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Research Article

PRECLINICAL EVALUTION OF ANTIUROLITHIATIC ACTIVITY OF *SWERTIA CHIRATA* STEMS

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ABSTRACT
The aim of our present study is to evaluate preventive effect of swretia chirata drug in experimentally induced urolithiasis model in rat. Rats were induced to produce renal stone by providing them 0.75% v/v ethylene glycol in drinking water for 28 days. In addition to this methanolic extract of *Swertia chirata* low dose and high dose i.e. 250 mg/kg and 500 mg/kg were administered along with ethylene glycol for 28 days. After 28 day, the urine, blood and kidney samples were collected from each animal and used for the estimation of various levels of promoters, inhibitors and antioxidant parameters. The ethylene glycol feeding resulted in an increased level of promoters with a decreased level of inhibitors as compare to normal control rats. All these conditions were significantly reversed with treatment of *Swertia chirata.* Histopathological analysis also reveals deposition of calcium oxalate crystals and disruption of tubular cells and juxtaglomerular cells. That deposition and disruption were also reduced in rats treated with *Swertia chirata*. These data suggest preventive effect of *Swertia chirata* against urolithiasis.

KEYWORDS: Urolithiasis, *Swertia chirata*, Ethylene glycol, Cystone, Hyperoxaluria, Histopathology.

INTRODUCTION

Urolithiasis is a worldwide problem. It is estimated that 12% of world population experiences renal stone disease with a recurrence rate of 70-80% in men and 47-60% in women.[1, 2, 3] Kidney stone formation or urolithiasis is a complex process that occurs due to imbalance between promoters and inhibitors in the kidneys.[4] It is a succession of several physicochemical events including supersaturation, nucleation, growth, aggregation and retention within the kidneys.[5] Calcium-containing stones, especially calcium oxalate monohydrate, calcium oxalate dihydrate and basic calcium phosphate are the most commonly occurring stones. Other types of stone include uric acid stone, struvite stone, cystine stone, silicate stone, protease related stone, DHA stone etc.

Drug treatments as well as invasive procedures are available for elimination of kidney stone. Drug treatment include use of diuretics, antibiotics, anti-inflammatory agents, muscle relaxants, analgesics etc., Invasive procedure include Extra corporeal shock-wave lithotripsy, nephrolithotomy, ureteroscopy etc. Surgery procedure causes complications and increase chances of recurrence.

The overuse of synthetic drug results in higher incidence of adverse drug reactions. This motivated humans to return to natural herbs for safe remedies. Herbal drugs can be easily available and are also economical for patient. It does not produce any type complications like synthetic drugs to patients. ***"Swertia chirata"*** is suggested to remove and reduce total number of urinary stones from kidney. Thus it may prove a potent drug having strong antiurolithiatic activity with minimum cost and fewer side effects than that of other available drug treatment. [6]

In the present study, the objective was to evaluate and validate the preventive antiurolithiatic activity of methanolic extract of *Swertia chirata* stems in ethylene glycol induced urolithiasis in rats.

MATERIALS AND METHODS

Chemicals

Cystone was procured from commercial source. Diagnostic kits for various bio chemical analysis were procured from Creast Biosystem, Goa, Beacon Diagnostics Pvt. Ltd. Navsari, Siemens Healthcare Diagnostics Ltd. Baroda and Span Diagnostics Ltd. Surat.

Animals

Male albino wistar rats weighing between 250-300 gm were selected for antiurolithiatic activity. They were kept under standard laboratory conditions (temperature: 25 ± 5 °C), humidity (55 ± 5%) and maintained on 12-h light: 12-h dark cycle. They were provided with regular rat chow and drinking water ad libitum.

Experimental protocol

Healthy male Wistar albino rats (30 rats) were divided into five groups containing six rats in each and the study was conducted for 28 days to evaluate the preventive efficacy of drug.

Group 1: Normal control (normal feed and water)
Group 2: Disease control (0.75% v/v EG in drinking water)
Group 3: Standard (0.75% v/v EG in drinking water +750mg/Kg cystone; p.o)
Group 4: Treatment (0.75% v/v EG in drinking water + 250 mg/kg of MeSC; p.o)
Group 5: Treatment (0.75% v/v EG in drinking water + 500 mg/kg of MeSC; p.o)

Preparation of drug extract

The drug was shade dried and grounded by mixture. Then mixed with methanol and kept in sohxlet apparatus for 24 hr. on the next day the extract was filtered and concentrated by evaporation.

