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INVESTIGATION OF TRADITIONAL MEDICINAL PLANTS AND FORMULATION IN THE TREATMENT OF UROLITHIASIS

Miss. Ashvini Arun Kakad, Dr. Rucha A Ingle, Dr. Kailash Biyani

Anuradha College of Pharmacy, Chikhali, Maharashtra, India

Abstract

Urolithiasis, the formation of stones within the urinary tract, is a prevalent disorder with multifactorial etiology, often resulting in severe clinical complications and high recurrence rates. The present study, titled *"Evaluation of Some Traditional Medicinal Plants and Its Formulation in Urolithiasis,"* explores the therapeutic potential of selected traditional medicinal plants in the prevention and management of urolithiasis. The study involved the selection of plant parts based on traditional use—leaves of *Chrysanthemum morifolium* and roots of *Taraxacum officinale*. These were authenticated and successively extracted using solvents of increasing polarity, including petroleum ether, ethyl acetate, methanol, and hydroalcoholic solutions. Preliminary phytochemical screening of all extracts was carried out using standard protocols to identify major bioactive constituents. The hydroalcoholic extract of *Chrysanthemum morifolium* (HACM) and ethyl acetate extract of *Taraxacum officinale* (EATO) were selected for further pharmacological evaluation based on their rich phytochemical profiles. Acute toxicity studies were conducted in healthy albino mice (30–50 g) following OECD guideline 425, which indicated that the extracts were safe up to 2000 mg/kg. Consequently, two doses (100 mg/kg and 300 mg/kg) were chosen for subsequent in vivo studies. The antiurolithiatic potential of the selected extracts was assessed using the calcium oxalate-induced urolithiasis model in rats. Evaluation parameters included urine analysis (volume, pH, oxalate, calcium, and magnesium), serum markers (uric acid, creatinine, and blood urea), kidney homogenate enzyme levels (ACP, ALP, ALT, AST, LDH), and histopathological examination of kidney tissues. The results demonstrated that pretreatment with HACM and EATO, particularly at 300 mg/kg, significantly reduced the formation of calcium oxalate crystals and restored biochemical parameters toward normal. These findings suggest that both extracts exhibit promising antiurolithiatic activity and support their traditional use in the management of urinary calculi.

Keywords: Urolithiasis, Traditional medicinal plants, *Chrysanthemum morifolium*, *Taraxacum officinale*,

1. Introduction

1.1 Background of Urolithiasis

Urolithiasis, commonly known as kidney stone disease, refers to the formation of calculi or stones in the urinary tract, including the kidneys, ureters, bladder, or urethra. It is a multifactorial disorder resulting from the supersaturation of urine with insoluble salts and minerals such as calcium oxalate, calcium phosphate, uric acid, struvite, or cystine. These salts crystallize and aggregate, forming stones of varying sizes and shapes. Urolithiasis remains a global health problem, affecting millions of individuals annually and contributing significantly to morbidity due to acute pain, recurrent infections, hematuria, urinary obstruction, and potential renal impairment (1). Globally, the prevalence and incidence of urolithiasis are on the rise due to changes in dietary habits, sedentary lifestyles, increasing obesity, metabolic syndrome, and dehydration. Epidemiological studies suggest a lifetime risk of kidney stone development ranging from 10% to 15%, with a higher predominance in males compared to females. Moreover, recurrence is common, with approximately 50% of patients experiencing a second episode within 5–10 years. This chronic and recurrent nature necessitates long-term management and preventive strategies (2).

