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EFFICACY OF *ANDROGRAPHIS PANICULATA* ON ETHYLENE GLYCOL INDUCED NEPHROLITHIASIS IN ALBINO RATS

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ABSTRACT
Nephrolithiasis is the third most common disorder of the urinary system with high recurrence rate. Clinical treatment of lithiasis is costly and can cause harmful side effects and hence nowadays phytotherapy have higher clinical significance in kidney stone management. Aim of the study is to investigate the antilithiatic property of the methanol extract of *Andrographis paniculata* (MEAP) in experimentally induced nephrolithiatic rats. Nephrolithiasis was induced in Wistar albino rats by administering the dose of 0.75% ethylene glycol for a period of 28 days. The experimental animals were divided into nine groups. Group I served as normal control. Group II received standard antilithiatic drug and group III as the lithiatic control. Group IV and V indicated as preventive regime, received 400mg/kg and 200mg/kg body weight MEAP and group VI, VII, VIII and IX indicated curative regime of which VI and IX received 400mg/kg extract and VII and VIII at 200mg/kg body weight of MEAP. At the end of the experimental period, serum (creatinine, blood urea, BUN, uric acid) urine (protein, calcium and phosphorus) and kidney (calcium and phosphorus) were analyzed. All the elevated biochemical parameters in EG received group were declined in the MEAP treated groups at dosage of 200 and 400mg/kg. Urinary protein, phosphorus and calcium also declined in both MEAP treatment groups than the lithiatic groups. Serum creatinine declined significantly in high dose received group than its low dose in both post and co treatment groups. A dose dependent effect was observed in all the serum parameters except BUN. Kidney phosphorus and calcium of preventive regime which received MEAP at a high dose of 400mg/kg showed a clear dose dependent effect than the curative regimes. The result of the present study suggests the usefulness of MEAP against nephrolithiasis.
Keywords: Nephrolithiasis, Phytotherapy, *Andrographis paniculata,* Ethylene glycol.

INTRODUCTION

Lithiasis is the formation of calculi or stone which is the concentration of mineral salts in parts of the body such as ureter, urinary bladder, kidney and gallbladder.[1] Nephrolithiasis or renal stone formation is worldwide in distribution and a common disorder estimated to occur in approximately 12% of population with a recurrence rate of 70-80% on males and 47-60% in females.[2] Urinary stone diseases have afflicted human kind since antiquity and can persist with serious medical consequences throughout a patient's life time. Kidney stones occur more frequently with increasing age and among men. The recurrence of urolithiasis represents a serious problem as patients who have formed one stone are more likely to form another. Once recurrent, the subsequent relapse risk of lithiasis is raised and the interval between recurrences is shortened.[3] *Andrographis paniculata* is one of the most commonly used medicinal herb in traditional Indian medicine belongs to the family Acanthaceae. Synonyms of the plant are Creat, Green chiretta, King of Bitters, Bhoonimba and Kirayat. The herb is widely distributed in tropical Asian countries. This versatile herb has been used to treat wide variety of diseases. The leaves and root of *Andrographis paniculata* is used indigenously in medicine particularly as bitter tonic curing fevers, dysentery and eliminating intestinal worms. The plant is used to relieve gripping, irregular stools and loss of appetite in case of infants. *Andrographis paniculata* is recognized for its reported blood purifying properties and has been used in traditional Asian medicine to treat conditions of impurities in blood such as boils, scabies and chronic fever.[4] Chinese medicine uses this plant to treat laryngitis, dysentery and coughs. Leaves are suggested to have antiviral, anticlotting and antibacterial properties.[5] Other than these, several properties of *Andrographis paniculata* have been revealed. The plant has reported with hepatoprotective effect[6], hyperglycemic activity[7], anticarcinogenic effect[8], cardiovascular activity[9], Anti HIV activity[10], renoprotective effect[11].

MATERIALS AND METHODS

Collection and Preparation of plant extract

The plant *Andrographis paniculata* was collected from the Botanical Garden, University Campus, Kariavattom (80 37' 36N, 76^{0}50$^{'}$ 14E) Thiruvananthapuram, India. It was authenticated by the Department of Botany, University of Kerala, India. The whole plant material was thoroughly washed, shade dried and powdered with a mechanical grinder and stored in airtight containers. The methanol extract of the plant material was extracted using soxhlet apparatus and was concentrated using rotary vacuum evaporator.

