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Research Article

EVALUATION OF ANTI-UROLITHIATIC ACTIVITY OF POLYHERBAL FORMULATION BY USING *IN-VIVO* MODEL

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ABSTRACT

The present study was aimed to evaluate the anti-urolithiatic activity of polyherbal formulation (*i.e.* Lithout Tablets) by using *in-vivo* model. For the preparation of polyherbal formulation *i.e.,* Lithout tablets, Wet granulation method was used and the *in-vivo* anti-urolithiatic activity was evaluated by ethylene glycol induced urolithiasis in rat model. The results of the formulation complies the IPQC tests and the results of *in-vivo* anti-urolithiatic activity are determined by ANOVA followed by Dunnett's test. From the present study, it can be concluded that animals treated with standard (Cystone) drug and Lithout tablet in dose of 200 mg/kg of body weight exerted significant anti-urolithiatic activity and protection against tubular interstitial damage in kidneys and liver in ethylene glycol induced urolithiatic rats.

Keywords: Anti-urolithiasis, Wet granulation, ANOVA, Cystone, Lithout tablets.

1. INTRODUCTION

The most important excretory organ in the human body is the kidney. It has function not only to excrete the metabolic waste products, but also to maintain the acid base balance and endocrine functions like erythropoietin production. In the current days people face so many problems of kidney in which most common are urolithiasis and nephrotoxicity [1]. Urolithiasis has common names such as renal calculus/ calculi or kidney stone [2]. When urinary constituents normally in solution are getting precipitates, calculi or stones form in the kidney and bladder. The solutes involved in the formation of kidney stones are usually oxalates and phosphates. After 30 years of age, males are more prone to dedvelop kidney stone and the condition is often recurrent. Kidney damage happens when some stones become too large to pass through the ureter and may obstruct the outflow of urine. Others pass to the bladder and are either excreted or increase in size and obstruct the urethra. Usually in developing countries and often in children, the stones produce in the bladder [3].

During their lifetime, about 5-12 % of world's population develops kidney stones. The chances of high risk for kidney stones in the people have grown up to 40 per cent in 2000 and will grow to 50 per cent by 2050. Out of ten, one people develops kidney stones and is responsible for 10 admissions of every 1000 hospital admissions, out of which the kidney failure patients are approximately 10 per cent causes due to kidney stones [4].

1.1. Pathophysiology & Pathogenesis of Urolithiasis [5-7]

The formation of renal stones is a consequence of increased urinary supersaturation with subsequent formation of crystalline particles. Stone growth starts with the formation of crystals in supersaturated urine which then adhere to the urothelium, thus creating the nidus for subsequent stone growth. These grow to erode the urothelium, forming a nucleus for calcium oxalate deposition. The sequence of events that trigger stone formation includes nucleation, growth, aggregation, and retention of crystals within the kidneys.

1.1.1. Crystal Nucleation

The first step in the formation of kidney stone begins by the formation of nucleus (termed as nidus) from supersaturated urine retained inside the kidneys. Nucleation may be formed in the kidney through free particle or fixed particle mechanism. In supersaturated solutions, if promoters exceed that of inhibitors, nucleation starts.

1.1.2. Crystal Growth

Crystals in urine stick together to form a small hard mass of stone referred as crystal growth. Stone growth is accomplished through aggregation of preformed crystals or secondary nucleation of crystal on the matrix-coated surface. Once a nidus has achieved, the overall free energy is decreased by adding new crystal components to its surface. The total free energy of the cluster is increased by the surface energy. The process of stone growth is slow and requires longer time to obstruct the renal tubules.

1.1.3. Crystal Aggregation

All models of CaOx urolithiasis concede that crystal aggregation is probably involved in crystal retention within the kidneys, since aggregation of crystals can have a considerable effect on particle size and aggregated crystals are commonly found in urine and renal stones.

1.1.4. Crystal-Cell Interaction

The attachment of grown crystals with the renal tubule lining of epithelial cells is termed as crystal retention or crystal-cell interaction. These structural and functional studies of crystal-cell interactions in culture indicate that COM crystals rapidly adhere to microvilli on the cell surface and are subsequently internalized. Crystal-cell interaction results in the movement of crystals from basolateral side of cells to the basement membrane.

1.1.5. Endocytosis of CaOx Crystals

Endocytosis or engulfment of crystals by renal tubular cells is the earliest process in the formation of kidney stones. Studies on tissue culture crystal-cell interactions indicated that COM crystals rapidly adhere to microvilli on the cell surface and subsequently internalized. Polyanion molecules present in tubular fluid/urine such as glycosaminoglycans, glycoproteins, and citrate may coat crystals and inhibit the binding of COM crystals to cell membrane.

1.1.6. Cell Injury and Apoptosis

Exposure to high levels of oxalate or CaOx crystals induces epithelial cellular injury, which is a predisposing factor to subsequent stone formation. CaOx crystal depositions in the kidneys upregulate the expression and synthesis of macromolecules that can promote inflammation. Crystals may be endocytosed by cells or transported to the interstitium. It has been suggested that injured cells develop a nidus which promotes the retention of particles on the renal papillary surface.

1.1.7. Genetic Basis of Kidney Stone Formation

Environmental factors interacting with underlying genetic factors cause rare stone disease. The production of promoters and inhibitors of crystallization depends on proper functioning of the renal epithelial cells. Cellular dysfunction affects the supersaturation of urinary excretion by influencing ions such as calcium, oxalate, and citrate.

1.1.8. Randall's Plaques

Randall's plaques appear to be the precursor's origin of urinary stone development although it is unclear whether it involves in all stone types or not. The majority of CaOx stones are found to be attached with renal papillae at the sites of Randall's plaque. It is located at the interstitial basement membrane in loop of Henle.

