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ANTILITHIATIC ACTIVITY OF POLYHERBAL EXTRACT ON ETHYLENE GLYCOL INDUCED UROLITHIASIS IN MALE WISTAR RATS

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ABSTRACT:

Kidney stones are common in all kinds of urolithiasis. The study focuses on Polyherbal extracts containing Boerhavia diffusa and Celosia argentea and Plumeria rubra seed pod was prepared and evaluated for antilithiatic byin vivo method. The traditional system of medicine includes the use of many herbal medicines for the treatment of kidney stone due to the presence of Flavonoids, triterpenoids, glycosides, and other chemicals having different mode of action. This study was conducted on Ethylene glycol-induced rats to examine the antilithiatic potential of selected herbs.

Objective:

To examine the Antilithiatic activity of Polyherbal extract on urine and serum parameters in Ethylene Glycol induced urolithiasis in male Wistar rats.

Method:

In this study Wistar rats were kept in metabolic cage separately from the day of starting the experiment until entire duration of 28 days of experiment, having six rats in each group. Urolithiasis was generated by administering ethylene glycol (0.75% v/v) in drinking water orally. Cystone 750mg/Kg is used as a standard for comparing the activity of plant extracts. Polyherbal extracts were used in two concentrations i.e. 200 and 400mg/Kg for 28 days.

Results:

It was found from urine analysis that, in disease control animals' calcium, phosphorus and oxalate excretion were significantly increased, while the magnesium level decreased when compared with Normal group animals. Also from serum analysis it was found that the treatment of rats with Extract 1 and 2 (200 and 400 mg/kg, p.o.) and Cystone (750 mg/kg, p.o.) exhibited significant ($p<0.05$; $p<0.01$; $p<0.001$) increment in Serum Calcium, Serum Phosphorus, Serum BUN, Serum Creatinine and increment in Serum Uric acid when compared with DC rats. Thus polyherbal extract of 400mg/Kg of all three plants was found to be effective for treating Kidney stones.

KEYWORDS: Polyherbal, Antilithiatic, Serum calcium, phosphorus, uric acid.

I. INTRODUCTION:

Kidney stone is common in all kinds of urolithiasis. The kidney stone develops due to decrease in volume of urine formation or increase in excretion of stone forming components like urate, cysteine, xanthine, calcium oxalate and phosphate. Urolithiasis is a common health problem with increasing prevalence of up to 20% all over the globe. The increased prevalence of the disease occurs due to the lifestyle changes such as lower dietary intake of vegetables or fruit, higher consumption of animal proteins, salt, sweetened beverages, and inadequate fluid intake. Amongst the nephrolithiasis calcium oxalate stone occurs most commnly.[1]For curing urinary stone disorders, numerous treatment plans have been established in recent decades. There are currently numerous techniques available within the NHS for removing lower pole kidney diseases like percutaneous nephrolithotomy (PCNL), extracorporeal shockwave lithotripsty (ESWL) and flexible urinoscopy (FURS) with laser lithotripsy. Commonly in about 35% cases stone develops in the lower pole of the kidney.[2] However, the majority of these treatments require surgery called Lithotripsy, making them costly and painful. Due to this, many individuals prefer using traditional herbal remedies to cure urinary stones. In vitro evaluation using dissolution method or titrimetry method is necessary to evaluate effectiveness of herbal medicines.[3, 4]Bladder calculi are usually a manifestation of an underlying pathological condition, including voiding dysfunction or presence of foreign body within the bladder. Vesical calculi are seen mostly in men, but in developing world, it is found in prepubescent boys. Only bladder calculus is commonly seen (75%) rather than multiple stones[5.] Ayurveda, Traditional Chinese medicine (TCM), Siddha, and Unani are having many such traditional medical systems that have described the use of various herbal treatments to treat urinary stone problems.[6-7] Urolithiasis has been regarded as one of the eight most problematic disorders, and in Ayurveda urinary stones are called as mutraashamari (mutra-urine; ashma-stone; ari-enemy) (mahagad). Four different forms of urinary calculi, including phosphatic stones (sleshmaashmari), urate stones (pittaashmari), oxalate stones (vataashmari), and spermolith or seminal concretions (sukraashmari), have been recorded in Ayurvedic writings. Herbal remedies, alkaline drinks, and surgical techniques, Ayurvedic medicine to treat and manage urinary stones.[8-9] Allopathic drugs are having many

side effects. As majority of drugs are excreted through kidney it will worsen the case, so it is better to prefer herbal medicines in treating such ailments as they are generally do not have side effects.