Experimental animal

Healthy adult male albino rats of Wistar strains were used for the present study. The animals were housed in polypropylene cages maintained in controlled temperature (27±2°C) with a 12hr light/dark cycle. They were provided with standard rat chow and drinking water *ad libitum*. The ethical clearance has been obtained from the Institutional Animal Ethical Committee prior to the beginning of the experiment (Approval no: IAEC-KU-23/2011-12-ZOOL-GP (3).

Experimental design

Induction of urolithiasis and study protocol

In the present study, urolithiasis was induced in experimental rats by free access to drinking water containing 0.75% Ethylene Glycol (EG) for 28 days. 2% Ammonium Chloride (AC) was given along with EG for the first few days to enhance the stone formation. The healthy male albino rats of

Wistar strains were used for the present study. Animals were weighed and randomly divided into control and experimental groups. EG and AC in drinking water was fed to groups II-IX for the induction of renal calculi till the 28th day. Dose for the methanol extract of plant was selected as 200 and 400mg/kg bodyweight. Group IV and V received the methanolic extract of *Andrographis paniculata* (MEAP) at a dose of 400mg/kg and 200mg/kg body weight respectively after treatment with EG/AC in drinking water. They were served as preventive regimes (PR). Group VI-IX was taken as curative regimes (CR). Among the CR, group VI and VIII received aqueous fruit extract of MEAP (400mg/kg and 200mg/kg body weight) from 15th day till 28th day. Group VII and IX received MEAP (200mg/kg and 400mg/kg body weight) from 1st day till 28th day. All drugs were given once daily by oral route using gastric tube. All animals were provided with standard feed and water *ad libitum.* The experimental groups are mentioned below

Group I: Normal control- received regular standard rat feed and drinking water *ad libitum.*

Group II: Antilithiatic control- received standard antiurolithiatic drug cystone (750mg/kg body weight).

Group III: Lithiatic control- received 0.75% EG and AC in drinking water till 28th day.

Preventive regime

Group IV: received at a dose of 400mg/kg body weight of MEAP (1-28 days)

Group V: received at a dose of 200mg/kg body weight of MEAP (1-28 days)

Curative regime

Group VI: received MEAP (15th-28th) at a dose of 400mg/kg body weight.

Group VII: received MEAP (1st-28th) at a dose of 200mg/kg body weight.

Group VIII: received MEAP (15th-28th) at a dose of 200mg/kg body weight.

Group IX: received MEAP (1st-28th) at a dose of 400mg/kg body weight.

Assessment of lithiatic activity

Collection and Analysis of urine and serum

During the experimental period, the rats were hydrated with 5ml of distilled water orally and kept separately in metabolic cages. Animal had deprived of food and water during 24hour urine collection period. The urine samples were collected at the end of the experimental period, centrifuged and a drop of concentrated HCl was added to the supernatant before being stored at 4°C. Supernatant was analyzed for protein, phosphorus and calcium. After the experimental period, animals were sacrificed by cervical decapitation and blood was collected by heart puncture, Serum was analyzed for creatinine, urea, blood urea nitrogen and uric acid.

Kidney homogenate analysis

The animals were sacrificed by cervical decapitation and the abdomen was cut opened to remove both kidneys from each animal. The isolated kidneys were cleaned off to remove the extraneous tissues and one of the kidneys from each animal was dried at 80°C in hot air oven. Dried kidneys were boiled in 10ml of 1N HCl for 30min and homogenized. The homogenate was centrifuged and the supernatant was analyzed for stone forming constituents such as kidney phosphate and calcium.

Statistical analysis

The values were expressed as mean ± SD. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multi comparison test. P values ≤ 0.05 were considered as significant.