For treatment of urolithiasis, there are many marketed formulations available but they have serious side effects like allergic reaction, kidney failure, and irregular heartbeat and also cause muscle cramps, increased blood sugar, increased cholesterol, diarrhoea, etc. Also another treatment of urolithiasis includes extracorporeal shock wave therapy, ureteroscopic stone removal and laser lithotripsy. These medical procedures require hospitalisation with associated cost and also none of them give satisfactory results.

Because of all above mentioned side effects and complications, there is a need to use herbal formulation. The present study gives the benefit of polyherbal formulation to add the new effective formulation to treat the kidney stone.

2. MATERIAL AND METHOD

2.1. Material

2.1.1. Plant Material

The powders of plants *i.e.*, Pashanbhed (*Bergenia ligulata*), Gokhru (*Tribulus terestris*), Manjishtha (*Rubia cardifolia*), Punarnava (*Boerhavia diffusa*), Hirda (*Terminalia chebula*), Gulvel (*Tinospora cardifolia*), Varuna (*Crataeva nurvala*) were obtained from local market of Pune city.

2.1.2. Chemicals

Ethylene glycol, Sodium chloride, Sodium phosphate, Sodium citrate, Sodium sulphate, Calcium chloride.

$2H_2O$, Ammonium hydroxide, Hydrochloric acid, Sulphuric acid were obtained from Research Lab Fine Chem Industries; Magnesium sulphate. $7H_2O$ were obtained from S. D. Lab Chemical Center; Ammonium chloride, Potassium chloride, Potassium permanganate was obtained from Poona Chemical Laboratory and Sodium oxalate was obtained from Research Lab Poona.

2.1.3. Animals

Wistar rats, weighing 200-250 gm of either sex and Swiss albino mice, weighing 25-30 gm of either sex were obtained from animal house of PDEA's Seth Govind Raghunath Sable College of Pharmacy, Saswad. The experimental protocol was approved by the Institutional Animal Ethics Committee. (Protocol Approval No. SGRS/IAEC/09/2018-19).

2.2. Method

2.2.1. Formulation of Lithout Tablet by Wet Granulation Method

All the ingredients were weighed and mixed by doubling up method. Little amount of water was added to the mixture to form a dough. The dough was passed through 44# mesh sieve to form granules. Dried the formed granules and again passed through 80# mesh sieve. The granules were punched in tablet punching machine to form the tablets [8].

Table 1: Composition of Lithout tablet

Sr.No.	Inredients	Quantity (mg)
1	Pashanbheda	35.7
2	Gokhru	35.7
3	Manjishtha	35.7
4	Punarnava	35.7
5	Hirda	35.7
6	Gulvel	35.7
7	Varuna	35.7

2.2.2. Acute oral toxicity study

Female swiss albino mice, weighing 25-30 m were selected. All animals were fasted for 24 hr prior to dosing of test drug. Dosing was started from 2000mg/ kg to 5000mg/kg as per OECD guideline 425.

2.2.3. In-vivo Anti-urolithiatic activity of Lithout tablets by ethylene glycol induced urolithiasis in rats

The rats were randomly divided into nine groups (n=6). Group I served as a vehicle control and maintained on regular rat food and drinking water *ad libitum* and received distilled water. All the remaining groups received calculi inducing treatment for 28 days, comprised of 0.75% v/v ethylene glycol with 1% w/v ammonium chloride in drinking water *ad libitum* for 3 days to accelerate lithiasis followed by only 0.75% v/v ethylene glycol for 25 days. Group II served as lithiatic control and received calculi inducing treatment for 28 days, comprised of 0.75% v/v ethylene glycol with 1% w/v ammonium chloride in drinking water *ad libitum* for 3 days to accelerate lithiasis followed by only 0.75% v/v ethylene glycol for 25 days. Groups III served as standard group and received Cystone at dose of 200 mg/kg from 14th day to 28th day of calculi induction. Group IV, V & VI served as preventive treatment group and received Lithout at doses of 100, 200 & 400 mg/kg respectively from 1st day to 28th day of calculi induction. Groups VII, VIII & IX served as curative treatment group and received Lithout at doses of 100, 200 & 400 mg/kg respectively from 14th day to 28th day of calculi induction. The Lithout tablet was dissolved in distilled water & was given once daily by oral route (0.5 ml/100 g). The three dose levels, 100, 200 and 400 mg/kg were used to evaluate the antiurolithiatic potential of Lithout tablet (a polyherbal formulation) against ethylene glycol induced urolithiasis in rats.

2.3. Evaluation Parameters

2.3.1. Characterization of Lithout tablets

2.3.1.1. Angle of Repose (θ)

To estimate the flow properties of granules, the funnel method was used to measure the angle of repose. When granular materials are poured onto a horizontal plane; a conical pile is formed. The internal angle between the surface of the pile and the horizontal surface is known as the angle of repose. The tan of the angle of repose was measured by height (H) of the cone and diameter of the base cone (D) [14].

$\text{Tan } \theta = h/r$

Where, θ = Angle of repose, h = Height of the cone, r = Radius of the cone.