Biochemical analysis

All the treatments were done once daily by orally for 28 days. All animals were kept in individual metabolic cages and urine samples of 24 h were collected on 28th day. Urine was analyzed for calcium, phosphorus, oxalate magnesium, uric acid, and creatinine content. After the experimental period, at end of 29th day blood was collected from the retro-orbital under anaesthetic conditions and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 15000 rpm for 20 min and analyzed for Urea, Uric acid, Creatinine, Calcium, Magnesium, Potassium, Sodium and Phosphorous content. The abdomen was cut open to remove both kidneys from each animal. One kidney was preserved in 10% neutral formalin for histopathology purpose. Another

kidney was divided into two parts, one part was used for estimation of antioxidant parameters and another was dried in oven and used for estimation of other parameters.

Statistical analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance ANOVA. $P < 0.05$ was considered significant.

RESULTS

Some changes were observed in disease control group as compare to normal control group. Body weight, diuresis and kidney weight were significantly reduced in disease control group as compared to normal control group. These physiological changes were significantly prevented in standard group (750 mg/kg; p.o.) as well as both low (MeSC-LD; 250 mg/kg; p.o.) and high (MeSC-HD-500 mg/kg; p.o.) dose treatment groups. (Table no. : 1). pH of urine was found to be acidic in normal control while model control urine pH was found to be alkaline. Whereas these shift of pH from acidic to alkaline was prevented by standard and treatment groups.

Various urolithiasis promoters' level which enhances chances of stone formation was evaluated in urine, serum and kidney homogenate samples. Levels of these promoters of stone formation were found to be increased in disease control as compare to normal control. These rises in levels of promoters was significantly prevented in standard group and two treatment groups by treating these groups with standard drug cystone, MeSC- LD and MeSC- HD respectively. (Table no. 2, 3, 4).

After completion of 28 day treatment, level of citrate was found to be reduced in disease control. There was significant reduction found in level of magnesium in urine as well as serum samples (Table no.5). Such reduction of inhibitors levels was significantly prevented with standard and test treatments.

Ethylene glycol induced disease control group showed marked increase in level of BUN and decreased levels of urea nitrogen and creatinine in urine as compared to normal control (Table no. 6). Analysis of various biological samples revealed that treatment with standard drug cystone, MeSC-LD and MeSC- HD significantly prevented changes in level of renal function markers like BUN, urea nitrogen and creatinine clearance.

Ethylene glycol was given continuously for 28 days to disease control group. So, level of protein and catalase was decreased significantly. While level of MDA was increased in disease control group as compare to normal control. (Table no. 7). Whereas treatment with standard drug cystone, MeSC-LD and MeSC- HD prevented significant rise in MDA level and increased levels of catalase and protein.

Histopathology of kidney section was performed to observe oxidative changes and crystal deposition in different groups. In normal control, (figure 1) intact nephron structure with normal glomerular capsule and juxtaglomerular cells can be observed. But in case of ethylene glycol induced model i.e. disease control (figure 2) nephron structure was disrupted and demonstrated by ruptured visceral podocytes (squamous epithelial cells) and parietal layer cells (squamous epithelial cells) of glomerular capsule along with damaged juxtaglomerular cells. Standard and treatment groups (figure 3, 4 and 5) significantly prevented the rupture of kidney cells as compare to disease control group.

DISSCUSSION

In the present study, 0.75% ethylene glycol induced urolithiasis in rats is used. According to various survey, it is observed that chances of kidney stone formation is more in men as compare to women. So, chances of stone formation in female rats are less than male rats. So, we had selected male wistar rats for study.

Ethylene glycol is rapidly absorbed and metabolized in the liver via alcohol dehydrogenase or aldehyde dehydrogenase to glycolic acid. Glycolic acid is oxidized to glyoxylic acid, which is further oxidized to oxalic acid by glycolate oxidase or lactate dehydrogenase, thus promoting hyperoxaluria. Since most of the stones are always composed of calcium oxalate, ethylene glycol model was selected to induce urolithiasis. Calcium, oxalate and uric acid are the major promoters of formation of kidney stone. Some factors like crystalluria, retention, infection, inhibitors etc. also affect the formation of stone.[7] Stones with smaller size can easily travel through the urinary system followed by excretion. But large size stones may lead to obstruction in urine passage and results in pain during urination. Due to pain, food consumption may be decreased, so these lead to decrease in weight of body. In disease control group, similarly significant loss of body weight was observed. In case of standard and treatment group, animals were treated with standard drug cystone (750 mg/kg body weight) and treatment group receive methanolic extract of *Swretia chirata* low dose (250 mg/kg body weight) and methanolic extract of *Swretia chirata* high dose (500 mg/kg body weight). These standard and treatment drugs showed effective diuretic activity, which could have resulted in the prevention of stone formation and thus also the prevention of weight loss in animals.

