Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 3, 2013

Research Article

EFFECT OF POLYHERBAL FORMULATION ON ETHYLENE GLYCOL INDUCED UROLITHIASIS

N.THANGARATHINAM[1*], N.JAYSHREE[1], A.VIJAY METHA[2], L.RAMANATHAN[2]

[1]Department of Pharmacognosy, College of Pharmacy, Madras Medical College, [2]Retort Pharmaceuticals, Madhavarm, Chennai, Tamil Nadu, India. Email: ashwinipuja23@gmail.com

Received: 21 Apr 2013, Revised and Accepted: 29 May 2013

ABSTRACT

The effect of Polyherbal Syrup (Aqueous decoction of *Aerva lanata, Astercantha longifolia, Cucumis sativus, Cumimum cyminum, Hemidesmus indicus, Lagenaria siceraria* and *Tribulus terrestris)* against ethylene glycol–induced urolithiasis in male Wistar albino rats was carried out. Urolithiasis was induced in rats by administering 0.75% ethylene glycol in drinking water for 28 days and was manifested by high urinary calcium, phosphorus and low urinary volume, pH, magnesium content and high uric acid, creatinine, urea and BUN in serum. Simultaneous administration of Polyherbal syrup (100 and 200 mg/kg/oral) and standard drug hydrochlorothiazide 150µg/kg orally for 28 days along with ethylene glycol (0.75%) reduced urinary calcium, phosphorus and increased urinary volume, pH and magnesium and reduced uric acid, creatinine, urea and BUN in serum. The histopathological studies confirmed the induction of urolithiasis as microcrystal deposition was observed in sections of kidney from animals treated with ethylene glycol. This was reduced after treatment with the Polyherbal syrup. These observations enable us to conclude that Polyherbal syrup is effective against ethylene glycol–induced urolithiasis.

Keywords: Polyherbal syrup, Urolithiasis, Ethylene glycol, Hydrochlorothiazide, Histopathology studies.

INTRODUCTION

Urolithiasis (Renal stone formation) is a recurrent disorder predominant in males. The present-day medical management of urolithiasis is either costly or not without side effects. Hence, the search for antiurolithiatic drugs from natural sources has assumed greater importance. [1]. Urolithiasis has a very old history. It has been found in tombs of Egyptian mummies dating back to 4000 BC and in the graves of North American Indians from 1500- 1000 BC. It is a serious, debilitating problem in all societies throughout the world, affecting approximately 12% of the population, and men are three times more prone than women. It is more prevalent between the ages of 20 to 40 in both sexes. Etiology is multifactorial and is strongly related to dietary lifestyle habits or practices. Increased rates of hypertension and obesity, which are linked to urolithiasis, also contribute to an increase in stone formation [2].

Through there is a substantial progress in the understanding of the pathophysiology and management of urolithiasis, there is no satisfactory method available for complete cure of urolithiasis. Hence an herbal with antiurolithiatic effect would be great interest. This may have lesser side effects.

Table 1: Composition of Polyherbal formulation

S. No.	Botanical Name	Family	Part Used	Quantity per Liter
1	*Aerva lanata*	Amaranthaceae	Whole plant	80 g
2	*Astercantha longifolia*	Acanthaceae	Seeds	80 g
3	*Cucumis sativus*	Cucurbitaceae	Seeds	80 g
4	*Cumimum cyminum*	Umbelliferae	Fruits	50 g
5	*Hemidesmus indicus*	Asclepiadaceae	Root	50 g
6	*Lagenaria siceraria*	Cucurbitaceae	Climbers	80 g
7	*Tribulus terrestris*	Zygopyllaceae	Fruits	80 g

Traditional medical practitioners prescribe a combination of herbal products for synergistic action [3]. One Polyherbal formulation made up of ingredients with claims of diuretic and antiurolithiatic activity was formulated (Table-1). This Polyherbal formulation was evaluated for antiurolithiatic activity.

MATERIALS AND METHODS

Plant materials

All the plants as mentioned in Table-1 were identified and authenticated by comparing with the standards used in Retort Pharmaceuticals, Madhavaram, Chennai-60.