1.2 Pathophysiology and Risk Factors

The formation of kidney stones involves a complex interplay of physicochemical processes, including supersaturation of stone-forming constituents, nucleation, growth, aggregation, and retention within the renal tubules. Factors contributing to stone formation include low urine volume, hypercalciuria, hyperoxaluria, hyperuricosuria, hypocitraturia, urinary tract infections, and anatomical abnormalities (3). Dietary habits—such as high intake of animal proteins, oxalate-rich foods, salt, and sugar—also exacerbate the risk. In contrast, adequate fluid intake and consumption of citrate-rich foods are protective. Oxidative stress and inflammation have recently emerged as significant contributors to the pathogenesis of urolithiasis (4). The deposition of crystals within renal tissue can lead to tubular injury, activation of pro-inflammatory cytokines, and oxidative damage, perpetuating the cycle of crystal retention and stone growth. These insights have spurred interest in therapeutic agents with antioxidant and anti-inflammatory properties for the prevention and treatment of urolithiasis (5).

1.3 Conventional Treatment Modalities

The treatment of urolithiasis depends on the size, location, composition of stones, and the severity of symptoms. Small stones (<5 mm) often pass spontaneously with conservative management including hydration, analgesics, and medical expulsive therapy. Larger or obstructive stones may require interventional procedures such as extracorporeal shock wave lithotripsy (ESWL), ureteroscopy, percutaneous nephrolithotomy (PCNL), or even open surgery. While effective in removing stones, these interventions are associated with several limitations, including high costs, procedural risks, stone recurrence, and potential damage to renal tissue. Pharmacological options such as thiazide diuretics, allopurinol, and potassium citrate are used to prevent recurrence, but they often come with side effects and limited efficacy in certain populations. This therapeutic gap highlights the need for alternative and complementary approaches that are safe, affordable, and effective for both treatment and prevention (6).

1.4 Traditional Medicinal Plants in Urolithiasis Management

The use of traditional medicinal plants for the treatment of urolithiasis has been deeply rooted in various systems of traditional medicine, including Ayurveda, Unani, Siddha, Traditional Chinese Medicine (TCM), and ethnomedicine practiced by indigenous communities. These plants are believed to possess multiple beneficial properties such as diuretic, antispasmodic, litholytic, antioxidant, anti-inflammatory, and nephroprotective effects, which collectively help dissolve or expel urinary stones and prevent their recurrence (7).

Several ethnobotanical surveys and pharmacological investigations have identified a wide range of medicinal plants with potential antiurolithiatic activity. Notable examples include *Crataeva nurvala* (Varuna), *Tribulus terrestris* (Gokshura), *Bergenia ligulata* (Pashanabheda), *Phyllanthus niruri* (Bhumyamalaki), *Boerhaavia diffusa* (Punarnava), *Aerva lanata*, *Didymocarpus pedicellata*, and *Asparagus racemosus*. These plants contain bioactive constituents such as flavonoids, saponins, alkaloids, phenolics, and glycosides that contribute to their pharmacological effects (8).

Modern scientific studies have demonstrated that these phytochemicals can inhibit crystal formation, reduce urinary supersaturation, modulate renal function, and attenuate oxidative stress and inflammation. For instance, *Phyllanthus niruri* has shown promising litholytic and nephroprotective activity in both preclinical and clinical studies. Likewise, *Tribulus terrestris* and *Bergenia ligulata* have been extensively studied for their diuretic and anti-crystallization effects (9).

1.5 Need for Scientific Validation and Formulation Development

Despite the widespread traditional use and anecdotal evidence supporting the efficacy of these plants, there is a pressing need for systematic scientific validation through phytochemical standardization, in vitro and in vivo pharmacological screening, and clinical trials. Standardization ensures consistency in the quality, safety, and efficacy of herbal products, which is critical for their integration into modern therapeutic regimens (10).

Additionally, formulating these herbal agents into appropriate dosage forms—such as capsules, tablets, syrups, or decoctions—is essential for enhancing patient compliance, bioavailability, and therapeutic outcomes. Advances in pharmaceutical technology, such as the use of nanoparticle carriers, phytosomes, and controlled-release systems, may further improve the delivery and efficacy of plant-based antiurolithiatic agents (11).

Developing polyherbal formulations that synergistically combine the beneficial effects of multiple plants is another promising strategy. Such formulations, guided by traditional knowledge and modern scientific insights, can target multiple pathophysiological pathways of urolithiasis and offer a holistic therapeutic solution (12).