II. MATERIAL AND METHODS:

a. Procurement and Authentication of drugs:

The plants used in the present study consists of whole plant of Boerhavia diffusa Linn, were collected from local area of Khandala, Maharashtra, Matured pods of Plumeria rubra Linn was collected from Rajuri, Junnar Maharashtra. Seeds of Celosia argentea Linn, was purchased from Mankarnika Ayurvedic medical store, Chinchwad Pune. Plants and seeds were authenticated by Dr. R.K. Chaudhary, Scientist from Agharkar Research Institute, Autonomous body under DST, GOI, and Pune.

The collected plant material was washed thoroughly in running tap water, rinsed using distilled water and shade dried for seven days then dried and clean plants were grounded by using laboratory herbal grinding mill. Coarse powder (60#) of dried plants were stored in air tight container for preparing aqueous extract for further in vivo study.

Chemicals: Ethylene Glycol was procured from GS Lab, Pune Maharastra, India. Biochain assay Kit was procured from Ajinkya Enterprises, Pune.

Animals:

Wistar albino male rats weighing between 180 and 250 g were selected for the antiurolithiatic activity. The animals were allowed to stay in metabolic cages so as get accustomed to standard laboratory conditions. Animals were fed with standard rat diet and provided access to drinking water ad libitum. Animal care and animal experiments were conducted in accordance with CPCSEA/IAEC approval no: 1401/PO/RE/S/11/IAEC/2020-21/07/02. Rats used for the study were obtained from the animal house of an authorized local supplier Lachmi Biofarms Pvt. Ltd. Pune Maharastra, India. Rats were housed in groups of six in clean cages. The bedding material of the cages was changed twice weekly.

b. Preparation of extract of polyherbals.

Aqueous extract of Boerhavia diffusa, Plumeria rubra seed pods and Celosia argentea seeds was prepared using Soxhlet extraction method for the period of 48 Hrs. The extract was prepared in two concentrations 200 and 400mg/Kg for in vivo study.

c. Acute toxicity studies

Acute Toxicity Studies: Acute oral toxicity of the Polyherbal formulation was carried out as per the guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423. The principle involves a stepwise procedure with the Use of a minimum number of animals per step to obtain sufficient information on the acute toxicity of the test substance to enable its classification. Healthy male Wistar

rats (3 animals/dose) were used for the experiment. Overnight fasted rats were orally fed with the Polyherbal formulation in increasing dose was 5mg, 50mg, 300 mg and 2000 mg/kg body weight, The animals were observed for their behavioral (alertness, restlessness, irritability, and fearfulness), neurological (spontaneous activity, Reactivity, touch response, pain response, and gait), and autonomic (defecation and urination) profiles continuously for 24 h. After a period of 24 h, the animals were observed for 14 days for mortality. [10]

d. Ethylene Glycol Induced Model of Urolithiasis in male Wistar Rats

The study was so designed to find out effect of aqueous Polyherbal extract of B. diffusa, P. rubra and C. argentea against ethylene glycol induced urolithiasis. Animals were divided into five groups each group containing six animals, kept in metabolic cages individually to collect urine. Group I is serves as Normal group, Group II to V receives ethylene glycol as stone inducer for 28 days. After administration of the same group III treated with cystone 750 mg/Kg as a standard antilithiatic drug (14, 15), Group IV and V were treated with 200 and 400mg/Kg aqueous extract of B. diffusa, P. rubra and C. argentea. After experimental period of 28 days, 24 hrs urine was collected from receivers of metabolic cage and further tested for urine parameters using biochain assay kit. Blood was collected from retroorbital plexus after making the animal unconscious.