Preparation of Polyherbal Syrup

The seven dried raw materials were coarsely powdered. The dried powder was mixed with 4000 ml (4 Lit) of water and the mixture was boiled until the total volume become one fourth of the original volume. The mixture was cooled and filtered (Decoction). Filtrate was taken to prepare final herbal syrup. One part of decoction was mixed with five parts of 35% simple syrup (1:5). Add suitable preservatives and flavorings agents were added [4,5].

Drugs and Chemicals

All Chemical and reagents use for study was AR and Laboratory grade. Diagnostic kits for various bio chemical analysis was procured from Crest Biosystems (Goa).

The protocol for conducting the acute toxicity studies (female wistar rats) and *in vivo* studies (male Wistar rats) was approved by the Institutional Animal Ethical Committee (IAEC) of the C.L.Baid Metha College of Pharmacy, Chennai.

Approved number: IAEC/I/06/CLBMCP/2013/15.2.13

Acute toxicity studies

Acute toxicity studies were carried out as per OECD guidelines (No: 423)[6] using female Wistar rats it was found that the Polyherbal syrup was safe up to 2000mg/kg dose..

Induction of Experimental Urolithiasis [6,7,8,9]

Calcium oxalate urolithiasis was induced in experimental animals by administering ethylene glycol 0.75% (0.75 ml of ethylene glycol in 100ml of drinking water) to rats for a period of 28 days for the production of calcium oxalate stone in rats.

Experimental animals

Male albino rats of Wistar strain weighing between 150-200gm were used. The animals were fed with commercial rat feed pellets (Amrut laboratory animal feed Ltd, Bangalore) and were given water *ad libitum*. Animals were housed in plastic cages with filter tops under controlled conditions of 12:12 light dark cycle, 50% humidity and 28°C.

The rats were divided into five groups each containing 6 rats and the following treatment protocol was followed:

Group - I	Control rats - Received normal pelleted diet
Group – II	**Urolithiasis induced rats -** Received 0.75% ethylene glycol in water for 28 days
Group - III	**Standard drug hydrochlorothiazide treated rats-** Urolithiasis induced rats received hydrochlorothiazide (150 µg / kg body weight) by oral administration for subsequent 28 days at a rate of 1.0 ml / rat / day**.**
Group - IV	**Polyherbal syrup treated rats -** Urolithiasis induced rats received Polyherbal syrup (100 mg / kg body weight) by oral administration for subsequent 28 days.
Group - V	**Polyherbal syrup treated rats** - Urolithiasis induced rats received Polyherbal syrup (200 mg / kg body weight) by oral administration for subsequent 28 days.

Assessment of Antiurolithiatic Activity

Collection and analysis of urine

Rats were kept separately in metabolic cages and urine samples of 24 h were collected on 28th day. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C.

Urine samples were analyzed for calcium[10], phosphorus[11], and magnesium[12].

Serum analysis

At the end of the experiment, blood samples were collected from the retro-orbital plexus under anesthetic conditions and analyzed for Urea[13], Uric acid[14], Creatinine[15] and BUN[13].249

To confirm the incidence of lithiasis, the animals were sacrificed and their kidneys were subjected to histopathological studies.

Histopathological studies

The kidneys were fixed rapidly with 10% neutralized formalin (pH 7.4). Sections of kidney fixed in paraffin were prepared and stained with hematoxylin and eosin and observed for pathological changes.

Statistical analysis

The results are expressed as mean ± SEM. Statistical analysis was carried out using one-way ANOVA followed by Dunnet test. A value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

***In vivo* antiurolithiatic activity of polyherbal syrup on ethylene glycol induced urolithiasis in rats**

From the acute toxicity study, it was found that the Polyherbal syrup was safe up to a dose of 2000mg/kg. Hence, the therapeutic dose was taken as 200 mg/kg/b.w and 100 mg/kg/b.w for the Polyherbal syrup.

Ethylene glycol (EG) is rapidly absorbed and metabolized in the liver via alcohol dehydrogenase/ aldehyde dehydrogenase to glycolic acid. Glycolic acid is oxidized to glyoxylic acid, which, in turn, is further oxidized to oxalic acid by glycolate oxidase. High doses of EG (>2,500 mg/kg body wt), particularly when given as an oral bolus, cause the saturation dependent accumulation of glycolic acid in the plasma. So glycolate oxidase (GO) is one of the rate limiting enzymes in the metabolism of EG.

