense of BUN and creatinine level. Increased level of these biomarkers impregnates the imparted kidney potential. Results of the present investigation clarifies that there is a significant increase in serological parameters such as blood urea nitrogen (BUN), creatinine and uric acid in EG treated rats. Oral administration of MAC at the dose of 200 and 400 mg/kg shown marginal decrease in serum biochemistry profile of all the rats subjected to MAC treatment.

5. Conclusion

Alternate medicines comprises of medicinal herbs known to contain multiple chemical constituents, which could offer a synergistic actions likely to offer more effective and safer remedy particularly in urolithiasis. Therefore, there is a need to look for an alternative therapy, especially herbal remedies, for the management and treatment of urolithiasis.

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