2.3.1.2. Bulk Density

Bulk density is defined as the mass of a powder divided by the bulk volume. The bulk density of a powder depends primarily on particle size distribution, particle shape and the tendency of the particles to adhere to one another. After the initial volume was observed, the cylinder was allowed to fall under its own weight on to a hard surface from the height of 2.5cm at 2 sec

interval. The taping was continued until no further change in volume was noted.

$$\text{LBD (Loose Bulk Density)} = \frac{\text{Weight of the powder}}{\text{Volume of the powder}}$$

2.3.1.3. Tapped density

The measuring cylinder containing a known mass of blend was tapped for a fixed time and the min. Volume (Wt) occupied in the cylinder was measured; the tapped density (pt) was calculated by using the following formula [15]:

$$\text{TBD (Tapped Bulk Density)} = \frac{\text{Weight of the powder}}{\text{Tapped volume of the powder}}$$

2.3.1.4. Hausner's ratio

Hausner's ratio is an indirect index of ease of powder flow. It is calculated by the following formula:

Hausner's ratio (H) = T/B

When T is the tapped density and B is bulk density. Lower H (<1.25) indicates better flow properties than higher ones (>1.25).

2.3.1.5. Percentage Compressibility

Percent compressibility of powder mix was determined by Carr's compressibility index calculated by following formula.

$$\text{Carr's Index \%} = \frac{\text{TBD} - \text{LBD}}{\text{TBD}} \times 100$$

Where, LBD = Loose Bulk Density, TBD = Tapped Bulk Density

2.3.1.6. Weight variation

According to USP weight variation test, 20 tablets were selected randomly from formulation and weighed individually and average weight was calculated. Not more than two individual weights deviated from the average weight by more than percentage given in the official limits in pharmacopoeia and none deviated by more than twice that percentage.

2.3.1.7. Hardness

The hardness of the tablets was determined using digital hardness tester. It is expressed in kg/cm^2. Five tablets were randomly picked and hardness of the tablets from formulation was determined. The mean was calculated from readings.

2.3.1.8. Friability

The friability of tablets was determined using Roche Friabilator. It is expressed in percentage (%). 20 tablets were initially weighed ($W_{initial}$) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes or run up to 100 revolutions. The tablets were weighed again (W_{final}).

The % friability was then calculated by:

$$F = \frac{W_{initial} - W_{final}}{W_{initial}} \times 100$$

% Friability of tablets less than 1% are considered acceptable.

2.3.1.9. Disintegration test

Disintegration is evaluated to ensure that the drug substance is fully available for dissolution and absorption from the gastrointestinal tract. In disintegration test, measured using tablet disintegration test apparatus (Electrolab, India) using distilled water without disk at room temperature (37±2°C) [16].

2.3.1.10. Dissolution

Dissolution was carried out using USP dissolution apparatus II (rotating paddle apparatus). Dissolution was carried out in 900 ml of suitable dissolution medium. Temperature of the dissolution medium was maintained at 37±2°C and the agitation intensity was 50 rpm. The time interval at which all the contents of tablets get dissolved was recorded.

2.3.2. In-vivo anti-urolithiatic activity

2.3.2.1. Collection and analysis of urine

All animals were kept in individual metabolic cages and 24 h urine samples were collected on 0, 7, 14 and 28th day of calculi inducing treatment. After measurement of urine volume, all urine samples were analyzed for pH, calcium, oxalate and phosphate content. The urine oxalate level was measured using the method of Hodgkinson [9].

2.3.2.2. Serum analysis

After urine collection of 28th day, blood was obtained from the retro-orbital sinus under anaesthetic condition and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000 rpm for 10 min and analyzed for creatinine, uric acid and blood urea nitrogen (BUN) [10].

2.3.2.3. Kidney and Liver histopathology

The abdomen was cut open to remove both kidneys and liver from each animal. Isolated kidneys and liver were cleaned off extraneous tissue and rinsed in ice-cold physiological saline. One of the kidneys and liver was fixed in 10% neutral buffered formalin, processed in a

series of graded alcohol and xylene, embedded in paraffin wax, sectioned at 5 µm and stained with H and E (Haematoxylin and Eosin) for examination under polarized light. The slides were also observed to estimate tubulointerstitial damage index [11, 12].

2.3.2.4. Statistical analysis

All the results were expressed as mean ± SEM. The results were analyzed statistically using one-way analysis of variance (ANOVA) followed by Dunnett's comparison test. p-values were calculated against vehicle and lithiatic control groups and $P < 0.05$ was considered significant [13].

3. RESULTS AND DISCUSSION

3.1. Acute Oral Toxicity

The test drug (Polyherbal Lithout Tablet) was orally administered to the animals. A single dose of 5000 mg/kg produces toxic effect on the mice. Out of five, three animals died. Thus dose was reduced to 2000 mg/kg. The lower dose of 2000 mg/kg does not affect the animals. All the five animals were survived. Thus the one tenth dose of 2000 mg/kg *i.e.* 200 mg/kg was selected as a therapeutic dose and the sub-therapeutic and super-therapeutic dose were selected as 100 mg/kg and 400 mg/kg respectively.

Table 2: Effect of single oral dose administration of Lithout tablet in mice

Dose	Death	Mortality	Symptoms of toxicity
2000 mg/kg	0/5	No Death	No Behavioural Change
5000 mg/kg	3/5	Death	Death

3.2. Characterization of Lithout tablets

Characterization results of prepared tablets were as given in Table 3.