Table 2: Estimation of urine volume and pH

Group	Treatment	Volume of urine	pH of urine
Group I	Control(Normal control)	2.3 ± 0.08	7.1 ± 0.06
Group II	Induced (Ethylene glycol 0.75%)	1.28 ± 0.08*	6.51 ± 0.06*
Group III	Standard (Hydrochlorothiazide 150 µg/kg)	2.71 ± 0.13*	8.5 6± 0.14*
Group IV	100 mg/kg/day formulation	2.38 ± 0.13	8.26 ± 0.11
Group V	200 mg/kg/day formulation	2.88 ± 0.06*	8.35 ± 0.13*

The values are expressed as Mean ± SD, n=6, * $p < 0.001$ as compared to control

The glomerular filtration rate (GFR) is an important parameter for ensuring renal function and it gets decreased in urolithiasis due to the obstruction to the outflow of the urine by stones in urinary system, which leads to a rise in nitrogenous waste products like urea, creatinine, and uric acid in blood.

From Table 2 it is seen that the urinary pH of control is neutral, ethylene glycol induced rats pH is reduced compared with control group. Treatment with hydrochlorothiazide 150 µg/kg/day was found to increase the urine pH (8.56±0.14), whereas, group receiving Polyhebral formulation 100 mg/kg/day and polyherbal formulation 200 mg/kg/day was also found to increase the urinary pH in a dose dependent manner but more significantly at 200mg/kg ($p<0.001$).

Calcium and phosphate play a vital role in renal calculogenesis. In the present study, chronic administration of 0.75% (v/v) ethylene glycol aqueous solution for 28 days resulted in hypercalciuria in male Wistar rats. Phosphate and calcium excretion were significantly increased (P <0.001), whereas magnesium decreased in urine and kidney of EG treated animals group II as compared to group I.

Table 3: Estimation of calcium, magnesium and phosphate

Group	Group	Calcium	Phosphorus	Magnesium
Group I	Control(Normal control)	8.51 ± 0.05	5.46 ±0.06	2.73 ± 0.02
Group II	Induced (Ethylene glycol 0.75%)	11.93 ± 0.01*	7.56 ± 0.04*	1.44 ± 0.01*
Group III	Standard (hydrochlorohiazide 150 µg/kg)	8.96 ± 0.01*	5.98 ± 0.01*	2.44 ± 0.02*
Group IV	100 mg/kg/day formulation	8.72 ± 0.01*	6.18 ± 0.44*	2.54 ± 0.03*
Group V	200 mg/kg/day formulation	8.24 ± 0.02*	5.22 ± 0.01*	2.45 ± 0.05*

The values are expressed Mean±SD, n=6, *$p < 0.001$ as compared to control

However, supplementation with polyherbal formulation at 100 and 200 mg/kg body weight and hydrochlorothiazide 150 µg/kg significantly (*P* <0.001) lowered the elevated levels of phosphate and calcium in urine and kidney as compared to the untreated group II animals. Magnesium level in the standard and test group V came close to normal and was comparable to the levels in the rats belonging to the untreated group II.

In calculi-induced rats (Group II), marked renal damage causes evaluation of serum levels of creatinine, uric acid and Blood Urea Nitrogen (BUN). However, polyherbal formulation restored the elevated serum levels of creatinine, uric acid and BUN . The treatment polyherbal formulation (Group IV and V) and standard hydrochlorothiazide (Group III) significantly (*P* <0.001) lowered the elevated serum level of uric acid as compared to group II.

Table 4: Esimation of Serum Constituents

Group	Group	Urea	Uric acid	Creatinine	BUN
Group I	Control(Normal control)	9.46 ± 0.01	2 ± 0.009	0.24 ± 0.02	4.41 ± 0.01
Group II	Induced (Ethylene glycol 0.75%)	15.59 ± 0.02*	4.94 ±0.01*	0.63 ± 0.03*	6.96 ± 0.02*
Group III	Standard (hydrochlorothiazide 150 µg/kg)	9.75 ± 0.02*	2.92 ±0.01*	0.43 ±0.03*	4.55 ± 0.02*
Group IV	100 mg/kg/day formulation	9.87 ± 0.01*	2.92 ±0.01*	0.44 ±0.02*	4.55 ± 0.02*
Group V	200 mg/kg/day formulation	9.7 ± 0.02*	2.87 ±0.01*	0.33 ± 0.02*	4.52 ± 0.02*

The values are expressed as Mean ± SD, n=6,*p < 0.001 as compared to control.

