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Efficacy of Herbmed Plus in urolithic rats: An experimental study

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ABSTRACT

Background: In Ayurveda, several herbs and formulations are available for the treatment of Urolithiasis. However, they are not systematically evaluated for their safety, efficacy, indication and limitations. Herbmed Plus is one such herbal formulation that has been known for the management of urinary tract disorders. An attempt has been made to evaluate its efficacy on Urolithiasis.
Objective: To evaluate the efficacy and safety of Herbmed Plus in urolithic rats.
Materials and methods: A total of 60 Wistar albino rats were used for this study. The male and female rats were divided into five groups: disease control, test (dose 90 mg/kg), standard I (Cystone), standard II (Alkaston insta) and normal control (six in each group). Urolithiasis was induced using ethylene glycol 0.75% in drinking water for 28 days. The rats with urinary oxalate crystals were dosed with oral test or standard treatments for 28 days.
Results: All the animals appeared normal and showed no clinical signs of toxicity. None of the groups reported mortality or adverse effect on body weight and food consumption. The treatment with test drug showed improvement in the SGPT level and urine output (5.4 vs 3.47 mL/24 h). A drastic reduction in number of crystals were observed in male 0.5 vs 22 and female rats 0 vs 22.7 in test and disease group. The kidney lactate dehydrogenase, alkaline phosphatase, urinary phosphorus and calcium oxalate level decreased in the test and standard drug groups as compared to disease groups. Microscopy of the urine samples showed reduction in the number of crystals after treatment compared to the urolithic group. Increase in citrate levels in urine in all the treatment groups indicated anti-urolithiatic activity. The test group showed a 69.70% recovery in males and 47.57% recovery in female rats compared to the disease control group.
Conclusion: Herbmed Plus showed a significant reduction in oxalate synthesizing enzymes suggesting anti-urolithiatic activity and anti-inflammatory and regenerative property in cellular injury caused by crystal deposits.

1. Introduction

Urolithiasis is one of the major disorders affecting all age groups with a significant burden on healthcare system worldwide. There has been a significant increase in the prevalence of urolithiasis over time [1]. Nearly half of patients with a history of urolithiasis will have a recurrence within 10 years [2]. There is a significant variation in the incidence, prevalence, stone composition and location of the stone and factors including geographical region, age, sex, climate, and food habits contribute to this variation. Additionally, socio-economic conditions may also impact the incidence, stone composition and location [3].

Surgical management is considered as a treatment of choice for patients with urolithiasis who do not respond to pharmacological treatment or have large stones which may not be suitable for pharmacological treatment. Recently, in the past few decades there has been significant development and advancement in surgical techniques to successfully manage stone disorders; however, pharmacological treatment may delay or avoid surgeries. In Ayurveda, the Indian system of medicine, several herbs, and formulations are described for the treatment of urolithiasis. However, the majority of them are not evaluated systematically by using acceptable guidelines for their safety, efficacy, indications, and limitations [4]. Herbmed is a herbal formulation derived from the bark of *Crataeva nurvala Buch-Ham* (commonly known as *Varun*) and stem of *Musa paradisiaca Linn* (known as *Kadali* or banana

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roots) [5]., As per Ayurveda, these herbs are known for beneficial effects in the management of urinary tract disorders and particularly for the treatment of urolithiasis [6].

This formulation was modified to Herbmed Plus by supplementing with *Achyranthes aspera Linn* (Aghada) whole plant and seeds of *Hordeum vulgare Linn* (Yav). According to Ayurveda these herbs are converted into *Varun bhavitkadalibhavit*, *Kshars* of *Kadali*, *Aghada* and *Yav* respectively and formulated in certain proportion at GMP certified facility in the capsule form [6,7]. The formulation is intended for oral administration by dissolving in water. In this study, the efficacy of Herbmed Plus was evaluated in urolithiasis induced albino rats (daily for 28 days) as per the protocol reported by Soundararajan et al. [8].

2. Material and methods

2.1. Animals

In this experimental study, Wistar albino rats (males, n = 30; females, n = 30) weighing between 170 g and 210 g, aged 10–11 weeks were randomly selected. The rats were acclimatized for four weeks before enrolling in the study. Three rats of the same sex were housed in each cage under a controlled temperature of 20–24 °C with relative humidity between 30 and 70% and 12 h of light and dark cycle without any stressful stimuli. Animals were provided with pellets of balanced animal food (Nav Maharashtra Oil Mills, Pune) and water were provided *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee of the National Toxicology Center, Pune (Ethics committee approval number: RP-151). Every effort was made to minimize animal suffering and reduce the number of animals used.

