Hardik Soni. *et al.* / *International Journal of Phytopharmacology*, 5(1), 2014, 15-20.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

EVALUATION OF ACUTE TOXICITY AND ANTI-UROLITHIATIC ACTIVITY OF URAL CAPSULE

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ABSTRACT

Urolithiasis is the third most common disorder of urinary system with high recurrence rate. The present day medical management of urolithiasis is either quite expensive or not without side effects. Invasive treatment procedures for urolithiasis always include risk of serious complications along with high cost to the patient. Hence the search for anti-urolithiatic drugs from natural sources has been of greater importance. Ural capsule is the patent & proprietary Ayurvedic formulation used for the treatment of kidney stone. Majority of its ingredients are reported individually for the treatment of urolithiasis in Ayurvedic texts. However, no such evidence was found available which proves safety and efficacy of such combinations. Hence, present study was conducted to evaluate toxicity and anti-urolithiatic efficacy of Ural Capsule. Healthy male Wistar rats were used in the present study and were divided randomly into 3 groups. Group I was considered as normal control and was fed 1% Carboxy Methyl Cellulose (CMC) suspension. Group II was considered as lithiatic control and served 0.75% ethylene glycol in drinking water *ad libitum* for 28 days. Group III was considered as Ural capsule treated (UCT) and received 0.75% ethylene glycol in drinking water *ad libitum*. After 14th day and 28th day blood and urine sample were collected. Bio-chemical parameters like calcium, phosphorus, creatinine and uric acid were estimated in serum and urine. Histopathology of kidney was also carried out. Ural capsule treatment showed highly significant effect on urine and serum biochemical parameters. No mortality was observed during acute oral toxicity study at 2000 mg/kg and 5000 mg/kg. From the available data it can be concluded that the treatment of Ural capsule showed significant anti-urolithiatic effect on ethylene glycol induced urolithiasis in rats. It can be a safe and effective therapy for the treatment of kidney stone.

Key words: Ural capsule, Ethylene glycol, Urolithiasis, Acute toxicity study.

INTRODUCTION

Urolithiasis is the third most common disorder of the urinary tract, the others being frequently occurring urinary tract infections and benign prostatic hyperplasia (Hiatt and Friedman, 1982). The worldwide incidence of urolithiasis is quite high (Anderson *et al*., 1967) and in spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi. Most patients still have to undergo surgery to get rid of this painful disease. Hyperoxaluria is the main initiating factor for urolithiasis (Robertson and Peacock, 1980).

Kidney stones ailments have been affecting human beings since antiquity. Ancient *vedic* literature describes stones as *Ashmari*. The occurrence of these stones has been increasing in rural and urban societies (Misra A and Kumar Ashwani, 2000). A large population of India suffers from urinary tract and kidney stones, formed due to the deposition of calcium, phosphate and oxalates. These chemicals start accumulating over a nucleus, which ultimately takes a shape of stone. These stones may persist or increase in size in certain period of time, lead to secondary complications causing serious consequences to patient's life. (Misra A and Kumar Ashwani, 2000). One of the negative sides of urolithiasis

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is its high recurrence. Thus, a protective system is required including extracorporeal shock wave lithotripsy and medicament treatment. Unfortunately, such treatments remain expensive, in most cases invasive and with side effects. Therefore, it is worthwhile to look for an alternative to these conventional methods by using medicinal plants or indigenous system of medicine. (Fouad A, 2003).