Table No 1: Anti-urolithiasis in vivo experimental design

Group	Treatment	No. of Animals
I	Regular diet and water	6
II	Ethylene Glycol (0.75% v/v) for 28 days	6
III	Ethylene Glycol (0.75% v/v) for 28 days + standard drug 750mg/kg (15-28th day)	6
IV	Ethylene Glycol (0.75% v/v) for 28 days + Effective extract 200mg/kg (1-28th day)	6
V	Ethylene Glycol (0.75% v/v) for 28 days + Effective extract 400mg/kg (1-28th day)	6

III. RESULTS AND DISCUSSION:

a. Acute Toxicity Study

At a dose of 2000 mg/kg the extract of B. diffusa did not exhibited any toxic effects as shown by the experimental animals. Also, the dose of up to 2000 mg/kg body weight extract of C. argentea and P. rubra was well tolerated by experimental animals and therefore 200 mg/kg, 400mg/kg body weight (i.e. $1/10^{th}$ and $1/20^{th}$) doses were selected for the study. More research is needed to determine persistent toxic effects.

b. In-Vivo Anti-Urolithiaitic Activity

Urine Parameters

i) Estimation of urine volume and pH

Table No. 2: Estimation of urine volume and pH

PARAMETERS	GROUP-I	GROUP-II	GROUP-III	GROUP-IV	GROUP-V
Urine Volume	2.57±0.11	1.60±0.23###	5.40±0.14***	3.35±0.54***	5.19±0.32***
pH	7.25±0.04	5.7±0.13**	7.29±0.45***	6.30±0.24***	7.24±0.04***

Values are expressed as Mean ± SEM, n=6.

*P ≤ 0.05,

P ≤ 0.01 and*P ≤ 0.001 compared with disease control

From Table no. 02, it can be seen that Urolithiasis induced animals shows decreased in urine volume (Group II), whereas the treated groups (II, III, IV) shows increased in urine volume when compared with Group II rats.

In ethylene glycol induced rats pH was reduced when compared with normal control group. Treatment with standard drug 750 mg/kg and ethanolic extracts of plants were found to increase the urine pH in a dose dependent manner.

ii) Estimation of Calcium, Oxalate, phosphorus and magnesium in Urine

Table No. 3: Estimation of calcium, oxalate, phosphorus and magnesium in urine

PARAMETERS	GROUP-I	GROUP-II	GROUP-III	GROUP-IV	GROUP-V
Calcium	9.54±0.10	13.56±0.08###	10.24±0.11***	12.81±0.15**	10.36±0.169***
Oxalate	1.86±0.07	4.39±0.17###	2.11±0.07***	3.8±0.12**	2.55±0.10***
Phosphorous	5.15±0.20	7.13±0.17###	5.18±0.14***	6.33±0.14**	5.28±0.11***
Magnesium	4.16±0.08	1.73±0.12###	3.33±0.11***	2.11±0.09*	3.18±0.07***

Values are expressed as Mean ± SEM, n=6.

*P ≤ 0.05,

P ≤ 0.01 and*P ≤ 0.001 compared with disease control

From table no. 03, in disease control animals calcium, phosphorus and oxalate Excretion were significantly increased, while the magnesium level decreased when compared with group I animals. When supplement with plant extract significantly lowered the elevated levels of calcium, phosphorus and oxalate when compared with group II animals, and restore the magnesium level when compared with normal animals.

Serum Parameters

Effect of PHF 200 and PHF 400 on Serum Calcium (mg/dl) in Ethylene induced Urolithiasis in Rats

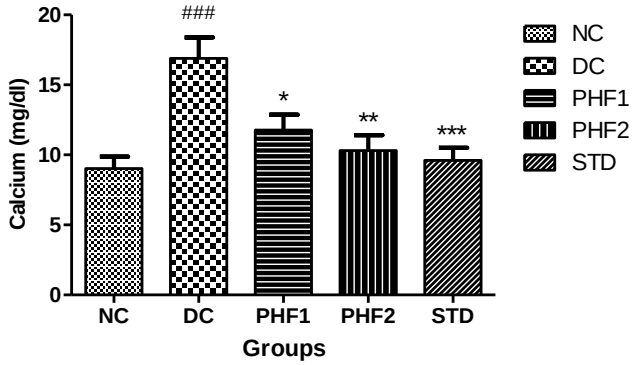

Figure 1: Effect of PHF 200 and PHF 400 on Serum Calcium (mg/dl) in Ethylene induced Urolithiasis in Rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The effects of PHF 1 and PHF 2 on Serum Calcium in EG induced Urolithiasis in Rats in Figure 1. The treatment of rats with EG (0.75 %, w/v) induced significant ($p<0.001$) elevation in Serum Calcium when compared with DC rats. However, the treatment of rats with PHF 1 and 2 (200 and 400 mg/kg, p.o.) and Cystone (750 mg/kg, p.o.) exhibited significant ($p<0.05$; $p<0.01$; $p<0.001$) increment in Serum Calcium when compared with DC rats.