Generally urine has acidic pH which is useful to prevent crystallization of stone forming promoters by dissolving them into urine. But in case of chronic hyperoxaluric rats in disease control group, pH was found to be alkaline. Alkaline pH promotes the crystallization in urine and thus stone formation.[8] Treatment with standard and test drugs significantly prevented the alkalinization of pH and thus, significantly prevented the precipitation of stone forming promoters like calcium, oxalate, uric acid etc.

It has been observed that increase in level of urinary inorganic phosphate in excretion elevates calcium phosphate crystal formation. This induces deposition of calcium oxalate crystals.[9] Ethylene glycol induces increase in level of inorganic phosphate as also observed in our disease control group. But it was significantly prevented in test groups. The uric acid crystals adsorb glutamic acid and other organic compounds and promote calcium oxalate crystals growth.[10] In disease control group there was significant increase in level of uric acid in urine and serum samples as compare to normal control group. This increase in uric acid level was significantly prevented in standard group and test groups.

After ingestion of ethylene glycol, level of promoters increases in kidney, which leads to formation and aggregation of stone in kidney[11]. So, disease control animals have more number of crystal depositions as compare to normal control group animals. This deposition of promoters in kidney increases weight of dry kidney. This aggregation of promoters causes water accumulation. Weight of dry and wet kidney was found to be increased in ethylene glycol induced disease control group. This increase in weight of kidney was significantly reduced in standard and treatment groups.

Various studies suggested that magnesium inhibits formation of stones by binding to oxalate and forming soluble complexes. So, salts of magnesium are inhibitors of stone formation.[12] Thus, it reduces the concentration of calcium oxalate.[13] Ethylene glycol does not change magnesium

concentration. But standard drug and test drug treatment significantly increased level of magnesium in various biological samples like urine, serum and kidney as compare to disease control group. Standard and test group have also increased level of magnesium as compare to normal control group.

Citrate is also an important urolithiatic inhibitor. Citrate forms soluble complexes with calcium and inhibits aggregation and precipitation of calcium oxalate and phosphate stones. Groups which were treated with standard and test drug showed significant increase in citrate level as compare to disease control. This may be the probable reason for reduced crystallization of calcium oxalate in standard and test groups.[14]

Stone possess obstruction in excretion of urine. Oxalate and calcium deposition in high amount possess nephrotoxicity. Waste nitrogenous substances like BUN accumulation possess decreased excretion of urea nitrogen and creatinine clearance in urine.[15] From different experimental reports, it was observed that calcium oxalate crystals causes direct oxidative stress.[16] Generation of reactive oxygen species causes damage to glomerulus and tubules. Damage of kidney due to ethylene glycol in disease control was also confirmed by histopathological study of kidney samples.

Catalase is considered as a first line defensive antioxidant enzyme since it regulates H_2O_2 levels, which can lead to hydroxyl radical excess through the metal catalyzed Fenton (Fe/Cu) and Haber-Weiss reactions.[17] Oxidative stress can be compensated by elevated antioxidant enzymes in the kidney.[8] The liberation of xanthine oxidase during uric acid formation is the key operator for the elevated release of hydrogen peroxides.[10] Catalase being the only enzyme regulating potent hydroxyl radical formation, its decreased activity in urolithiasis may lead to excessive H_2O_2 accumulation in the kidney and thus, more hydroxyl radical formation as also observed in disease control group.[16] Administration of standard drug cystone (750 mg/kg body weight) and treatment group receive methanolic extract of *Swretia chirata* low dose (250 mg/kg body weight) and methanolic extract of *Swretia chirata* high dose (500 mg/kg body weight) resulted in significant decrease in lipid peroxidation level and so significantly prevented decrease in the catalase levels in kidney as compared to the disease control animals. This suggests its efficacy in preventing free radical-induced damage. This may be reason for protection to the kidney which was revealed by histopathological study after treatment of tests and standard samples.

From the obtained data we can conclude that *Swretia chirata* is effective in prevention of aggregation of crystals and which may be result of combination of various effects like diuresis, decrease in promoter's level, increase in inhibitors level. From the data we can observe that both low dose and high dose of *Swertia Chirata* showed a good antiurolithiatic activity. However, high dose showed more significant effect at various stages.

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Table no 1: Effect of ***Swretia chirata*** in on various **physical parameters** in ethylene glycol induced urolithiasis preventive study.