Ayurveda, an indigenous system of Indian medicine, offers vast scope for the successful treatment of urolithiasis. Hence, the present study was undertaken to investigate overall safety along with anti-urolithiatic activity of patent & proprietary Ayurvedic formulation – Ural capsule. Ural capsule consists extract of *Tribulus terrestris* Linn (Gokshur) fruit (Anand R *et al.,* 1994; Sangeeta D *et al.,* 1993), *Berginia ligulata* Wall (Pashanbhed) root (Joshi VS *et al.*, 2009; Bashir S *et al.,* 2009), *Boerhaavia diffusa* Linn (Punarnava) root (Jalard *et al.*, 2011; Pareta S *et al.,* 2010), *Crataeva nurvala* Buch-Ham (Varun) bark (Sumankumar M *et al.,* 2011; Atanu Bhattacharjee *et al.,* 2012), *Dolichos biflorus* Linn (Kulathi) seed (Garimella TS *et al.,* 2001) and *Achyranthes aspera* Linn (Apamarg) whole plant (Anshu Aggarwal *et al.,* 2012); powders of Hajrool yahood Bhasma (Rasatantrasar, 1980a), Kasis Bhasma (Rasatantrasar, 1980b), Chandraprabha vati (Rasatantrasar, 1980c) and Shuddha shilajit (Shastri SD, 2006). It is manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara. Major ingredients of Ural capsule are individually well reported in Ayurvedic texts and scientific research publications for variety of activities like diuretic, anti-urolithiatic, anti-inflammatory etc. However, no such evidence was available which proves the safety and efficacy of such combinations. Therefore, present study was focused on evaluating toxicity and anti-urolithiatic efficacy of Ural capsule.

MATERIALS AND METHODS

Test Drug and Experimental Dose

Ural capsules were emptied to receive powder which was then triturated with 1g CMC followed by addition of 100mL distilled water to make suspension. For acute toxicity study 2000mg/kg and 5000mg/kg single dose was administered orally. For anti-urolithiatic activity, dose of the test drug (Ural capsule) was fixed by extrapolating the human therapeutic dose to laboratory animals based on body surface area ratio as per the table of Paget and Barnes. Test drug was administered at 450 mg/kg/day (p.o).

Experimental Animals

Healthy Swiss albino mice (20-25g) of either sex were taken for acute toxicity study and Wistar albino male rats (200-250 g) were taken to assess anti-urolithiatic activity. Both were procured from S.K.Patel College of Pharmaceutical Education And Research, Ganpat University, Kherva, Mehsana, Gujarat, India. All the experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) under the specified protocol (IAEC/SKPCPER/2010-02/18 & 20) for acute toxicity study and anti-urolithiatic activity respectively. Animals were housed in polypropylene cages, maintained under standardized condition (12-hrs light/dark cycle, 24°C, 35 to 60% humidity) and provided free access to standard pellets diet and purified drinking water *ad libitum.* The animals were deprived of food for 24 hrs before experimentation but allowed free access to water throughout.

Acute Oral Toxicity Study

For acute toxicity study on mice, 'Fixed dose' method of the Organization for Economic Cooperation and Development (OECD, 2000) guideline 420 was followed. The test drug was administered by gavages (orally) at single doses of 2000 mg/kg and 5000 mg/kg. The animals had free access to water and food throughout the experiment, except for the fasting period before the oral administration of the single dose of the test drug. The general behavior of rats was continuously monitored for 2hours. After period of 24 hours, 72 hours, 7 days and 14 days they were observed for changed in body weight and lethality or death.

Evaluation of Anti-urolithiatic activity

Experimental procedure

Anti-urolithiatic activity was evaluated by reported method with minor modification (Verma NK *et al.,* 2009). The acclimatized animals were divided into three groups having six animals in each group. The animals of Group I was considered as "Normal control" and was fed 1% Carboxy Methyl Cellulose (CMC) suspension. The Group II was considered as "Lithiatic control" and served 0.75% ethylene glycol in drinking water *ad libitum* for 28 days. The Group III was considered as "Ural capsule treated" (UCT) and received 0.75% ethylene glycol in drinking water *ad libitum*, along with Ural capsule (450 mg/kg/day) suspension by oral route for 28 days. The 24 hours urine was collected from rats housed in metabolic cages on 14^{th} and 28^{th} days. The volume and pH of urine were noted on these days. Urine calcium, phosphorous, creatinine and uric acid were also estimated from collected urine on 14^{th} and 28^{th} day. Blood samples were collected on 14^{th} and 28^{th} days and biochemical parameters like serum calcium, phosphorous, creatinine and uric acid were evaluated. At the end of the study, animals were sacrificed. Kidneys were isolated from all groups of animals and stored for histopathological study in 10% formalin solution.