1.6 Research Gap and Rationale

Although numerous plants have been traditionally employed and preliminarily investigated for their antiurolithiatic potential, there remains a gap in translating these findings into standardized, evidence-based, and clinically validated formulations. Most existing studies are limited to animal models, lack detailed mechanistic insights, and often overlook the pharmacokinetics, toxicity, and long-term safety of herbal agents (13).

Furthermore, limited availability of comprehensive phytochemical profiles, standard markers for quality control, and robust clinical trials hinder the mainstream adoption of herbal medicines for urolithiasis. This underscores the necessity of a multidisciplinary research approach integrating ethnobotany, pharmacology, phytochemistry, toxicology, and pharmaceutics (14).

The present research aims to bridge this gap by systematically investigating selected traditional medicinal plants used for urolithiasis, evaluating their pharmacological activity, identifying their bioactive compounds, and developing scientifically validated formulations. The study will focus on both mono- and polyherbal approaches, guided by traditional formulations and modern design principles (15).

2. Material Methods

2.1 Preparation of extracts:

Chrysanthemum morifolium Teraxacum officinale plant material were successively extracted by maceration using petroleum ether, ethyl acetate, methanol, hydro - alcohol and water as solvents (16).

2.2 Preliminary phytochemical analysis of extracts

All these extracts were subjected to preliminary phytochemical analysis using established method. Based upon results of preliminary phytochemical analysis, the most potent extract having prominent presence of potential phytoconstituents were selected for further pharmacological screening (17).

2.3 Pharmacological screening of the extracts for antiurolithiatic activity

Swiss albino mice and Wistar rats were used for acute oral toxicity study and antiurolithiasis study respectively. They were maintained at 25 ± 2° C and relative humidity of 45 to 55% and under standard environmental conditions (12 hr. light 12 hr. dark cycle). The animals had free access to food and water. Institutional Animal Ethical Committee (IAEC) approved the protocol. All experiments were carried out between 12:00- 16:00 hour (18).

2.4 Calcium oxalate induced urolithiasis

Experimental design

Thirty Six rats were divided into six groups, each containing six animals (n=6) and were subjected to groupwise specific treatment as per the details tabulated below for the period of 28 days (19).

Group	Treatment	Dose and route
I (normal control)	Distilled water	10 ml/kg p.o.
II induction control)	Ethylene glycol + Distilled water	0.75 % v/v in Drinking water + 10mg/kg, p.o.
III	Ethylene glycol + HACM	0.75 % v/v in Drinking water + 100 mg/kg p.o.
IV	Ethylene glycol + HACM	0.75 % v/v in Drinking water + 300 mg/kg p.o.
V	Ethylene glycol + EATO	0.75 % v/v in Drinking water + 100 mg/kg p.o.
VI	Ethylene glycol + EATO	0.75 % v/v in Drinking water + 300 mg/kg p.o.

Table 1 : Drug treatment schedule for Calcium oxalate induced urolithiasis in rats.

On 28th day, following parameters were evaluated:

1. **Urine analysis:** Animals were placed individually in metabolic cages for 24 hrs and collected urine was subjected to evaluations.
   a) Measurement of urine volume
   b) Determination urine pH
   c) Estimation of urinary oxalate level
   d) Estimation of urinary calcium level
   e) Estimation of urinary magnesium level

a) Urine volume

Animals were placed in separate metabolic cages for 24 h and total urinary volume was measured using the measuring cylinder and reported in ml (20).