Effect of PHF 200 and PHF 400 on Serum Phosphorus (mg/dl) in Ethylene induced Urolithiasis in Rats

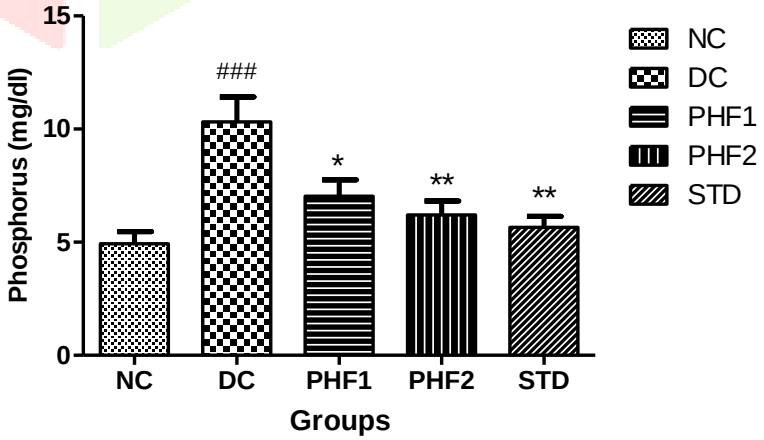

Figure 2: Effect of PHF 200 and PHF 400 on Serum Phosphorus (mg/dl) in Ethylene induced Urolithiasis in Rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The effects of PHF 1 and PHF 2 on Serum Phosphorus in EG induced Urolithiasis in Rats in Figure 2. The treatment of rats with EG (0.75 %, w/v) induced significant ($p<0.001$) elevation in Serum Phosphorus when compared with DC rats. However, the treatment of rats with PHF 1 and 2 (200 and 400 mg/kg, p.o.) and Cystone (750 mg/kg, p.o.) exhibited significant ($p<0.05$; $p<0.01$; $p<0.01$) increment in Serum Phosphorus when compared with DC rats.

Effect of PHF 200 and PHF 400 on Serum BUN (mg/dl) in Ethylene induced Urolithiasis in Rats

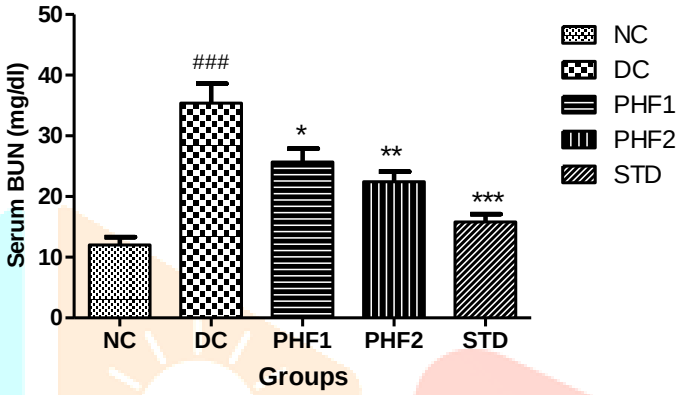

Figure 3: Effect of PHF 200 and PHF 400 on Serum BUN (mg/dl) in Ethylene induced Urolithiasis in Rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The effects of PHF 1 and 2 400 on Serum BUN (mg/dl) in EG induced Urolithiasis in Rats in Figure 3. The treatment of rats with EG (0.75 %, w/v) induced significant ($p<0.001$) elevation in Serum BUN when compared with DC rats. However, the treatment of rats with PHF 1 and 2 (200 and 400 mg/kg, p.o.) and Cystone (750 mg/kg, p.o.) exhibited significant ($p<0.05$; $p<0.01$; $p<0.001$) increment in Serum BUN when compared with DC rats.