Table 3: Results of Optimized formulation

Sr. No.	Parameters	Result
1	Angle of repose ɵ	27.9°
2	Bulk density	2.28 gm/ml
3	Tap density	0.33 gm/ml
4	Hausners ratio	1.19
5	Percentae compressibility	15.15
6	Weight variation	pass
7	Hardness	6.24 kg/cm^2
8	Friability	0.02%
9	Disinteration time	30 sec.
10	Dissolution rate at 25 min.	73.30%

All the results mentioned in the table 3, complies the IPQC standards

3.2.1. In-vivo anti-urolithiatic activity (Preventive Study)

The Lithout tablet was prepared by using aqueous wet granulation method and all the in process quality control tests were performed. A Lithout tablet was also evaluated for acute oral toxicity study. In the present study, to evaluate anti urolithiatic activity of Lithout tablets, we have performed *in-vivo* study. There are several drugs which are used to induce the kidney stone in experimental animals such as ethylene glycol, cisplatin, gentamicine, etc. Among those, the more commonly used inducer ethylene glycol was used for *in-vivo* study. The *in-vivo* anti-urolithiatic activity was evaluated by ethylene glycol induced urolithiasis model. Urine volume of positive control group decreases due to deposition of calcium oxalate crystals in the kidney. When animals in groups III, IV, V, VI treated with standard drug (Cystone), 100 mg/kg, 200 mg/kg and 400 mg/kg Lithout tablet respectively the urine volume was increased significantly (*** $p<0.001$) from 14th & 28th day in both preventive and curative study. Similarly, urine pH also decreased in positive control group and it was significantly ($^{***}p<0.001$) increased in groups III, IV, V, VI treated with standard drug (Cystone), 100 mg/kg, 200 mg/kg and 400 mg/kg Lithout tablet respectively on 14th & 28th day in both preventive and curative study. As the ingestion of ethylene glycol in animals increased day by day the subsequent increase in urinary calcium takes place. When animals in groups III, IV, V, VI treated with standard drug (Cystone), 100 mg/kg, 200 mg/kg & 400 mg/kg Lithout tablet respectively urinary calcium was increased significantly ($^{***}p<0.001$) on 14th and 28th day in both preventive and curative study. Urinary oxalate was decreased significantly ($^{###}p<0.001$) in positive control group as compared to normal control. Significant ($^{***}p<0.001$) rise in urinary BUN was takes place on 14th and 28th day in both preventive and curative study in groups III, IV, V, VI treated with standard drug (Cystone), 100 mg/kg, 200 mg/kg & 400 mg/kg Lithout tablet respectively. There was significant ($^{###}p<0.001$) reduction of urinary phosphate content in positive control group as compared to normal control group. When animals in groups III, IV, V, VI treated with standard drug (Cystone), 100 mg/kg, 200 mg/kg & 400 mg/kg Lithout tablet respectively urinary phosphate content was increased significantly ($^{***}p<0.001$) on 14th and 28th day in both preventive and curative study.

Serum creatinine level of all the drug treated groups *i.e.* 100 mg/kg, 200 mg/kg and 400 mg/kg Lithout tablet and also standard drug (Cystone) group was decreased significantly ($^{***}p<0.001$) as compared with positive control group. Similarly serum BUN was decreased significantly ($^{***}p<0.001$) in 100 mg/kg, 200 mg/kg and 400 mg/kg Lithout tablet and standard drug (Cystone) groups as compared with positive control group. Also serum uric acid level lowers significantly (*** $p<0.001$) in 100 mg/kg, 200 mg/kg and 400 mg/kg Lithout tablet and standard drug (Cystone) groups as compared with positive control group in both preventive and curative study.

Table 4: Effect of Lithout tablet on urine volume of urolithiatic rats

Parameter Urine Volume (ml/24 hr)	Group I Normal	Group II Positive Control	Group III Standard (Cystone)	Group IV 100 mg/kg	Group V 200 mg/kg	Group VI 400 mg/kg
0 Day	2.27±0.07	2.30±0.02	2.31±0.03	2.92±0.03	2.28±0.02	2.30±0.02
7 Day	2.30±0.06	1.81±0.04$^{###}$	1.86±0.03	1.72±0.02	1.90±0.02	1.94±0.03*
14 Day	2.33±0.07	1.48±0.03$^{###}$	2.08±0.03***	1.79±0.03***	1.90±0.02***	2.03±0.02***
28 Day	2.34±0.03	1.15±0.03$^{###}$	2.13±0.02***	2.02±0.04***	2.07±0.04***	2.14±0.02***

Table 5: Effect of Lithout tablet on urine pH of urolithiatic rats

Parameter Urine pH	Group I Normal	Group II Positive Control	Group III Standard Cystone)	Group IV 100 mg/kg	Group V 200 mg/kg	Group VI 400 mg/kg
0 Day	7.67±0.05	7.67±0.05	7.72±0.06	7.71±0.05	7.76±0.05	7.81±0.05
7 Day	7.64±0.06	6.73±0.07$^{###}$	6.65±0.06	6.60±0.02	6.88±0.05	6.94±0.05*
14 Day	7.68±0.07	6.45±0.04$^{###}$	7.11±0.04***	6.79±0.03***	7.15±0.03***	7.27±0.01***
28 Day	7.71±0.04	5.27±0.04$^{###}$	7.47±0.02***	6.98±0.04***	7.32±0.02***	7.49±0.02***

Table 6: Effect of Lithout tablet on urine calcium of urolithiatic rats

Parameter Urine Calcium	Group I Normal	Group II Positive Control	Group III Standard (Cystone)	Group IV 100 mg/kg	Group V 200 mg/kg	Group VI 400 mg/kg
0 Day	3.97±0.07	4.16±0.14	3.94±0.09	4.22±0.07	4.16±0.07	4.04±0.05
7 Day	4.10±0.07	6.07±0.16$^{###}$	5.56±0.17**	6.05±0.01	5.54±0.02**	5.47±0.01***
14 Day	4.11±0.09	7.32±0.02$^{###}$	4.98±0.07***	5.31±0.08***	5.24±0.09***	5.19±0.09***
28 Day	4.16±0.07	7.43±0.02$^{###}$	5.55±0.10***	6.70±0.07***	5.47±0.12***	4.75±0.05***