Urine pH

Uric acid crystals were found to deposit most frequently in the concentrated acidic urine. Thus, the acidity of the urine was tested using the pH meter (21).

b) Urinary oxalate

One ml of urine was acidified by concentrated HNO_3 to solubilize crystals and then adjusted to pH 7 by NaOH in the presence of color indicator, the bromothymol blue. About 2ml of saturated $CaSO_4$ and 14 ml of pure ethanol were added to precipitate oxalate overnight. The samples were centrifuged at 450 × g for 10 min and then filtered on filter paper. The precipitate obtained was solubilized in 10 ml of water acidified by 2ml of concentrated sulfuric acid. The samples were titrated by a solution of $KMnO_4$ (22).

c) Urine calcium: *o-cresolphthalein complexone method*

It was estimated by using commercially available standard kit of Biolab diagnostics Pvt. Ltd. Tarapur (India). Determination of urine calcium was done by using CHARIOT prince autoanalyser (23).

Kidney homogenate analysis:

After blood collection, the animals were sacrificed by cervical dislocation, the abdomen was cut open and kidneys were removed. Isolated kidneys were cleaned off extraneous tissue and rinsed in ice-cold physiological saline. The kidneys of animals were finely minced and 10 % homogenate was prepared in Tris-Hcl buffer (0.02 mol/l, pH 7.4) (24). The homogenate using kidneys of three animals was prepared and analyzed according to the method of King (1965a), King & Armstrong (1934), Reitman & Frankel (1957) and King (1965b)

3. Results

Table 2 Preliminary phytochemical screening of *Chrysanthemum morifolium*

Sr. No	Constituents	Pet Ether Extract	Ethyl Acetate Extract	Methanolic Extract	Hydro alcoholic extract	Aqueous Extract
1	Phytosterols	+	-	-	-	-
2	Glycosides	-	-	++	-	-
3	Carbohydrates	-	+	-	++	+
4	Flavonoids	-	+	+	+++	++
5	Alkaloids	+	-	-	-	-
6	Tannins	-	-	+	+	+
7	Proteins	-	-	-	-	-
8	Saponins	-	-	-	+	-
9	Triterpenoids	+	-	-	-	-

Table 3 Preliminary phytochemical screening of *T. officinale*

Sr No	Constituents	Pet Ether Extract	Ethyl Acetate Extract	Methanolic Extract	Hydro alcoholic extract	Aqueous Extract
1	Phytosterols	+	-	-	-	-
2	Glycosides	-	-	+	-	-
3	Carbohydrates	-	++	-	-	++
4	Flavonoids	-	++	-	+	-
5	Alkaloids	-	-	-	-	-
6	Tannins	-	-	+	+	+
7	Proteins	-	-	-	-	-
8	Saponins	-	+++	-	-	+
9	Triterpenoids	+	++	-	-	-

3.1 Calcium oxalate induced urolithiasis

3.1.1 Analysis of urine

The urine volume of rats of group II i.e. induction control showed significant increase ($p<0.01$) as compared to the rats of group I i.e. normal control. The pretreatment with both doses of all extract except EATC &HACM 100 mg/kg showed significant reduction in urine output when compared with induction control. Further HACM 300 mg/kg,and EATC 300 mg/kg and were found to be more effective ($p<0.01$) in this regard.

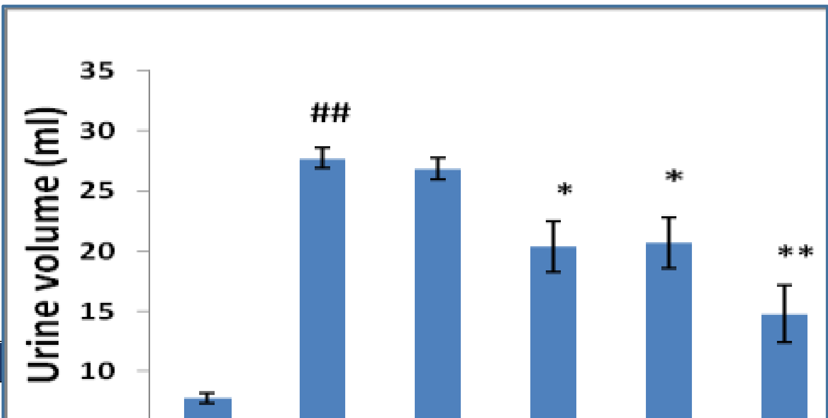

Figure 1: Effect of different extracts on volume of urine in calcium oxalate induced urolithiasis in rats.