Effect of PHF 200 and PHF 400 on Serum Creatinine (mg/dl) in Ethylene induced Urolithiasis in Rats

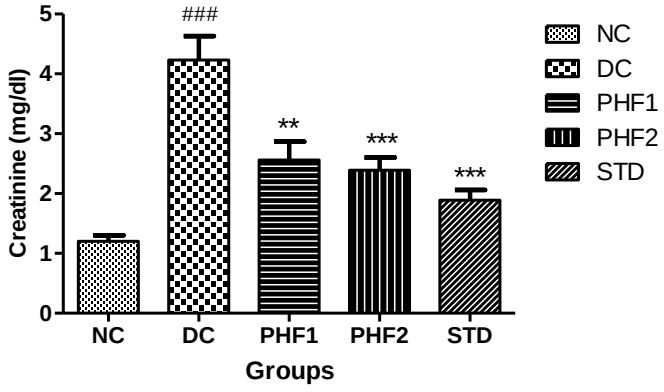

Figure 4: Effect of PHF 1 and PHF 2 on Serum Creatinine (mg/dl) in Ethylene induced Urolithiasis in Rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The effects of PHF 1 and 2 on Serum Creatinine in EG induced Urolithiasis in Rats in Figure 4. The treatment of rats with EG (0.75 %, w/v) induced significant ($p<0.001$) elevation in Serum Creatinine when compared with DC rats. However, the treatment of rats with PHF 1 and 2 (200 and 400 mg/kg, p.o.) and Cystone (750 mg/kg, p.o.) exhibited significant ($p<0.01$; $p<0.001$) increment in Serum Creatinine when compared with DC rats.

Effect of PHF 200 and PHF 400 on Serum Uric acid (mg/dl) in Ethylene induced Urolithiasis in Rats

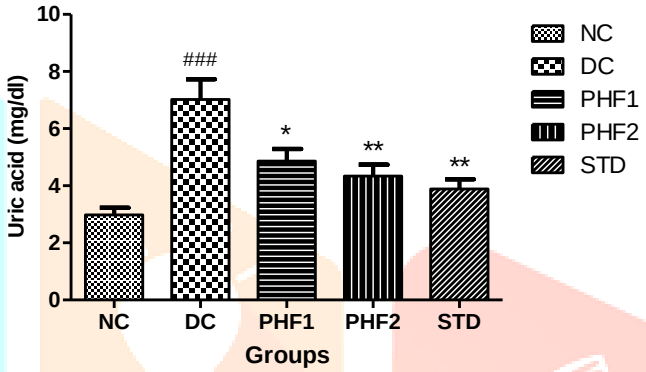

Figure 5: Effect of PHF 200 and PHF 400 on Serum Uric acid (mg/dl) in Ethylene induced Urolithiasis in Rats. Values are expressed as Mean ± SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's Kramer test. $^{###}p<0.001$ versus NC rats and $^{***}p<0.001$ versus DC rats.

The effects of PHF 1 and 2 on Serum Uric acid in EG induced Urolithiasis in Rats in Figure 5. The treatment of rats with EG (0.75 %, w/v) induced significant ($p<0.001$) elevation in Serum Uric acid when compared with DC rats. However, the treatment of rats with PHF 1 and 2 (200 and 400 mg/kg, p.o.) and Cystone (750 mg/kg, p.o.) exhibited significant ($p<0.05$; $p<0.01$; $p<0.001$) increment in Serum Uric acid when compared with DC rats.

IV. CONCLUSION:

The effect three medicinal plants i.e. Boerhavia diffusa, celosia argentea and Plumeria rubra studied by in vivo method for their litholytic activity. The polyherbal extract of 400mg/Kg was shown better effect it was found from urine analysis that, in disease control animals' calcium, phosphorus and oxalate excretion were significantly increased, while the magnesium level decreased when compared with Normal group animals. Also from serum analysis it was found that the treatment of rats with Extract 1 and 2 (200 and 400 mg/kg, p.o.) and Cystone (750 mg/kg, p.o.) exhibited significant ($p<0.05$; $p<0.01$; $p<0.001$) increment in Serum Calcium, Serum Phosphorus, Serum BUN, Serum Creatinine and increment in Serum Uric acid when compared with DC rats. Thus poyherbal extract of 400mg/Kg of all the three plants was found to be effective for the treatment of Kidney stone.

FUTURE SCOPE: Role of these plants in preventing reoccurrence is remains to be assessed. Imbalance in the level of parathyroid and Calcitonin hormone may contribute in reoccurrence of stone is to be assessed.

AUTHOR CONTRIBUTIONS. Guide and Co-guide who have contributed by guiding and providing facilities to conduct this research.

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CONFLICT OF INTEREST: There are no conflicts of interest in present study.

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