Table 7: Effect of Lithout tablet on urine oxalate of urolithiatic rats

Parameter Urine Oxalate	Group I Normal	Group II Positive Control	Group III Standard (Cystone)	Group IV 100 mg/kg	Group V 200 mg/kg	Group VI 400 mg/kg
0 Day	0.80±0.02	0.82±0.02	0.82±0.02	0.81±0.02	0.79±0.03	0.80±0.02
7 Day	0.82±0.02	1.34±0.03$^{###}$	1.31±0.02	1.11±0.04***	0.95±0.01***	0.87±0.01***
14 Day	0.83±0.02	2.52±0.18$^{###}$	1.26±0.02***	1.51±0.02***	1.23±0.02***	0.84±0.01***
28 Day	0.83±0.02	3.67±0.15$^{###}$	1.04±0.05***	0.93±0.02***	0.82±0.02***	0.77±0.01***

Table 8: Effect of Lithout tablet on urine phosphate of urolithiatic rats

Parameter Urine Phosphate	Group I Normal	Group II Positive Control	Group III Standard (Cystone)	Group IV 100 mg/kg	Group V 200 mg/kg	Group VI 400 mg/kg
0 Day	5.29±0.02	5.30±0.02	5.28±0.01	5.26±0.01	5.26±0.02	5.26±0.01
7 Day	5.27±0.02	6.35±0.05$^{###}$	6.36±0.02	6.12±0.03***	5.79±0.02***	5.57±0.03***
14 Day	5.29±0.02	7.44±0.03$^{###}$	6.16±0.03***	5.92±0.02***	5.61±0.01***	5.37±0.07***
28 Day	5.28±0.02	8.10±0.07$^{###}$	6.18±0.03***	6.30±0.02***	5.81±0.02***	5.47±0.02***

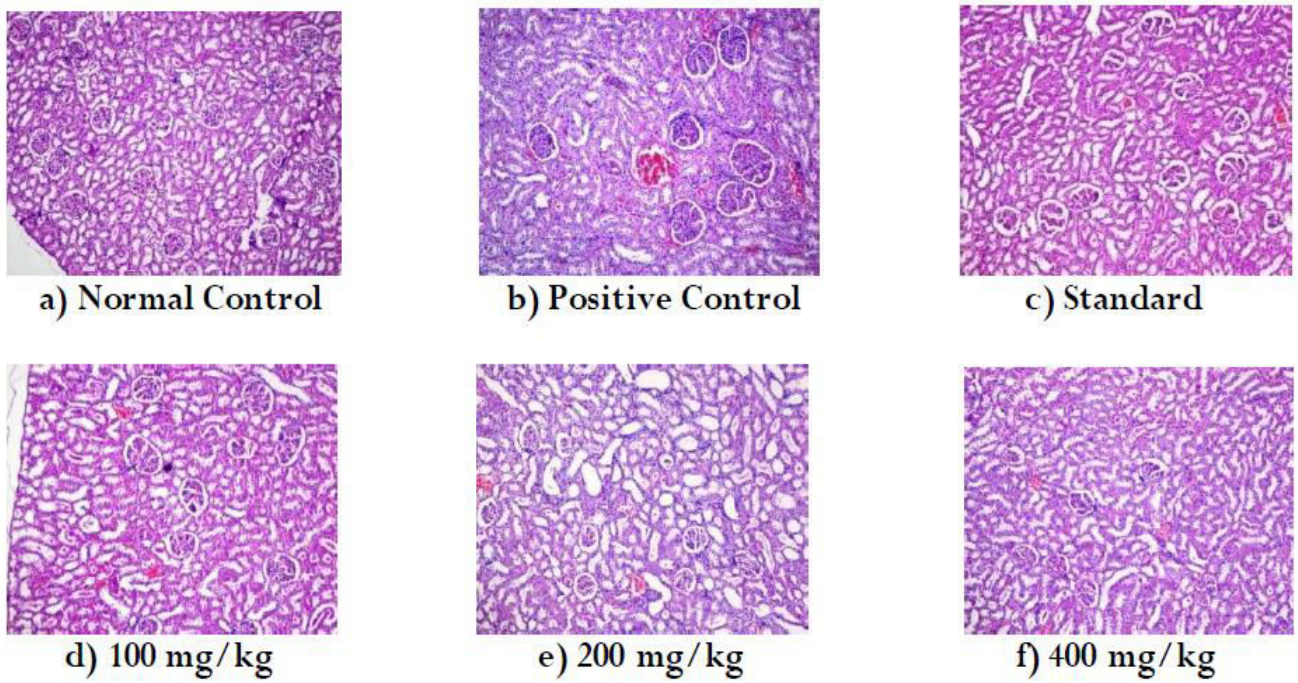
3.2.2. Histopathology Study

In this present study, animals in positive control group treated with ethylene glycol shows moderate (+++) pathological changes in kidney and liver in both preventive and curative study. Animals in group III treated with standard drug (Cystone) shows minimal (+) to NAD pathological changes in kidney and liver in both preventive and curative study. In case of preventive and curative study, animals in group IV treated with 100 mg/kg dose of Lithout tablet shows mild (++) pathological changes in kidney and mild (++) to moderate (+++) pathological changes in liver. Animals in group V treated with 200 mg/kg dose of Lithout tablet shows minimal (+) pathological changes in kidney and mild (++) pathological changes in liver in case of preventive study. Similarly, in case of curative study, there are mild (++) pathological changes in both kidney and liver. Animals in group VI treated with 400 mg/kg dose of Lithout tablet shows minimal (+) pathological changes in kidney and liver in both preventive and curative study.

Hence, from all the gathered data we can conclude that prepared polyherbal formulation *i.e.* Lithout tablet may have antiurolithiatic activity.