3.1.2 Effect of different extracts on urine pH in calcium oxalate induced urolithiasis in rats.

The urine pH was significantly decreased in ethylene glycol treated rats i.e. induction control as compared to rats of normal control group. This change in pH was significantly restored by both extracts at all doses except EATC & HACM 100 mg/kg. Overall EATC & HACM 300 mg/kg was found to be most significant ($p<0.01$).

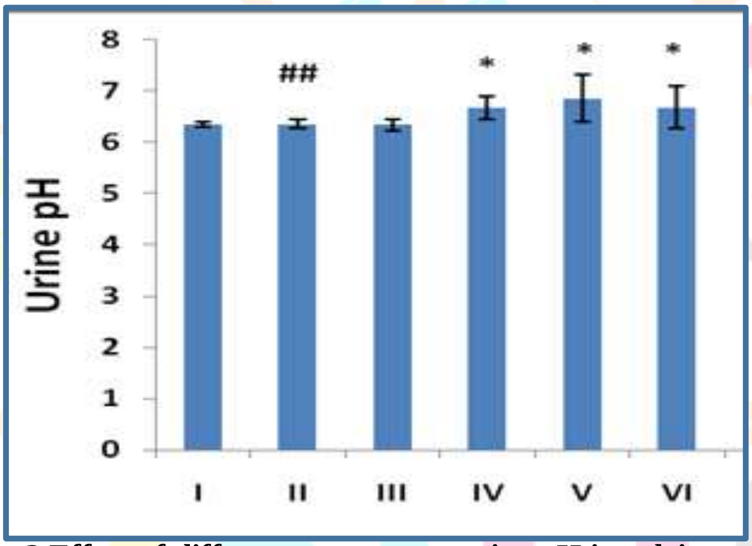

Figure 2 Effect of different extracts on urine pH in calcium-oxalate induced urolithiasis in rats.

3.1.3 Effect of different extracts on urine oxalate levels in calcium oxalate induced urolithiasis in rats

Oxalate levels in the induction control were significantly increased ($p<0.01$) when compared against normal control. All doses of all extract except HACM 100 mg/kg showed significant reduction in this elevated levels.

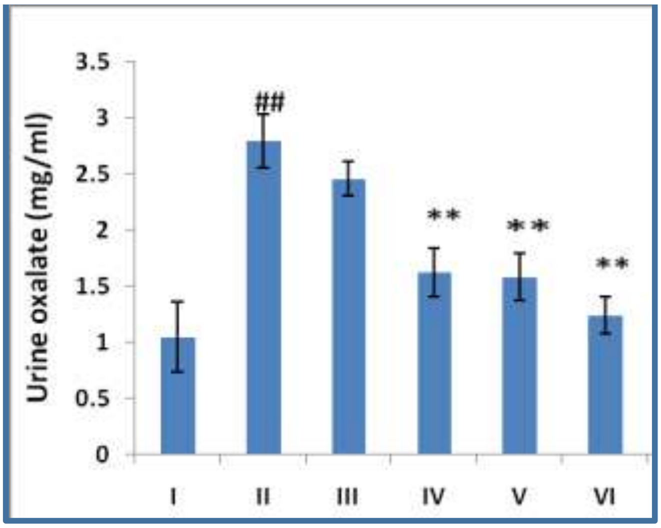

Figure 3 Effect of different extracts on urine oxalate levels in calcium oxalate induced urolithiasis in rats

3.1.4 Effect of different extracts on urine calcium levels in calcium oxalate induced urolithiasis in rats.

Ethylene glycol administration in induction control group resulted in significant ($p<0.01$) increase in the levels of urine calcium as compared to vehicle treated normal control group rats. The co-administration with different extract showed significant reduction in this elevated level except with lower dose of HACM. The results also exhibited dose dependent reduction ($p<0.01$).