RESULTS

The present study was designed to determine the effect of methanol extract of *Andrographis paniculata* on calcium oxalate induced kidney stone in male albino rats. From the acute toxicity study, MEAP was found to be safe, as there was no animal death and gross behavioral changes at a maximum dose of 2000mg/kg body weight. Hence, the therapeutic dose of plant extract was taken as 400mg/kg and 200mg/kg. The oral administration of 0.75% aqueous solution of EG to male Wistar albino rats resulted in elevated levels of urinary, serum and kidney parameters. However, supplementation with methanol extract of *Andrographis paniculata* (MEAP) lowered the levels of all these parameters and varied with dosage among treatment groups. When compared to the group II (cystone control), the lithiatic group had increase in serum creatinine concentration. A significant ($P<0.01$) reduction in creatinine was observed from group IV (post treatment) and groups, VII, VIII and IX (co treatment). Animals received 400mg/kg of body weight had significant ($P<0.01$) reduction in creatinine than 200mg/kg received groups ($P<0.01$). Among the co and post treated groups, animals received 400mg/kg body weight from 1-28 days (group IX) had very high significant reduction in serum creatinine (0.69±0.03). When compared to group I and group II, significant increase in blood urea was observed in EG treated animals. The elevated blood urea level was found to be decreased in all the MEAP treated animals. Both post and co-treatment groups exhibited significant decrease ($P<0.01$) in urea than lithiatic groups. Among the co-treatment groups, animals received the MEAP at 400mg/kg had significant reduction in blood urea concentration. Blood urea nitrogen (BUN) increased significantly ($P<0.01$) in lithiatic groups when compared to control and cystone treated animals. Highly significant reduction ($P<0.01$) in blood urea nitrogen was recorded from all the fruit extract treated groups of animals (group IV- group IX) when compared with the EG control groups. Co- treatment group (group VI) supplemented with 400mg/kg body weight exhibited a remarkable decrease (9.25±0.28) in BUN than other extract received groups (Figure 1-3). The analysis of the statistical values of serum blood urea nitrogen showed that the plant extract reduced the BUN in all the treatment groups than the standard anti-urolithiatic drug, cystone. The increased uric acid value in group III is decreased in all MEAP treated animals. Among all extract treated groups very high reduction in uric acid value was noticed from Group VI (400mg/kg body weight) of co- treated animals and the value is nearly equal to the cystone received animals [Table 1].

TABLE 1: EFFECT OF MEAP ON KIDNEY PHOSPHORUS AND CALCIUM

Treatment Groups	Phosphorus (mg/dl)	Calcium (mg/dl)
G I Normal control	2.54±0.42	5.60±0.63
G II Cystone control(750mg/kg)	3.82±0.10	1.07±0.21
G III Lithiatic control	14.77±1.29 a** *b***	12.47±0.83 *a***
G IV Post Treatment (400mg/kg)	10.10±0.75 *c***	2.32±0.71
G V Post Treatment (200mg/kg)	5.56±0.81 *d***	0.52±0.13 *c***
G VI Co Treatment (400mg/kg)(15-28 days)	2.98±0.06 *c*** *e***	0.71±0.26 *c***
G VII CO Treatment (200mg/kg)(1-28 days)	5.43±0.88 e**	0.45±0.06 *c**
G VIII CO Treatment (200mg/kg) (15-28 days)	8.65±0.67 *c*** *e***	1.54±0.28 *c**
G IX CO Treatment (400mg/kg) (1-28 days)	0.21±0.03 *c*** *e***	5.12±1.14 *c***

Values are expressed as mean ± SE. *a*-indicates significant difference with normal control groups, *b*- indicates significant difference with lithiatic control groups, *c*-indicates significant difference with cystone treated groups, *d*- indicates significant difference with CR, e-indicates significant difference with PR. *-P<0.05, **-P<0.01.