Collection and analysis of urine and blood

Before collection of urine and blood, animals were deprived of food and water for 24 hours. The urine and blood samples were collected on 14^{th} day and 28^{th} day. Urine volume and pH were measured immediately. Urine samples were centrifuged and supernatant were stored at 4^0C. Supernatant of urine was analyzed for urine calcium, urine phosphorous, urine creatinine and urine uric acid level. Serum was separated from collected blood samples and analyzed for serum calcium, serum phosphorous, serum creatinine and serum uric acid. All analysis were carried out by automated clinical chemistry analysis system (Merck, Germany).

Histopathology of kidney

Kidney from each animal was removed after sacrificing them under anesthesia. All were preserved in 10% formalin solution and were processed by paraffin technique. Section of approximately 5µm thickness was cut and stained by hematoxylin and eosin (H&E). Then sections were examined under microscope to evaluate cyto-architectural changes.

Statistical analysis

The results were expressed as mean values ± S.E.M. (standard error of mean) where each group contained six animals. Statistical comparison was carried out by analysis of variance (ANOVA). The statistical analysis was carried out with software, Graph pad Prism®, version 5.0. The results were considered statistically significant when $p < 0.05$.

RESULTS

In the acute oral toxicity study, the animals did not manifest any signs of toxicity or deaths at both dose level 2000 mg/kg and 5000 mg/kg. The body weights of all the mice were increased after the oral administration of Ural capsule where the marked % gains were observed on 7^{th} day and 14^{th} day (Table 1).

Lithiatic control animals showed statistically significant decrease ($p<0.01$) in urine volume as compared to normal control. No significant variation was observed in pH of lithiatic control group. Ural capsule treated group showed significant increase ($p<0.01$) in urine volume with reference to lithiatic control group but no significant variation was observed in pH of urine. (Table 2)

The level of urine calcium and phosphorus were significantly increased in lithiatic control group in 14^{th} day and 28^{th} day samples as compared to normal control group. Ural capsule treated group showed significant reversal effect on urine calcium and phosphorus level (Table 3). While other urine bio-chemical parameters like urine creatinine and uric acid were drastically reduced in 14^{th} day and 28^{th} day samples as compared to normal control group. Ural capsule treated group showed significant increase in both the parameters (Table 3).

Table 1. Effect of Ural capsule on the body weight of mice during acute toxicity study

Single Dose	Mean body weight (g)				
	0 day	7^{th} day	% Gain on 7^{th} day	14^{th} day	% Gain on 14^{th} day
2000 mg/kg	22.66 ± 0.88	27.00 ± 0.29	19.15 ± 3.49	33.33 ± 0.60	47.08 ± 7.12
5000 mg/kg	34.66 ± 1.01	37.67 ± 0.44	08.68 ± 3.33	40.00 ± 0.29	13.96 ± 5.21

Table 2. Effect of Ural capsule on Urine volume and pH

Groups	Urine volume (mL/100g/24hrs)	pH
Normal control	5.33 ± 0.03	7.49 ± 0.05
Lithiatic control	$2.89 \pm 0.08^{\#\#}$	7.42 ± 0.03
Ural capsule treated	$5.24 \pm 0.04^{**}$	7.45 ± 0.06

Table 3. Effect of Ural capsule on urine biochemical parameters

Groups	U. calcium (mg/dL)		U. phosphorus (mg/dL)		U. creatinine (mg/dL)		U. uric acid (mg/dL)	
	14^{th} day	28^{th} day	14^{th} day	28^{th} day	14^{th} day	28^{th} day	14^{th} day	28^{th} day
Normal control	4.59 ± 0.35	4.89 ± 0.63	5.10 ± 1.38	5.53 ± 1.38	57.68 ± 5.12	54.98 ± 5.35	3.35 ± 0.13	3.65 ± 0.19
Lithiatic control	$9.60 \pm 0.29^{\#\#\#}$	$11.60 \pm 0.24^{\#\#\#}$	$12.10 \pm 0.64^{\#\#\#}$	$12.73 \pm 3.19^{\#\#\#}$	$35.40 \pm 0.58^{\#\#}$	$31.23 \pm 0.28^{\#\#\#}$	$1.65 \pm 0.06^{\#\#\#}$	$1.76 \pm 0.03^{\#\#\#}$
Ural capsule treated	$9.00 \pm 0.45^{**}$	$9.60 \pm 0.34^{***}$	$10.20 \pm 1.31^{***}$	$10.16 \pm 0.49^{***}$	$48.40 \pm 2.21^{**}$	$49.68 \pm 3.62^{**}$	$3.13 \pm 0.12^{**}$	$4.00 \pm 0.20^{***}$

All the values are expressed as mean ± SEM (n=6) in each group. Where, $^{*}p<0.05$, $^{**}p< 0.01$, $^{***}p< 0.001$when compared to lithiatic control group. While, $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ when compared to normal control group.