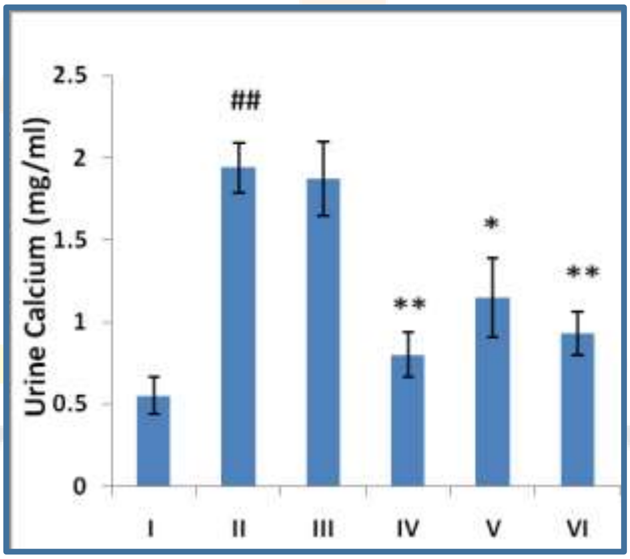

Figure 4: Effect of different extracts on urine calcium levels in calcium oxalate induced urolithiasis in rats.

3.1.5 Effect of different extracts on urine magnesium levels in calcium oxalate induced urolithiasis in rats.

Ethylene glycol administration for 28 days resulted into significant reduction ($p<0.01$) in the urine magnesium levels when compared to vehicle treated normal control rats. This reduction showed significant restoration with all doses of all extracts except E A T C 100 mg/kg.

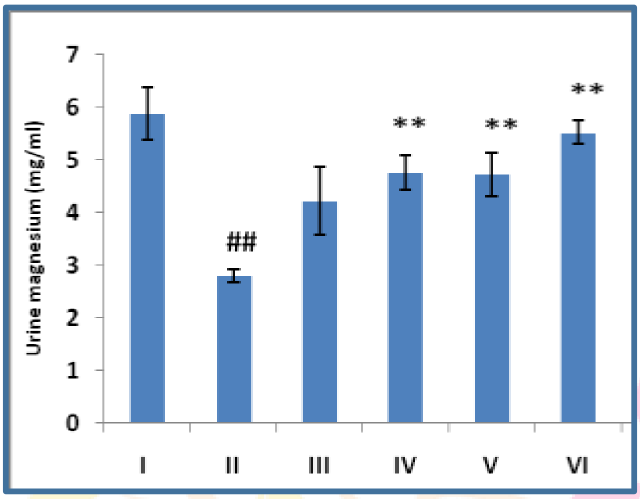

Figure 7: Effect of different extracts on urine magnesium levels in calcium oxalate induced urolithiasis in rats.

3.1.6 Serum analysis

28 days administration of ethylene glycol showed significant increase in the levels of uric acid in the serum as compared to control group rats. This increase was significantly reduced with simultaneous administration of extracts. There was mixed effects as far as significance was recorded. E A T C ,HACM showed dose dependent effect.

3.1.7 Effect of polyherbal formulation (PHF) on urine oxalate levels in calcium oxalate induced urolithiasis in rats.

Urine oxalate level was found to be significantly ($p<0.01$) increased in ethylene glycol treated group as compared to normal group. This elevated levels were significantly ($p<0.05$ and $p<0.001$) decreased by all doses of polyherbal formulation. The lower dose was less significant ($P<0.05$) than higher two doses which were found to be equipotent ($P<0.01$) in this regard.