HISTOPATHOLOGICAL EXAMINATION OF THE RAT KIDNY

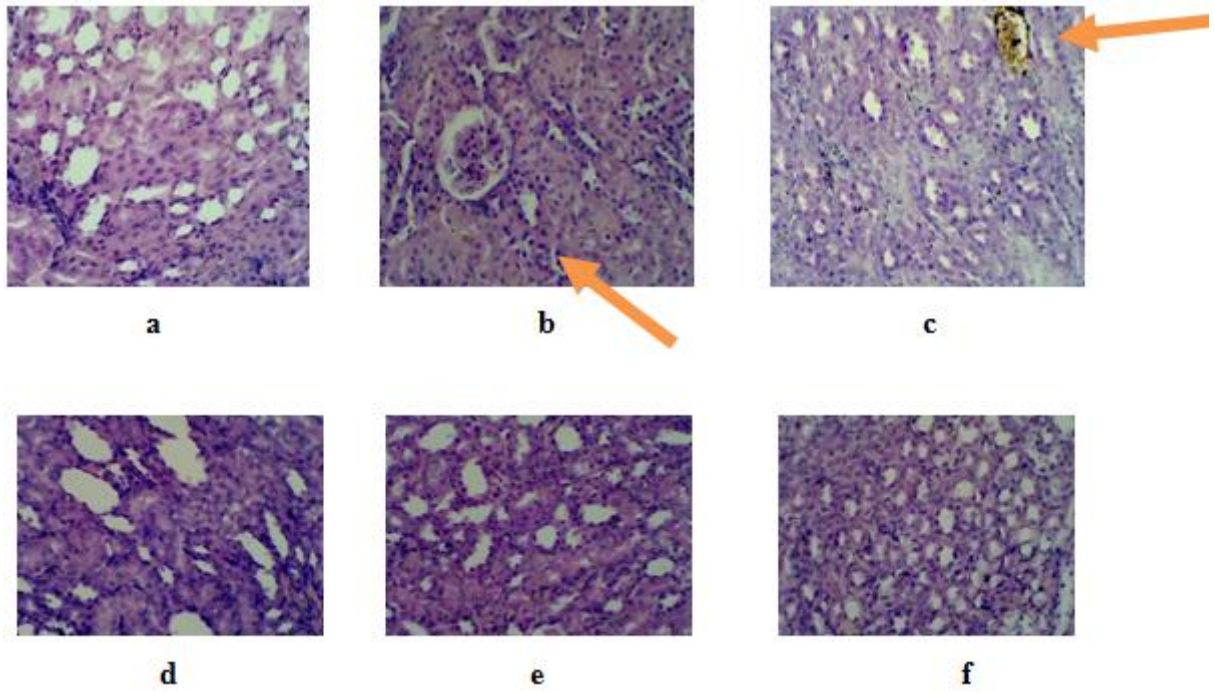

Fig 1: Histopathological examination of the rat kidney a - Vechicle control rat, b - Ethylene glycol induced rat, c- Standard thiazide, d - 100 mg/kg formulation, e - 200 mg/kg formulation

The antiurolithiatic effect was further confirmed by kidney histopatholological studies. Indeed, kidney sections of untreated rat showed abundant crystal deposition. Furthermore, renal epithelial cells had more tubular dilatation and damage shown by large spaces in the tissue. In treated rats, less crystal depositions were seen compared to untreated animals and the necrosis as well as the tubule dilatation was very limited**.** Renal stone deposition damages the renal tissue and detoriate the renal function.