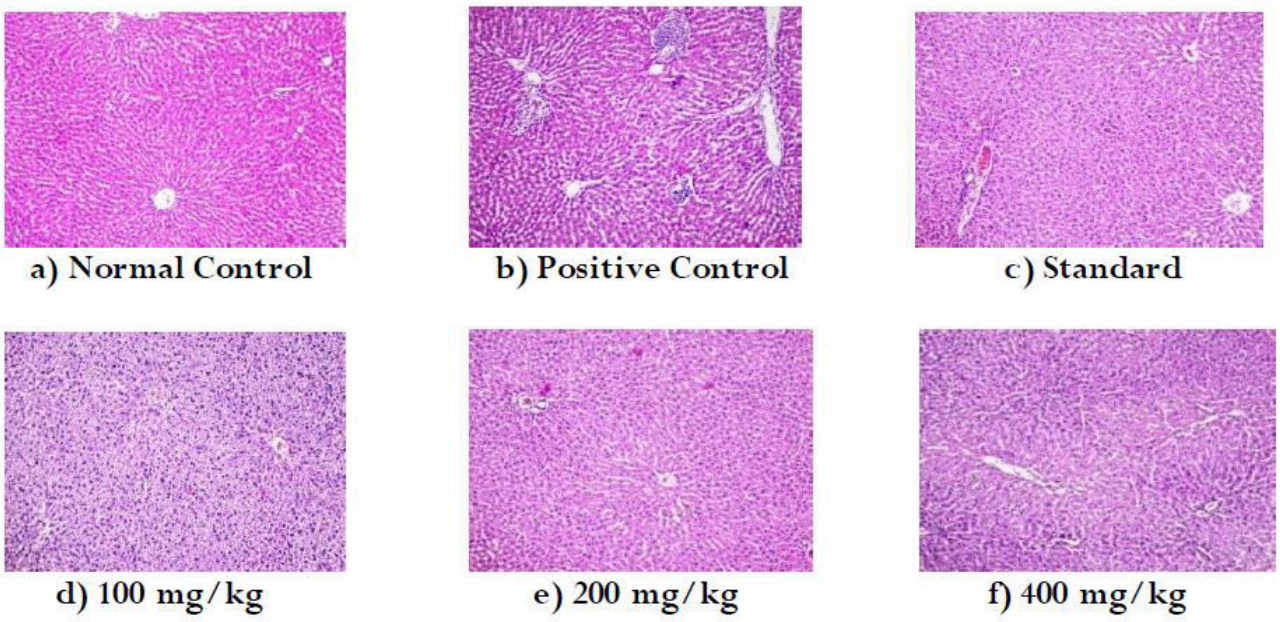
Table 9: Effect of Lithout tablet on serum parameters of urolithiatic rats

Parameters	Group I Normal	Group II Positive Control	Group III Standard (Cystone)	Group IV 100 mg/kg	Group V 200 mg/kg	Group VI 400 mg/kg
Creatinine	0.92±0.03	3.12±0.16$^{###}$	1.44±0.02***	1.99±0.06***	1.69±0.03***	1.46±0.013***
BUN	12.85±0.10	24.43±0.33$^{###}$	15.56±0.17***	22.18±0.25***	17.96±0.12***	15.23±0.17***
Uric Acid	1.75±0.07	6.66±0.15$^{###}$	3.17±0.09***	5.05±0.19***	3.18±0.12***	2.76±0.16***



a) Normal Control: The renal tubules showed normal epithelium b) Positive Control: Multiple foci of dilation of renal tubules with interstitial haemorrhages were observed c) Standard: The renal tubules showed focal degenerative changes in renal parenchyma d) Sub-therapeutic Dose 100 mg/kg: Multiple foci of dilation of renal tubules with interstitial haemorrhages were observed e) Therapeutic Dose 200 mg/kg: Focal cellular swelling and focal degenerative changes in renal parenchyma with focal accumulation of eosinophilic debris in lumen of tubules f) Supper-therapeutic: Focal cellular swelling and focal degenerative changes in renal parenchyma with focal accumulation of eosinophilic debris in lumen of tubules

Fig. 1 Effect of Lithout tablet on histopathology of isolated kidneys of urolithiatic rats in ethylene glycol induced urolithiasis model



a) Normal Control: There was absence of any inflammatory or metabolic pathological changes in the hepatocytes. b) Positive Control: cellular swelling with enlarged nucleus, focal necrotic changes of hepatocytes with loss of nucleus and cell borders was also noted. c) Standard: focal congestion of central veins. d) Sub-therapeutic Dose 100 mg/kg: Focal fatty infiltration was noted in hepatocytes. e) Therapeutic Dose 200 mg/kg: Hepatocytes showed cellular swelling with enlarged nucleus as well as diffuse degenerative changes with granular cytoplasm. f) Supper-therapeutic: Focal degenerative changes were noted with focal cellular swelling in few of the hepatocytes only.

Fig. 2: Effect of Lithout tablet on histopathology of isolated liver of urolithiatic rats in ethylene glycol induced urolithiasis model

3.2.3. Curative Study

Table 10: Effect of Lithout tablet on serum parameters of urolithiatic rats

Parameters	Group I Normal	Group II Positive Control	Group III Standard (Cystone)	Group IV 100 mg/kg	Group V 200 mg/kg	Group VI 400 mg/kg
Creatinine	0.92±0.03	3.12±0.16$^{###}$	1.44±0.02***	2.65±0.14**	1.90±0.02***	1.55±0.08***
BUN	12.85±0.10	24.43±0.33$^{###}$	15.56±0.17***	22.77±0.43***	19.22±0.17***	15.14±0.20***
Uric Acid	1.74±0.07	6.66±0.15$^{###}$	3.16±0.09***	4.82±0.16***	3.18±0.10***	2.76±0.12***

