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Research Article

### EVALUATION OF SAFETY & ANTI-UROLITHIATIC PROPERTY OF VARIOUS POLYHERBAL FORMULATIONS USING ETHYLENE GLYCOL-AMMONIUM CHLORIDE & POTASSIUM OXONATE INDUCED UROLITHIASIS IN RATS & MICE RESPECTIVELY

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#### ABSTRACT

The aim of present work was to evaluate safety & anti- urolithiatic property of various polyherbal formulations using ethylene glycol-ammonium chloride & potassium oxonate induced urolithiasis in rats & mice and to evaluate effect of poly herbal formulation on oxidative stress, i.e. SOD, catalase, LPO and to evaluate of poly herbal formulation on physical parameter i.e. body weight, urine PH, urine volume

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#### INTRODUCTION:

Kidney stone, also called as urolithiasis, when solid part or stone present in urinary track. When urine is supersaturated by salt, ions and minerals like calcium oxalate, Cystine, struvite and uric acid caused renal stone. Calcium have highest amount (60- 80 %) in stone. They have different size like small to large stone (staghorn stone). In some cases CaOx precipitation present in LOH membrane, its accumulate in renal papillae's sub epithelial space, it caused Randall's plaques. Symptoms of stone produces when it migrates down to urinary track or stay at same position. Various studies show that preliminary reason for stone formation is calcium phosphate cell which produces Nano bacteria. When low volume of urine, high amount of calcium excretion or both of this cause supersaturation of CaOx in urine lead to kidney stone<sup>1</sup>. When absorption of oxalate in intestine rise (enteric hyperoxaluria), higher oxalate contain meal intake, oxalate synthesis altered by genetic abnormalities (primary hyperoxaluria-1 and 2) and dysfunction of transport mechanism in renal part. Although, main reason for urine supersaturation is genetic abnormalities bonded to calcium outcome and also pH, this phenomena is the main core reason of the urolithiasis. In stone formation, growth and crystal-membrane interaction, protein has main function in matrix and organic matrix of stone. World's 10% of population are suffering from urolithiasis because of

industrialized part effect. Kidney stones are common in industrial countries with a yearly frequency of 1 % to 2 %. Diet plan, Urine alkalisation method, Analgesic drugs, Diuretics, Lithotripsy methods, Allopurinol and various Surgery basic steps for curing of CaOx and uric acid type stone. Commonest side effects of management are hypersensitivity, headache, GIT abnormalities and nausea. Acute trauma, bruising & damage to blood vessel of the kidney are side effect of ESWL. Herbal medicines are moderately safer compared to the allopathic medicines. In case of Allopathic medicines the gap between ED<sub>50</sub> & LD<sub>50</sub> is very low while comparing with the herbal medicines. Key objectives of herbal medicine management are cure without any adverse or side effects. Ayurveda has listed herbal medicines to cure or prevention of kidney stone diseases (mutrashmari) example like, *Tribulus Terrestris* (Gokshrua), *Crataeva Nurvala* (Varuna Tree), *Boerhaavia Diffusa* (Punarnava), *Hordeum vulgare* (Barely), *Bergenia Ligulata* (Pashanbheda), *Asparagus racemosus* (Shatamull), *Crataeva religiosa* (Bidasi), and *Dolichos Biflorus* (Kulthi). Our primary objective of study is to determine anti- urolithiatic property of polyherbal formulation on rats & mice<sup>2</sup>.

#### MATERIALS AND METHODS:

**Materials** Ethylene glycol (EG) 0.75% (v/v), Ammonium chloride 1% (w/v) and Potassium oxonate

**Method** Acute oral toxicity study of polyherbal formulations was carried out according to OECD 425 guideline in mice. Two methods were used to check anti-urolithiatic effect of polyherbal formulations i.e. Ethylene glycol (EG) 0.75% (v/v) uric acid, oxalates. Ammonium chloride 1% (w/v) and Potassium oxonate induced urolithiasis in rats and mice. Ammonium chloride was given first seven days continuously with ethylene glycol to accelerate process of renal stone formation. EG was continued for 28 days in all groups except normal control group. 5 various polyherbal formulations were treated in groups 3-7 and group -8 received standard drug. Blood and urine were collected at 14<sup>th</sup> & 28<sup>th</sup> days in order to measure

serum creatinine, uric acid, potassium, chloride, phosphorus, calcium, chloride and urine volume, pH, uric acid, oxalate, potassium, chloride, calcium, phosphate & oxalate. Second animal model employed to check anti-urolithiatic effect was Potassium oxonate induced hyperuricemia in mice. Potassium oxonate (250 mg/kg, p.o.) was given to all groups excluding normal control group. Polyherbal formulations were treated 60 minutes after potassium oxonate for consecutive 7 days in groups 4-7 and group-8 received standard drug. On 8<sup>th</sup> day blood and urine were collected in order to measure water & food intake, serum creatinine, uric acid, urine volume, pH 3-5.