Lithogenic treatment caused impairment of renal functions of the untreated rats as evident from the markers of glomerular and tubular damage: raised BUN, uric acid, urea and serum creatinine, that was lowered in a dose-dependent manner in animals receiving a treatment with polyherbal formulation. Tissue injury and inflammation in these animals is due to exposure to phosphate and Calcium phosphate crystals, leading to the generation of reactive oxygen species, development of oxidative stress, lipid peroxidation and depletion of antioxidant enzymes. Renal epithelial injury further promotes crystal retention, as epithelial injury exposes a variety of crystal adhesion molecules on epithelial surfaces and promotes stone formation. Probably antioxidant constituents of polyherbal formulation restore the renal antioxidant enzyme and prevent renal cell injury .

CONCLUSION

The present study indicating the presence of anti-urolithic effect in Polyherbal formulation. The Polyherbal formulation resulted in an increase in urinary volume, decrease in Calcium, and Phosphate excretion along with increased excretion of Magnesium. Further the Histopathological examination of renal tissues showed drastic reduction in stone formation. So, it is concluded that the Polyherbal formulation used in the present study is an effective drug in the management of urolithiasis and could be tried in the treatment of urolithiasis. However further studies are required for authentication in human beings.

ACKNOWLEDGEMENT

1) A. Vijay Metha (M.D) and L.Ramanadhan (G.M), Retort Pharmaceuticals, Madhavaram, Chennai-600 060 for his support in carrying out this study.
2) Dr.N.Jayshree, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600 003 for his guidance in doing this study.

REFERENCES

1. Patel RK, Patel SB, Shah JG Dabhi Kinnari J: Antiurolithiatic activity of *Daucus carrota* Linn.seeds on ethylene glycol - induced urolithiasis in rats. American Journal of Pharm Tech Research 2012;2 Suppl 1:2249-3387.
2. Goyal Parveen Kumar, Mittal Arun, Kumar Rishi: Evaluation of *Tinospora cardifolia* for antiurolithiatic potential. IJBMS 2011:9 Suppl 14:1-5.
3. Akila L, Ashok Kumar and Nirmala P: Effect of polyherbal formulation on ethylene glycol induced urolithiasis. International Journal of Pharma and Bio Sciences 2011;2 Suppl 4:7-24.
4. Patel Divyakant A, Patel Yogesh K, Shah Paresh B: Development and evaluation of herbal syrup from Neolamarckia cadamba (Roxb .) Bosser leaves. IRJP 2012; 3 Suppl 9: 201-202.
5. Tushar Brahmbhatt, Biren Shah, Upendra Patel, Hiren Kadikar: Development and evaluation of various oral Formulations for anti-asthmatic plant extract. IJPRBS 2012;1 Suppl 3:317-327.
6. Soundararajan P, Mahesh R, Ramesh T and Hazeena Begum V: Biopotency of *Aerva lanata* on membrane bound ATPases and marker enzymes in urolithiatic Rats. IRJP 2007;1:221-228.
7. Badmanaban R, Patel CN, Samdaniel P and Kamal Modh: Pharmacognostical studies on *Lagenaria siceraria* (Mol.) stand leaves. Int J Chem Sci 2009;7 Suppl 4: 2259-2264.
8. Sathya M, Kokilavani R: Antiurolithiatic activity of ethanolic root extract of *Saccharum spontaneum* on glycolic acid induced urolithiasis in rats. JDDAT 2012;2 Suppl 5:86-89.
9. Surendra K. Pareta, Kartik Chandra Patra, Papiya Mitra Mazumder, Dinakar Sasmal: *Boerhaavia diffusa* Linn aqueous extract as curative agent in ethylene glycol induced urolithiasis. Pharmacologyonline 2010;3:112-120.
10. Carmela T.M Masico, Jeff D, Alder and Jared Silverman: Bactericidal action of daptomycin against stationary-phase and nondividing staphylococcus aureus cells. Antibacterial Agents and Chemotherapy 2007:51 Suppl 12: 4255–4260.
11. Fiske CH, Subbarow Y: The colorimetric determination of phosphorous. J Biol Chem 1925;66:375.
12. Gindler E, Heth DA: Colorimetric determination with bound "calmagite" of magnesium in human blood serum. Clin.Chem 1971;17:662.
13. Fearon WR: The carbanido diacetyl reaction: A test for citrullin. Biochem J 1939;33:902.
14. Trinder P: Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann.Clin.Biochem 1969;6:24.
15. Bonsnes RW, Tauss HH: J.Biol.Chem1945;158,581.