PARAMETRS	NORMAL	CONTROL	STANDARD	MeSC [LD]	MeSC [HD]
CHANGE IN BODY WEIGHT (gm)					
	276.66±6.692	216.66±6.692##	248.33±4.029**	246.66±2.116**	245±2.244*
VOLUME OF URINE (ml)					
	17.66±0.211	15.5±0.429##	28.33±0.423**	26.0±0.448**	27.166±0.402**
Ph					
	6.5±0.224	8.66±0.211##	7.33±0.211**	7.5±0.224**	7.66±0.211*
WET KIDNEY WEIGHT (gm)					
	0.825±0.036	1.388±0.088##	0.775±0.024**	0.741±0.008**	0.83±0.007**
DRY KIDNEY WEIGHT (gm)					
	0.141±0.010	0.366±0.013##	0.186±0.010**	0.15±0.003**	0.175±0.004**

Table no. 2: Effect of ***Swretia chirata*** in on various **promoters** in ethylene glycol induced urolithiasis preventive study. [In **urine**]

PARAMETERS	NORMAL	CONTROL	STANDARD	MeSC [LD]	MeSC [HD]
CALCIUM (mg/dl)					
	5.771±0.248	7.395±0.535#	4.287±0.372**	4.997±0.304**	4.923±0.175**
OXALATE (mg/dl)					
	1.898±0.342	6.499±0.171##	0.454±0.051**	2.407±0.275**	2.666±0.197**
INORGANIC PHOSPHATE [IP] (mg/dl)					
	0.172±0.017	0.874±0.052##	0.151±0.018**	0.658±0.052*	0.425±0.053**
URIC ACID (mg/dl)					
	10.133±0.518	25.733±0.764##	9.252±0.433**	22.381±0.711*	20.008±0.775**

Table no 3: Effect of ***Swretia chirata*** in on various **promoters** in ethylene glycol induced urolithiasis preventive study. [In **serum**]

PARAMETERS	NORMAL	CONTROL	STANDARD	MeSC [LD]	MeSC [HD]
CALCIUM (mg/dl)					
	4.011±0.156	5.052±0.216##	3.096±0.130**	4.418±0.236^	3.962±0.206**
INORGANIC PHOSPHATE [I.P.] (mg/dl)					
	0.926±0.015	1.272±0.094##	0.774±0.029**	0.951±0.041**	0.775±0.065**
URIC ACID (mg/dl)					
	2.496±0.193	4.114±0.250##	1.807±0.139**	2.107±0.201**	1.940±0.233**

Table no 4: Effect of ***Swretia chirata*** in on various **promoters** in ethylene glycol induced urolithiasis preventive study. [In **kidney samples**]

PARAMETERS	NORMAL	CONTROL	STANDARD	MeSC [LD]	MeSC [HD]
CALCIUM (mg/g)					
	0.226±0.007	0.4±0.020##	0.191±0.003**	0.217±0.004**	0.212±0.005**
OXALATE (mg/g)					
	4.111±0.062	6.132±0.168##	1.173±0.111**	3.443±0.197**	1.926±0.220**
INORGANIC PHOSPHATE [I.P.] (mg/100 mg kidney)					
	4.529±0.078	8.937±0.052##	5.445±0.041**	5.806±0.005**	6.324±0.040**
URIC ACID (mg/100 mg kidney)					
	1.182±0.080	2.566±0.036##	1.491±0.055**	1.711±0.035**	1.898±0.059**

Table no 5: Effect of ***Swretia chirata*** in on various **inhibiters** in ethylene glycol induced urolithiasis preventive study. [In **urine** and **serum samples**]

PARAMETERS	NORMAL	CONTROL	STANDARD	MeSC [LD]	MeSC [HD]
CITRATE (mg/dl)					
	5.941±0.063	2.876±0.125##	4.550±0.229**	5.136±0.162**	4.631±0.169**
MAGNESIUM IN URINE (mg/dl)					
	1.246±0.161	0.685±0.130^	2.485±0.189**	0.825±0.217^	1.205±0.114^
MAGNESIUM IN SERUM (mg/dl)					
	0.931±0.038	0.625±0.037##	1.239±0.031**	0.828±0.032**	1.118±0.051**

Table no 6: Effect of ***Swretia chirata*** in **on renal function** in ethylene glycol induced urolithiasis preventive study. [In **urine** and **serum** samples]