## RESULTS AND DISCUSSIONS:

**Table 1:** Urolithiasis induce by Ethylene Glycol and Ammonium Chloride in Rats

Result of Body Weight

	Normal	Diseases	Calcirex	Calcirex	Cistonil	Cistonil	Cistonil	Standard
0 week	173.8±8	173.0±8	172.8±7	174.3±5	172.3±9	177.7±9	178.0±8	172.7±6
1st week	175.8±7	163.8±6	173±8	142.7±10	135±7	149.3±7	176.5±6	168.8±6
2nd week	188.7±8	177.7±6	172±18	167.3±10	164.3±6	174±11	195.8±6	190.2±7
3rd week	209.5±9	209.5±4	170±29	199.2±9	199±9	210.2±12	213±36	213±5
4th week	228.3±10	223.16±5	238.3±9	230.2±9	228.6±11	257.2±13	250.±11	240.6±8

Toxicity study was performed according to OECD 425 guideline. Polyherbal formulations found safe up to dose of 2000mg/kg

High level of serum calcium, potassium, chloride & acidic pH in DC group showed the production of renal stone and RTA. Whereas polyherbal formulations decreased serum calcium, potassium, chloride level nearer to normal group and made urine pH alkaline. Poly herbal formulations showed effect against kidney stone, RTA as well as oxidative stress.

**Table 2:** Effects on Serum Calcium (mg/dl)

Group	Serum Calcium (mg/dl)	
	14 <sup>th</sup> Day	28 <sup>th</sup> Day
Normal control	5±1	5.5±0.42
Disease control	16.5±0.5	18.5±0.7####
Calcirex capsule	8.5±0.5	9±0.36***
Calcirex syrup	15±1	15.1±0.6**
Cistonil capsule	15±11	15.6±0.4**
Cistonil syrup	13.5±0.5	15.8±0.8*
Cistonil tablet	10±2	8.3±0.5***
Standard	6.5±1.5	6.6±.33***

**Table 3:** Effects on Serum Creatinine

Group	Serum Creatinine (mg/dl)	
	14 <sup>th</sup> Day	28 <sup>th</sup> Day
Normal control	0.08±0.03	0.111±0.05
Disease control	1.431±0.11	1.49±0.13###
Calcirex capsule	0.669±0.13	0.402±0.05***
Calcirex syrup	0.628±0.09	0.49±0.08***
Cistonil capsule	1.11±0.24	0.802±0.1**
Cistonil syrup	1.237±0.03	1.039±0.12*
Cistonil tablet	0.562±0.07	0.462±0.10***
Standard	0.395±0.05	0.325±0.10***

**Table 4:** Effects on Serum Uric Acid

Group	Serum Uric acid (mg/dl)	
	14 <sup>th</sup> Day	28 <sup>th</sup> Day
Normal control	0.8±0.29	1.96±0.21
Disease control	6.7±0.53	7.02±0.41 <sup>###</sup>
Calcirex capsule	6.2±0.62	5.11±0.67 <sup>*</sup>
Calcirex syrup	6.2±0.24	5.16±0.41 <sup>*</sup>
Cistonil capsule	4.6±0.54	3.99±0.67 <sup>***</sup>
Cistonil syrup	5.9±0.26	5.87±0.34 <sup>ns</sup>
Cistonil tablet	3.9±0.51	3.28±0.35 <sup>***</sup>
Standard	3.69±0.43	3.24±0.21 <sup>***</sup>

**Effects on various urine parameters:**

Biochemical parameters (serum: calcium, creatinine, phosphate, potassium, chloride), urine analysis parameter (urine: pH, volume, calcium, phosphate, potassium, chloride, oxalate) found nearer to normal range in polyherbal formulation treated groups. So it shows polyherbal formulations having effectiveness against urolithiasis.

Our primary motto was to check the effect of various poly herbal formulations' effect on urolithiasis on different *in-vitro* models. We employed two different animal models i.e. Ethylene glycol model and potassium

oxonate induced urolithiasis. Both models showed significant urolithiasis and it was evident by acidic pH of urine in DC. So, it was affirmative signal for stone formation & also for kidney damage. On other side, treatment group showed basic or normal range pH value. So, it was proved treatment was worked properly. It can be used to treat renal stone (CaOx & Uric acid) & kidney damage. As per the acute toxicity study, poly herbal formulations were completely safe up to the dose of 2000 mg/kg. All animals were survived with purely healthy condition. There was no any abnormality or other side effect like condition observed.

**CONCLUSION:**

Toxicity study was performed according to OECD 425 guideline. Polyherbal formulations found safe up to dose of 2000mg/kg.

Biochemical parameters (serum: calcium, creatinine, phosphate, potassium, chloride), urine analysis parameter (urine: pH, volume, calcium, phosphate, potassium, chloride, oxalate) found nearer to normal range in polyherbal formulation treated groups. So it shows polyherbal formulations having effectiveness against urolithiasis.

Significant increased level in SOD & Catalase activity found & drop in LPO level was observed, which shows that polyherbal formulations have anti-oxidant activity.

As per the result, we concluded that various poly herbal formulations have potential to cure or treat renal stone like CaOx as well as uric acid stone & RTA. Cistonil tablet & Calcirex capsule were best poly herbal formulation among all to use in urolithiasis as treatm

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