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# EVALUATION OF ANTI-UROLITHIATIC ACTIVITY OF ETHANOLIC EXTRACT OF POLY HERBS IN ETHYLENE GLYCOL INDUCED UROLITHIASIS IN RATS

**S. Kalpana*, S. Nelson Kumar, S. Afroz Khan, P.Keerthana, J.Prasanna Lakshmi, E.S.Irfan, M. Lakshmi Amrutha, M.Janardhan Reddy.**

* Department of Pharmacology, P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India.

*Corresponding author E-mail: sana.kalpana35@gmail.com

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**ABSTRACT**

Urolithiasis is one of the most frequent diseases of the urinary tract in the world, displaying an increase in incidence and prevalence in all age groups and genders in the last decades. The aim of the present study is to evaluate the anti-urolithiatic activity of ethanolic extract of poly herbs in ethylene glycol induced urolithiasis in rats. Antiurolithiatic activity is assessed by ethylene glycol induced urolithiasis in rats. Healthy Male Wistar rats were selected and divided into five groups having six animals in each. Group-I served as normal, Group-II as control, Group-III as standard, Group-VI and Group- V as test. In the present study the ethanolic extract of seeds of polyherbs was evaluated for antiurolithiatic activity against 0.75% ethylene glycol and 1% ammonium chloride for induction of renal calculi by using cystone (750 mg/kg) as a standard drug for 28 days. There is a significant restoration of urine and serum parameters exhibiting antiurolithiatic activity of these plants.

## INTRODUCTION

Urolithiasis is defined as formation of stone in the urinary system i.e. in the kidney, ureter, and urinary bladder or in the urethra. Urolithiasis is one of the most frequent diseases of the urinary tract in the world, displaying an increase in incidence and prevalence in all age groups and genders in the last decades, especially in industrialized countries. (1)It determines large costs for the health care systems in the world. Conventional drugs used in the treatment of urolithiasis are often inadequate. Therefore, it is necessary to search alternative drugs for treatment of urolithiasis and to replace the currently used drugs which are doubtful of its efficacy and safety (2)It causes severe acuteback pain and occasionally leads to more severe complications, such as pyelonephritis Or acute renal failure. Kidney stone formation is a common urological problem with a lifetime prevalence of approximately 10% in men and 6% in women.(3)

## MATERIALS AND METHODS:

Preparation of extract:Seeds of *Macrotyloma uniflorum* , *Ocimum basilicum, hordeum vulgare* was taken, powdered in a grinder-mixer to obtain a coarse powder and then passed through 40 mesh sieves. About 200 gms of powder was extracted by using methanol by Soxhlet apparatus process up to 24hrs. The solution was filtered through Whatman filter paper and the resultant filtrate was distilled under reduced pressure for recovery of solvent. The dried extract thus obtained was kept in desiccators and used for further experiments.(4)

**EXPERIMENTAL DESIGN:**

The experimental design used to carry out the antiurolithiatic activity of ethanolic extract of Polyherbs in ethylene glycol induced urolithiasis in rats. Healthy Male Wistar rats (150-200 gms) were selected and divided into five groups having six animals in each. Group-I served as normal and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) & Ammonium chloride (1%) in drinking water was fed in Group-II to Group-V for induction of renal calculi for 28 days. Group-III received standard antiurolithiatic drug Cystone (750 mg/kg b. wt.) Group-VI and Group- V received ethanolic extract of PH 200 mg/kg b. wt. and 400 mg/kg b. wt. for 28 days. Extracts were given once daily by oral route [5].

**RESULTS:**

**Table 1: Effect of seed extract on calcium levels, serum creatinine and Effect on uric acid in ethylene glycol induced urolithiasis On 28th day**

| Groups | Treatment | Calcium (mg/dl) | Creatinine (mg%) | Uric acid (mg%) |
|---|---|---|---|---|
| I. | Normal (Received 1 % water) | 8.649 ± 0.763 | 1.678 ±0.263 | 2.99 ±0.326 |
| II. | Control (Received EG in distilled water) | 16.15 ± 0.82 | 6.394 ±0.286 | 8.246 ±0.286 |
| III. | Standard (Received EG in distilled water + Cystone (750 mg/kg) | 10.10 ± 0.88 | 2.307 ±0.268 | 4.623 ±0.341 |
| IV. | Test-1 (Received EGin distilled water + EEPH 200 mg/kg b. wt. (P.O) | 12.26 ± 0.79 | 4.329 ±0.568 | 6.208 ±0.203 |
| V. | Test-2 (Received EGin distilled water + EEPH 400 mg/kg b. wt. (P.O) | 11.0 ± 1.01 | 3.895 ±0.269 | 9.307 ±0.346 |