Table 11: Effect of Lithout tablet on urine volume of urolithiatic rats

Parameter Urine Volume (ml/24 hr)	Group I Normal	Group II Positive Control	Group III Standard (Cystone)	Group IV 100 mg/kg	Group V 200 mg/kg	Group VI 400 mg/kg
0 Day	2.27±0.07	2.30±0.02	2.31±0.03	2.29±0.02	2.26±0.03	2.30±0.03
7 Day	2.30±0.06	1.81±0.04$^{###}$	1.83±0.02	1.70±0.03	1.81±0.03	1.89±0.03
14 Day	2.33±0.07	1.48±0.03$^{###}$	2.08±0.03***	1.65±0.01**	1.85±0.01***	2.00±0.04***
28 Day	2.34±0.03	1.15±0.03$^{###}$	2.13±0.02***	1.92±0.01***	2.04±0.04***	2.15±0.02***

Table 12: Effect of Lithout tablet on urine pH of urolithiatic rats

Parameter Urine pH	Group I Normal	Group II Positive Control	Group III Standard (Cystone)	Group IV 100 mg/kg	Group V 200 mg/kg	Group VI 400 mg/kg
0 Day	7.67±0.05	7.67±0.05	7.72±0.06	7.59±0.03	7.60±0.03	7.67±0.04
7 Day	7.64±0.06	6.76±0.06$^{###}$	6.65±0.06	6.65±0.07	6.61±0.02	6.65±0.03
14 Day	7.68±0.07	6.45±0.04$^{###}$	7.11±0.04***	6.98±0.02***	7.11±0.03***	7.17±0.03***
28 Day	7.71±0.04	5.27±0.04$^{###}$	7.47±0.02***	7.19±0.03***	7.36±0.03***	7.48±0.02***

Table 13: Effect of Lithout tablet on urine calcium of urolithiatic rats

Parameter Urine Calcium	Group I Normal	Group II Positive Control	Group III Standard (Cystone)	Group IV 100 mg/kg	Group V 200 mg/kg	Group VI 400 mg/kg
0 Day	3.97±0.07	4.16±0.14	3.94±0.09	4.04±0.03	4.04±0.04	3.97±0.04
7 Day	4.10±0.07	6.07±0.16###	6.06±0.07	6.23±0.03	6.01±0.05	5.95±0.05
14 Day	4.11±0.09	7.32±0.02###	4.98±0.07***	6.12±0.08***	6.03±0.09***	5.94±0.09***
28 Day	4.16±0.07	7.43±0.02###	5.55±0.10***	6.14±0.06***	5.06±0.06***	4.79±0.05***

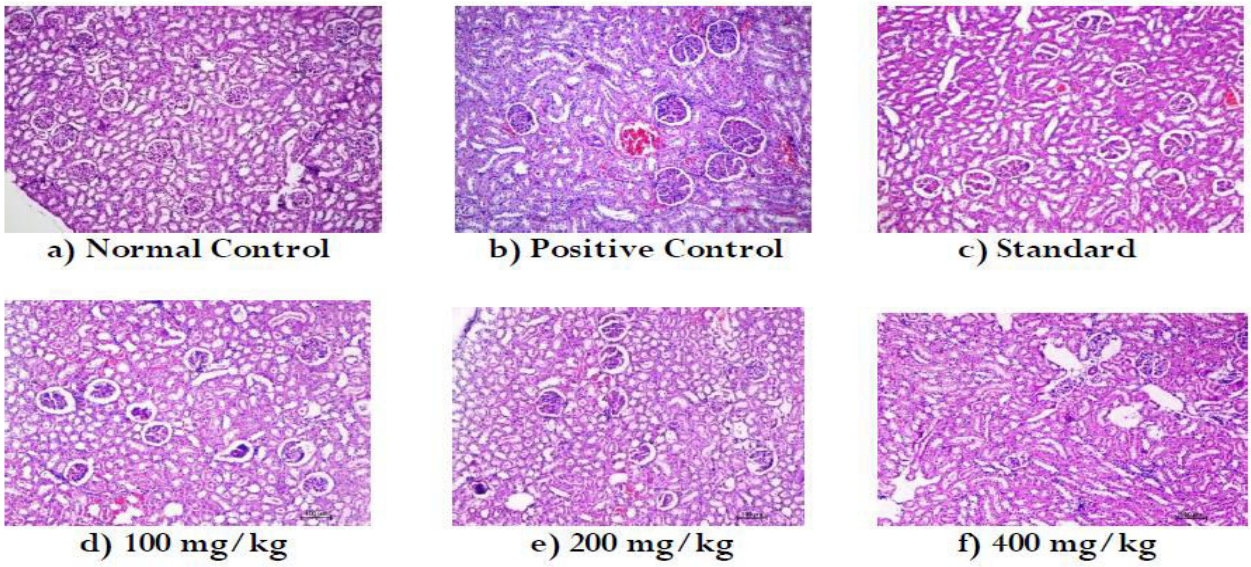
Table 14: Effect of Lithout tablet on urine oxalate of urolithiatic rats

Parameter Urine Oxalate	Group I Normal	Group II Positive Control	Group III Standard (Cystone)	Group IV 100 mg/kg	Group V 200 mg/kg	Group VI 400 mg/kg
0 Day	0.80±0.02	0.82±0.02	0.82±0.02	0.80±0.02	0.82±0.02	0.83±0.02
7 Day	0.82±0.02	1.34±0.03###	1.31±0.02	1.36±0.03	1.32±0.03	1.29±0.02
14 Day	0.83±0.02	2.52±0.18###	1.54±0.02***	1.51±0.02***	1.10±0.03***	0.90±0.02***
28 Day	0.83±0.02	3.67±0.15###	1.89±0.02***	1.25±0.01***	0.94±0.01***	0.85±0.02***