Table 4. Effect of Ural capsule on serum biochemical parameters

Groups	S. calcium (mg/dL)		S. phosphorus (mg/dL)		S. creatinine (mg/dL)		S. uric acid (mg/dL)	
	14th day	28th day	14th day	28th day	14th day	28th day	14th day	28th day
Normal control	9.88 ± 0.08	9.93 ± 0.09	4.40 ± 0.09	4.38 ± 0.04	0.51 ± 0.04	0.42 ± 0.31	2.47 ± 0.06	2.47 ± 0.09
Lithiatic control	10.35 ± 0.04$^{###}$	10.73 ± 0.06$^{###}$	5.43 ± 0.06$^{###}$	5.78 ± 0.14$^{###}$	0.95 ± 0.04$^{###}$	1.37 ± 0.03$^{###}$	5.60 ± 0.36$^{###}$	6.65 ± 0.18$^{###}$
Ural capsule treated	8.08 ± 0.13***	7.37 ± 0.08***	3.72 ± 0.60***	3.71 ± 0.06***	0.82 ± 0.03***	0.60 ± 0.37***	3.75 ± 0.12***	3.03 ± 0.09***

All the values are expressed as mean ± SEM (n=6) in each group. Where, *p<0.05, **p< 0.01, ***p< 0.001when compared to lithiatic control group. While, $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 when compared to normal control group.