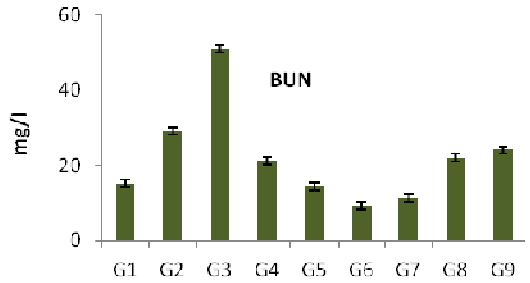

Figure 1: Effect of oral administration of MEAP on serum BUN

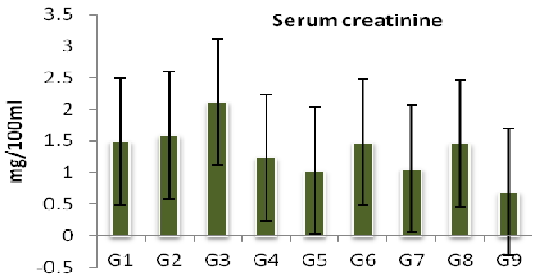

Figure 2: Effect of oral administration of MEAP on serum creatinine

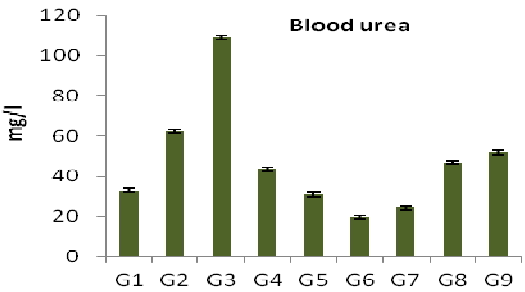

Figure 3: Effect of oral administration of MEAP on serum blood urea

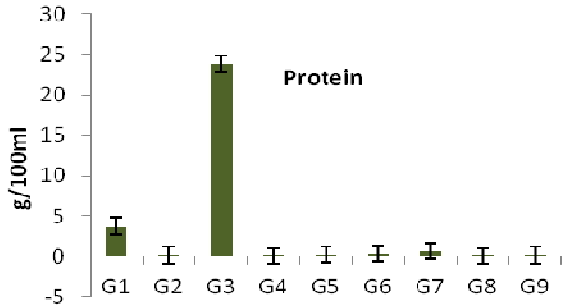

Figure 4: Effect of oral administration of MEAP on urinary protein

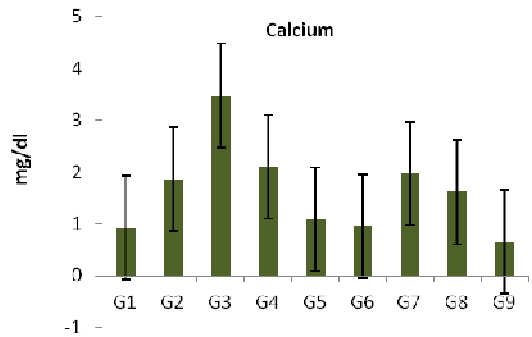

Figure 5: Effect of oral administration of MEAP on Calcium

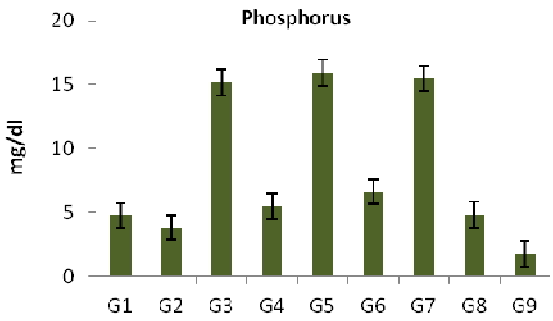

Figure 6: Effect of oral administration of MEAP on phosphorus

All urinary parameters found to be increased in group III lithiatic control animals. When compared to the lithiatic control group, a significant (P<0.01) decrease in the level of protein was noticed in both curative (group IV, group V) and preventive regimes (group VI, group VII, group VIII and group IX). The values were in the same range of standard anti urolithiatic groups. Among the post treatments, the group IV received the extract at a dose of 400mg/kg and had very low urinary protein. Among the co-treatment groups, group VIII and group IX had decreased urinary protein excretion. Urinary phosphorus concentration was found to be decreased in both regimes and significant (P<0.01) decrease was observed in preventive regimes (group IX and group VIII) than groups of curative regime. Significant (P<0.01) decrease in urinary calcium was observed in preventive and curative regimes of MEAP treated groups when compared to lithiatic control. Among the groups, VI, VII, VIII and IX of preventive regimes and V of curative regime had significant decrease in urinary calcium concentration. The decreased urinary calcium values were also noticed in group IV (2.10±0.59) but the values are statistically insignificant (Figure 4-6). Kidney analysis showed that there was a significant (P<0.01) increase in kidney phosphorus and calcium level was observed from lithiatic control groups when compared to control and cystone treated ones. Both preventive and curative regime had significant (P<0.01) reduction in kidney phosphate value when compared to group II. Co- treated groups of animals exhibited a remarkable significant decrease in phosphate concentration than the post treated animals. Among the co-treated groups, group IX received 400mg/kg MEAP from 1-28 days had very low kidney phosphate level (0.21±0.03) than its low dose. In the case of kidney calcium, group V (200mg/kg) of post treated animals had significant decrease in calcium when compared with EG control groups. In the co treatments, group VI, VII, VIII and IX showed significant (P<0.01) reduction in elevated kidney calcium in group III [Table 1].