Table 15: Effect of Lithout tablet on urine phosphate of urolithiatic rats

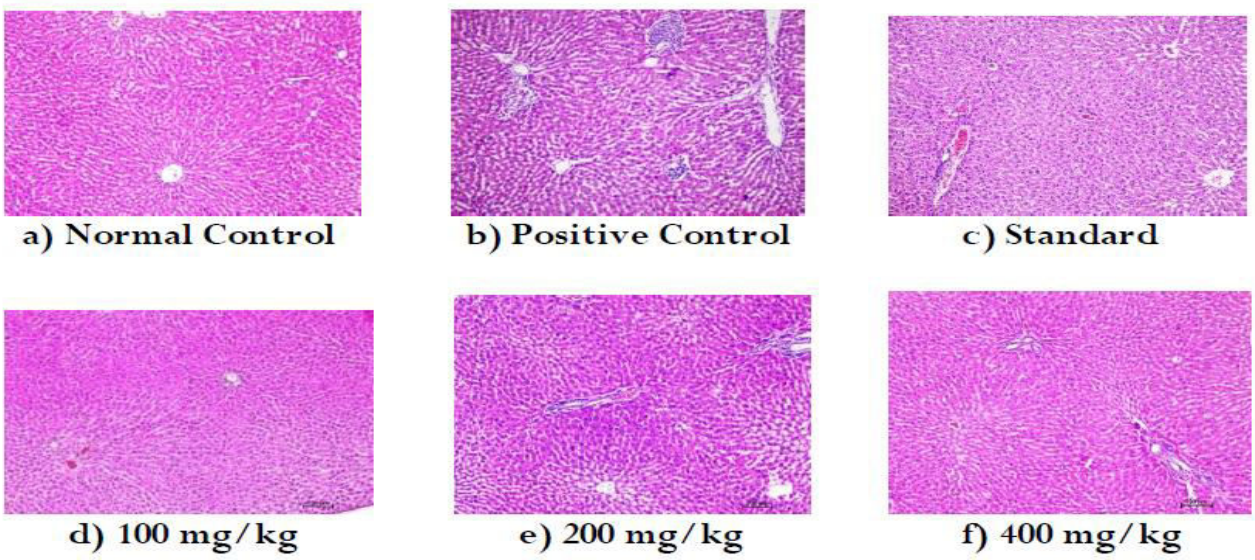
Parameter Urine Phosphate	Group I Normal	Group II Positive Control	Group III Standard (Cystone)	Group IV 100 mg/kg	Group V 200 mg/kg	Group VI 400 mg/kg
0 Day	5.29±0.02	5.30±0.02	5.28±0.01	5.27±0.02	5.26±0.01	5.27±0.01
7 Day	5.27±0.02	6.35±0.05###	6.31±0.03	6.30±0.02	6.25±0.02	6.27±0.02
14 Day	5.29±0.01	7.44±0.03###	6.79±0.03***	6.51±0.01***	6.01±0.07***	5.84±0.04***
28 Day	5.28±0.02	8.10±0.07###	5.50±0.01***	5.80±0.02***	5.55±0.02***	5.37±0.03***

3.2.4. Histopathological effects of tablets



a)Normal Control: There was absence of any inflammatory or pathological changes in kidney, b) Positive control: degenerative changes in renal tubules with loss of nucleus and vacuolar changes of damaged tubules, c) Standard: The renal tubules showed focal degenerative changes in renal parenchyma, d) Sub-therapeutic Dose 100 mg/kg: Multiple foci of dilation of renal tubules with interstitial haemorrhages were observed, e) Therapeutic Dose 200 mg/kg: Degenerative and Nephropathic changes were lesser than Low dose group, f) Super-therapeutic Dose 400 mg/kg: Degenerative and Nephropathic changes were very minimal and were of significantly lesser than Low dose and Mid dose group.

Fig. 3: Effect of Lithout tablet on histopathology of isolated kidneys of urolithiatic rats in ethylene glycol induced urolithiasis model



a) Normal control: There was absence of any inflammatory or metabolic pathological changes in the hepatocytes, b) Positive Control: Multi-focal areas of derangement of hepatic cords were observed c) Standard: Focal presence of degenerative changes of hepatocytes was noted d) Sub-therapeutic Dose 100 mg/kg: Hepatocytes showed cellular swelling with enlarged nucleus as well as diffuse degenerative changes with granular cytoplasmic changes in the parenchyma e) Therapeutic dose 200 mg/kg: Hepatocytes showed cellular swelling with enlarged nucleus as well as diffuse degenerative changes with granular cytoplasm f) Supper-therapeutic Dose 400 mg/kg: Focal degenerative changes were noted with focal cellular swelling in few of the hepatocytes only.

Fig. 4: Effect of Lithout tablet on histopathology of isolated liver of urolithiatic rats in ethylene glycol induced urolithiasis model

3.3. Statistical analysis

All the statistical analysis done by using ANOVA followed by Dunnett's test, where, *** $p<0.001$, ** $p<0.01$, * $P<0.05$ Group II compared with Group I. ### $p<0.001$, n = 6

4. CONCLUSION

In the present study, anti-urolithiatic activity of polyherbal Lithout tablet was evaluated by *in-vivo* model using ethylene glycol induced urolithiasis. From the present study, it can be concluded that animals treated with standard (Cystone) drug and Lithout tablet in dose of 200 mg/kg of body weight exerted significant anti-urolithiatic activity and protection against tubular interstitial damage in kidneys and liver in ethylene glycol induced urolithiatic rats.

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