Fig 1. Histopathology of kidney

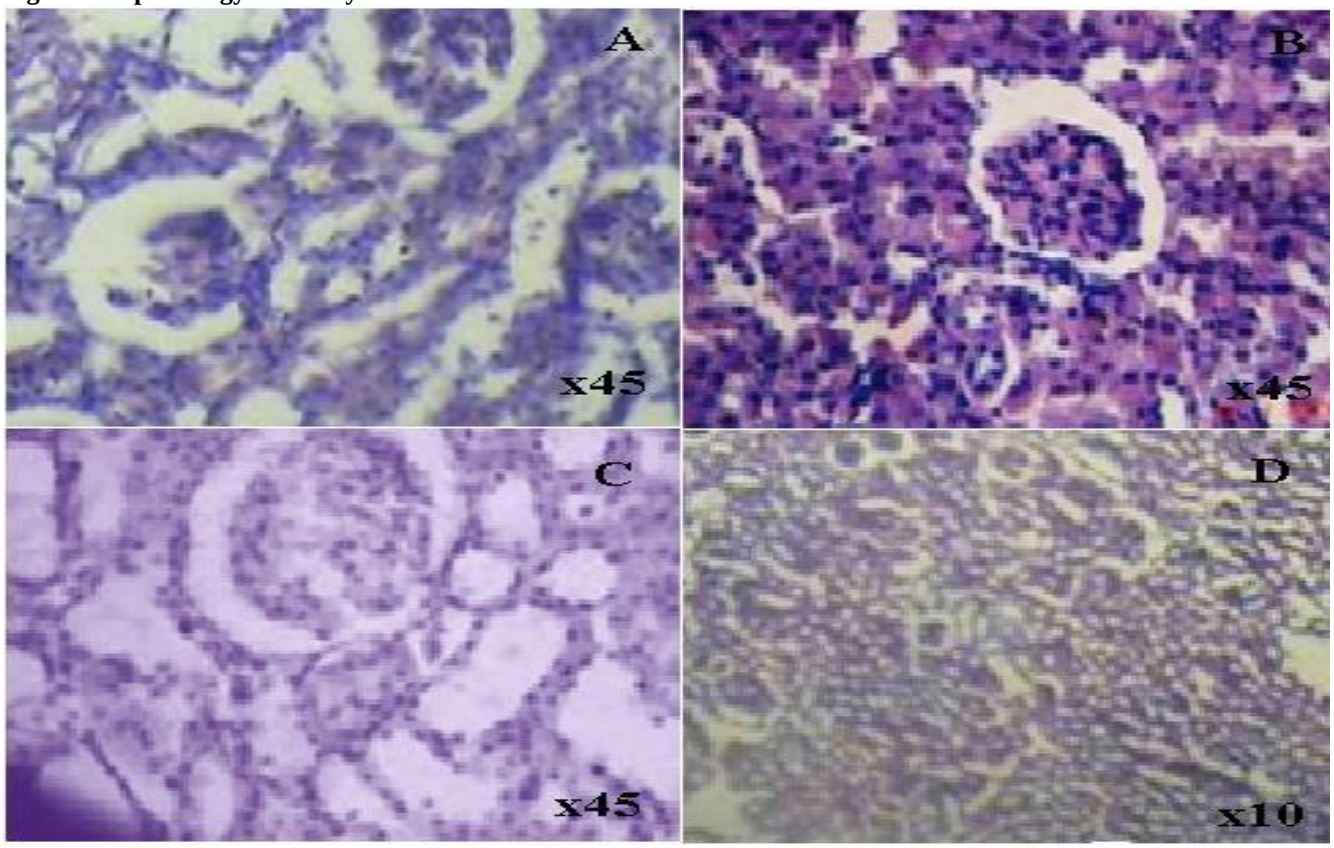

Figure 1A. Normal control; Figure 1B: Lithiatic control; Figure 1C & 1D: Ural capsule treated (450 mg/kg/day)

The level of serum calcium, s. phosphorus, s. creatinine and s. uric acid were significantly increased in lithiatic control group in 14th day and 28th day samples as compared to normal control group. Ural capsule treated group showed significant reversal effect on serum calcium and phosphorus level (Table 4). It also showed significant decrease in s. creatinine and s. uric acid in 14th day and 28th day samples as compared to lithiatic control (Table 4).

Section of kidney treated with ethylene glycol (lithiatic control) showed marked dilation of tubules, tubular damage and infiltration of inflammatory cells into the interstitial space (Figure 1B). Ural Capsule treated group showed improvement of the above symptoms and establish normal cyto-architecture of kidney cell. (Figure 1A, 1C & 1D).

DISCUSSION

The study has been conducted to evaluate efficacy of Ural capsule on ethylene glycol induced urolithiasis in rats. Urinary supersaturation with respect to stone-forming constituents is generally considered to be one of the causative factors in urolithiasis. In this context, the

changes in urinary oxalate levels are relatively much more important than those of calcium (Robertson and Peacock, 1980). In the present study, 0.75% ethylene glycol was induced which can develop condition like hyperoxaluria, which is known to be a more significant risk factor in pathogenesis of stone formation. Hyperoxaluria is usually the initiating factor of oxalate urolithiasis. (Runyan and Gershoff, 1965). It has been stated that high concentration of calcium and phosphorus in urine and serum initiate the precipitation process of calcium oxalate and calcium phosphate crystal (Verma NK *et al.*, 2009). In this study, Lithiatic control group showed similar type of results. Ural capsule treated group showed significant reduction in serum and urine level of calcium and phosphorus. The increase in urine volume may also help in minimizing the tendency for crystallization. Significant change in level of creatinine and uric acid were also reported during several investigations due to the effect of ethylene glycol (Atef M. Al-Attar, 2010). Ural capsule treated group showed significant reverse in serum and urine creatinine and uric acid level. Ural capsule treatment also restored the damage of kidney and maintained the normal cyto-architecture.

From the available data it can be concluded that the treatment of Ural capsule showed significant reversal effect on ethylene glycol induced urolithiasis in rats. It can be a safe and effective remedy for the treatment of kidney stone.

ACKNOWLEDGEMENT

Authors are sincerely thankful of the management of Vasu Healthcare Pvt. Ltd. for providing test sample and also thankful to S. K. Patel College of Pharmaceutical Education and Research, Ganpat University for providing necessary facilities and technical support for conducting this study.

CONFLICT OF INTEREST: Nil

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