4. Discussion

In the present study, all the extracts were screened using calcium oxalate (CaOx) induced urolithiasis by the way of administration of ethylene glycol (EG) which is highly recommended models. In this study, ethylene glycol induced calcium oxalate urolithiasis produced significant alterations in the various parameters as compared to control indicating successful induction of urolithiasis. This alteration induced increase in the urine volume, urinary levels of oxalate, calcium and magnesium(124). Reported studies indicated that, upon 14 days administration of ethylene glycol to the young Wistar rats resulted into the formation of renal calculi composed mainly of calcium oxalate. Stone formation in ethylene glycol fed is caused by hyperoxaluria, which causes increased excretion of oxalate. Renal calcium oxalate deposition by EG in rats is frequently used to mimic the urinary stone formation in humans. Ammonium chloride reported to accelerate the lithiaisis. Therefore, we evaluated the effect of different extracts on calcium oxalate urolithiasis using this model. In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats has more resemblance to that of human. Continuous ethylene glycol treatment to animals leads to renal obstruction followed by decreased in urinary output. In this study, the urine volume was increased with induction which may be due to sudden reflex action to obstruction. The treatment of different plant extracts further showed significant reduction in the elevated urinary output. This effect is surprising and need to be addressed for individual extract however effect may also be attributed to the variation associated with experimental design. Ethylene glycol treatment induces the metabolic acidosis which turns theurinary pH acidic. Ethylene glycol is rapidly absorbed and metabolized in the liver to glycolic acid this glycolic acid is oxidized to glyoxylic acid, which, further oxidized to oxalic acid. In our study, the ethylene glycol treatment resulted into lowering pH suggesting induction of condition favorable for formation of stone whereas treatment with different plants extracts restored pH of urine almost to the normal suggesting preliminary effect to prevent stone formation. In modern medicines also urinary alkanizers are commonly prescribed to prevent process of stone formation at early stage. The result confirmed use of extracts as symptomatic treatment. Consistent with some previous reports, significant risk factor in the pathogenesis of renal stone. It has been reported that oxalate play an important role in stone formation and has about 15-fold greater effect than urinary calciu. In the present study, urinary oxalate was increased in ethylene glycol induced urolithiatic rats. The reduction in oxalate excretion was observed on both plant extracts treatment. This decreased excretion of oxalate may be due to the inhibition of formation of oxalate by the drug.

5. Conclusion

Urolithiasis is the process of formation of stones anywhere in the urinary tract. It is a disorder of the urinary tract which is caused by a wide range of aetiological factors. The current study titled "Evaluation of some traditional medicinal plants and its formulation in urolithiasis" was conducted. Plants were selected from the traditional medicinal system. Plants selected for the study were leaves of *Chrysanthemum morifolium*, roots of *Teraxacum officinal*. The authenticated plant material was extracted successively with different solvent (Viz; petroleum ether, ethyl acetate, methanol and hydroalcohol). Both extracts were subjected to preliminary phytochemical analysis using established method (Khandelwal, 2003; Kokate, 1997). Based upon results of preliminary phytochemical analysis, the most potent extract having prominent presence of potential phytoconstituents were selected for further pharmacological screening. Acute toxicity study of selected extracts was performed in healthy albino mice (30-50gm) as per guidelines (AOT 425) suggested by the Organization for Economical Co-operation and Development (OECD). Acute toxicity study revealed that all the extracts were safe upto a dose of 2000 mg/kg of body weight. From this data and pilot study reports; two different doses 100 and 300 mg/kg were selected for this study. The extracts were screened for their activities against urolithiasis using preclinical models viz; Calcium oxalate induced urolithiasis model in rats. In calcium oxalate induced urolithiasis in rats model, all the extracts were evaluated for its effect on analysis of urine (Urine volume, urine pH, urine oxalate levels, urine calcium levels and urine magnesium levels), serum analysis (Serum uric acid levels, serum creatinine levels and blood urea levels), kidney homogenate analysis (ACP, ALP, ALT, AST and LDH levels) and histopathology of kidney in rats. In this model pretreatment with HACM and EATO showed most significant antiurolithiatic activity at the dose of 300 mg/kg.

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