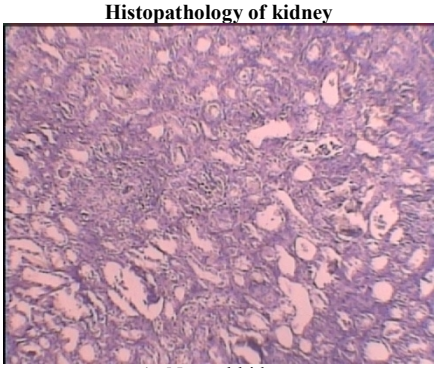
PARAMETERS	NORMAL	CONTROL	STANDARD	MeSC [LD]	MeSC [HD]
UREA NITROGEN IN URINE (mg/dl)					
	12.254±0.581	18.476±0.952##	10.283±0.565**	14.287±0.688**	11.039±0.497**
BUN (mg/dl)					
	40.135±0.711	61.606±0.665##	37.416±0.781**	41.601±0.748**	36.637±0.489**
CREATININE CLEARANCE IN URINE (mg/dl)					
	14.786±0.956	27.488±0.778##	13.566±0.787**	17.052±0.718**	15.859±0.775**
CREATININE CLEARANCE IN SERUM (mg/dl)					
	0.541±0.023	0.998±0.069##	0.390±0.028**	0.728±0.044**	0.640±0.056**

Table no 7: Effect of ***Swretia chirata*** in on **oxidative stress** in ethylene glycol induced urolithiasis preventive study. [In **kidney**]

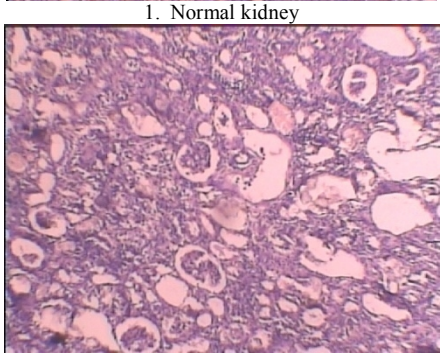
PARAMETERS	NORMAL	CONTROL	STANDARD	MeSC [LD]	MeSC [HD]
PROTEIN (mg/ml)					
	3.603±0.125	2.865±0.082##	3.549±0.155*	3.459±0.177*	3.518±0.135*
CATALASE (µmol/min/mg kidney)					
	77.620±0.308	43.802±0.272##	72.967±0.284**	58.202±0.164**	70.430±0.280**
MDA (nmol/mg)					
	0.843±0.088	1.618±0.117##	0.903±0.080**	1.092±0.087*	0.927±0.195**

All the values are expressed in terms of **mean ± SEM**; **n = 6** in each group.
Statistical analysis of various physical and biochemical parameters is carried out using the one-way **ANOVA** test.
#Significantly different from normal p≤0.05
Highly significantly different from normal p≤0.001
*significantly different from control p≤0.05
**Highly significantly different from control p≤0.001
^no significant difference from normal and control p≥0.05

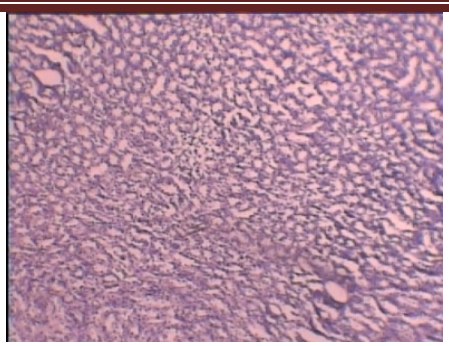
Histopathology of kidney



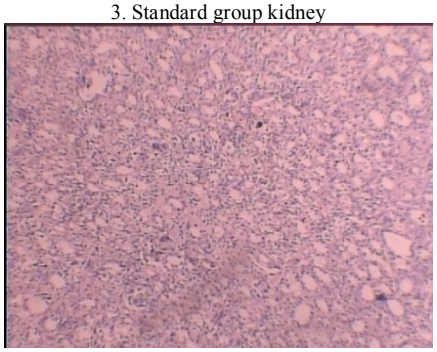
1. Normal kidney



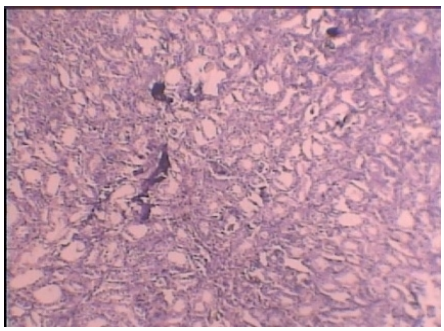
2. Disease control kidney



3. Standard group kidney



4. **MeSC- LD** kidney



5. **MeSC- HD** kidney

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