Data represents the Mean ± SEM values (n=6). Statistical significance: [a]P<0.05, [b] P<0.01, [c] P<0.001 with respect to Disease control on 28th day by One way ANOVA followed by Dunnetts: Compare all columns vs. Disease control

**Table 2: Effect of seed extract on, urinary output, Urine calcium levels, phosphate levels , and oxalate levels in ethylene glycol induced urolithiasis On 28th day**

| Groups | Treatment | urinary output (ml/day) | Calcium (mg/dl) | Phosphate (mg/dl) | Oxalate (mg/dl) |
|---|---|---|---|---|---|
| I | Normal | 28.41 ± 0.46 | 1.43 ±0.14 | 3.89 ± 0.19 | 2.65 ±0.18 |
| II | Control | 18.42 ± 0.78 | 1.20 ± 0.18 | 7.43± 0.36 | 5.63±0.46 |
| III | Standard | 29.67 ± 1.00 | 1.878 ±0.10 | 5.23± 0.29 | 3.27±0.18 |
| IV | Test-1 | 22.42 ± 1.29 | 2.19 ±0.13 | 6.87± 0.32 | 4.39±0.45 |
| V | Test-2 | 26.71 ± 1.35 | 1.69 ±0.37 | 5.58± 0.42 | 3.85±0.18 |

**Table 3: Effect of seed extract on SOD and CAT levels**

| Groups | Treatment | SOD (U/mg protein) | CAT (µM H2O2 consumed/mg protein) |
|---|---|---|---|
| I. | Normal | 12.547 ± 1.850 | 48.720 ± 3.106 |
| II. | Control | 5.158 ± 1.962 | 22.040 ± 2.646 |
| III. | Standard | 10.325 ± 2.296 | 42.667 ± 1.994 |
| IV. | Test-1 | 5.758 ± 2.745 | 25.210 ± 2.845 |
| V. | Test-2 | 8.601 ± 1.562 | 36.254 ± 1.554 |

Data represents the Mean ± SEM values (n=6). Statistical significance: [a]P<0.05, [b] P<0.01, [c] P<0.001 with respect to Disease control on 28th day by One way ANOVA followed by Dunnetts: Compare all columns vs. Disease control

**DISCUSSION AND CONCLUSION:**

The pathology of urolithiasis was assessed by measuring the urinary parameters include urinary volume, calcium, phosphate, oxalates and serum parameters include calcium, creatinine and uric acid along with Anti-oxidant parameters like CAT, SOD. In the present study, the urinary volume was significantly decreased in ethylene glycol treated animals due to obstruction of stones in the bladder. Cystone treated animals showed significant increase in urinary volume due to its potent diuretic activity. EEPH treated groups (Group IV-V) shows dose dependent increase in urinary volume, compared to disease group. There was a significant raise in calcium, uric acid and creatinine in serum, because of decreased glomerular filtration rate due to obstruction in the urine flow in urinary system with the deposition of calcium oxalate in renal tubule in control group. Cystone treatment showed significant reduction of these elevated levels. EEPH treated grouped animals showed significant reduction in such nitrogenous substances in serum.[6]

SOD and CAT were the most important enzymes in the enzymatic anti-oxidant defense system. SOD scavenges the superoxide anion to form hydrogen peroxide and diminishes the effect caused by the free radical and catalase (CAT), which decomposes hydrogen peroxide and protects the tissue from the highly reactive hydroxyl radical.(7). Depletion in the activities of these enzymes with was observed in disease control rats. Cystone treated animals showed significant increase in the enzyme activities of SOD and catalase. Administration of EEPH showed dose dependent increase in enzymatic activity. The antioxidant activity may be due to the presence of poly phenols such as flavonoids, tannins and saponins, contribute to induce antioxidant potential activity. In the present study the ethanolic extract of seeds of polyherbs was evaluated for antiurolithiatic activity against 0.75% ethylene glycol and 1% ammonium chloride for induction of renal calculi by using cystone (750 mg/kg) as a standard drug for 28 days. There is a significant restoration of urine and serum parameters exhibiting antiurolithiatic activity of these plants.

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