DISCUSSION

The study has been conducted to demonstrate the efficacy of *Andrographis paniculata* in reducing and preventing the renal lithiasis induced by ethylene glycol (EG). Administration of EG to experimental rats increased the risk of hyperoxaluria by the formation of high levels of calcium oxalate crystals in kidney. In the present study, male rats were chosen for the experimental study as the prominence for lithiasis is more in male sex and the urinary system show close resemblance to human being. The pathogen city and toxicity of ethylene glycol lies within its conversion to metabolites. Thus before metabolism, it is purely nontoxic. The mechanism of action of ethylene glycol in body involves four step processes and it takes place in the vital organ, liver. In the first step, ethylene glycol gets converted to glycoaldehyde, glycolate and glycoxylate. Cellular metabolic enzymes get berated by the activity of glycolate resulting in the formation of metabolic acidosis, a condition of poisoning by ethylene glycol. The final step involves the conversion of glycoxylate to oxalate. The oxalates were precipitated into calcium oxalate and deposited as crystals in the nephrons.[12] In urine there are number of crystalloids of different types such as oxalate, uric acid, calcium and cystine which are kept in solution by the presence of colloids like mucin. When there is an imbalance in the crystalloid- colloid ratio, formation of renal stones takes place by the action of promoters. The experimental animals received EG showed a progressive increase in oxalate and calcium excretion[13]. Similar results were obtained from the present study. The elevated calcium concentration has greater importance than that of the oxalate levels in urine.[14] Increased levels of urinary calcium thus promote nucleation and precipitation of calcium oxalate crystals and its subsequent crystal growth.[15] However, on treatment with MEAP, reduction in the calcium level was observed in a dose dependent manner. The extract performed an effective role in both preventing the stone formation and dissolving the pre-formed calcium oxalate stones. Mucoproteins direct the crystallization of urinary oxalic acid complexes with calcium and forms insoluble Caox stones.[16] Flavonoids act by disintegrating the mucoproteins and prevent calcium oxalate deposition and its excretion in kidneys.[17] MEAP revealed the presence of flavonoids as secondary metabolite. Their action may have reduced the calcium and oxalate deposition by pre-coating calcium oxalate crystals and disintegrating the mucoproteins. Urinary phosphate levels also attain an elevation in lithiatic group. The increase of urinary phosphate together with oxalate stress triggered the degree of stone formation resulting in calcium phosphate crystals, which leads to calcium phosphate deposition.[18] Biochemical changes in the urine and blood are expected to produce changes in the process of stone formation in the kidney tubules.[19] Administration of MEAP to the treatment groups, alleviated the phosphate level thus restoring its concentration and reducing the risk of lithiasis. Proteins are the predominant macromolecules in the urine[20]. The organic matrix of stones in kidney is highly rich in protein. They contribute a directive role in stone formation.[21,22] Urinary proteins modulate the process of crystallization so the presence of these proteins in crystal matrix indicates their involvement in modulating crystallization. A decrease in urinary protein level was observed in the animal groups which received MEAP which revealed the crystallization reducing property of MEAP. Serum biochemical parameters such as creatinine, urea, uric acid and blood urea nitrogen showed an escalating effect in the lithiatic control when compared to the treatment groups. This is due to the occurrence of severe renal damage in lithiasis. In the case of lithiasis, obstruction in the urinary outflow appears to be responsible for the decrease in glomerular filtration rate (GFR).[23] As a consequence, the waste products including the nitrogenous substances such as creatine, urea, uric acid and blood urea nitrogen accumulates and increases its concentration in blood. Treatment with MEAP significantly alleviated all the serum parameters in a dose dependant manner. In rats which received ethylene glycol observed an increase in the weight of kidneys. This condition may be due to water retention and inflammation of epithelium of nephrons as observed by Reza *et al*[24] On analyzing it is noticed to have a significant elevated level on kidney parameters such as calcium and phosphorus. From the study it is concluded that the antilithiatic activity of *Andrographis paniculata* at a dosage of 200mg/kg and 400mg/kg shows dose dependent curative effect. Qualitative chemical analysis of the plant extract of *Andrographis paniculata* revealed the presence of saponins and flavonoids. The antiurolithiatic effect is due to the ability of plant to disintegrate and disrupt calcium oxalate calculi. The major bioactive component of *Andrographis paniculata* is andrographolide which contribute the efficacy to the plant. Since there is no effective drug to treat recurrent urolithiasis,

the use of *Andrographis paniculata* in herbal therapy makes a wide scope. In conclusion the present study reports that the methanol extract of *Andrographis paniculata* exhibits a significant antilithiatic effect in ethylene glycol induced lithiasis in male wistar albino rats. It also suggests further findings on therapeutic and preventive effects of *Andrographis paniculata* on kidney calculus formation in human.

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