2.2. Methods

Urolithiasis was induced by treatment of ethylene glycol 0.75% in drinking water for 28 days. Male (n = 30) and female (n = 30) rats were divided in five groups (six rats per group); normal control, disease control, test, standard I and standard II. Normal control group was fed on regular rat food and drinking water *ad libitum*. All remaining groups received ehylene glycol 0.75% in drinking water for 28 days. At the end of 28 days, 24-h urine (i.e., 24-h of day 28) was collected individually from rats. The urine sample was analysed for the presence of oxalate crystals microscopically. The rats showing the presence of oxalate crystals were selected for the study. The groups studied were disease control, test (dose 90 mg/kg), standard I (Cystone [dose 750 mg/kg], a proprietary ayurvedic medicine manufactured by Himalaya company indicated for the treatment of urolithiasis) and standard II (Alkastoninsta [dose 210 mg/kg], a potassium magnesium citrate and commercial product of Intas India Pvt Ltd). Test, standard I and II were administered orally from 1st day of calculi induction and continued further for 28 days before sacrificing. One group of six males and six females were used as normal control.

2.3. Preparation of Herbmed plus

Preparation of *Varun swaras bhavit Varun churna: Varun Bharad* prepared from pulverization of dried stem bark of *Varun* was mixed with water and boiled (*quath*/*kadha*/water decoction). This *quath* was added to *Varun bharad* and kneaded to obtain a mixture of homogenized powder. The product obtained was dried in oven. This is called *V. swaras bhavit V. churna*.

Kadali swaras bhavit Varun: Stems of *M. Paradasiaca* were chopped into pieces and ground in a mixer to obtain aqueous extract, which was added to *V. swaras bhavit V. churna*. This was further ground to make a homogenous composition which was dried in oven to obtain a fine powder. This is called *K. swaras bhavit Varun*. Hence, *K. swaras* was used as *bhavna drvaya* for *V. churna* (There are no other terms known to us for *V. swaras bhavit V. churna* and *K. swaras bhavit Varun* that could be cited in traditional literature). While imparting '*bhavana*' the drug is triturated with *swaras* till it gets dried. We have dried it in oven and agree that it should not be done likewise.

M. Paradasiaca: Banana stems, roots and *kand* were used to prepare *Kadali Kshar*.

Acharynthes aspera- Whole plant of *Apamarg* consisting of flowers, leaves, seeds, roots and fruits (*panchang*) were used to obtain *Apamarg kshar*. *H. vulgare- Yav* grains are used to prepare *Yavkshar*.

Final formulation composition of Herbmed formulation was: Each 500 mg capsule contains *V. swaras bhavit V. churna* (250 mg), *Kadali Kshar* (75 mg), *Yav kshar* (100 mg) and *Apamarg Kshar* (75 mg).

2.4. Clinical signs

Clinical signs, toxic symptom, and mortality were recorded immediately at the half, 1, 2, 4 and 24 h and later twice a day thereafter up to 28 days to determine their general health, behaviour and moribund condition. Any abnormality observed during this period was recorded.

2.5. Bodyweight

Body weights of the animals were weighed individually on day 0, 7, 14, 21, 28 and on the day of sacrifice. Any circumstances of death would be recorded and a microscopic examination post-mortem would be performed. A gross necropsy would be performed on all animals that die during the course of the test and sacrificed at the termination of the test.

2.6. Haematology and blood chemistry

Blood samples were collected separately for haematology and clinical chemistry analysis. Haematology estimation was carried out using Mindray 2800 analyser (China) and Biochemical parameters were carried out using SERI BSA 3000(France) analyser with Kits from Coral, CREST Biosystems.

2.7. Urine analysis

Urine analysis was performed in the last week of the study before termination of the animals. Urine samples were collected using a battery of specially designed stainless-steel urine collection cages. Each rat was housed in this cage. Urine samples were collected over a period of 24 h. Urine analysis was performed using SIEMENS Multistix SG, Siemens Ltd, India. Urine microscopic examination was done for number of CaOx and crystals per high power field (HPF) were measured in various groups.

2.8. Organ weight

After sacrifice of the animals the adrenals, heart, kidneys, liver, spleen, brain, lungs and testis/ovaries were weighed and recorded.

2.9. Determination of tissue enzymes

The liver and kidneys were dissected and part of the tissue was homogenized for enzyme analysis by biochemistry analyzer and kits.

Table 1
Data on mean body weight of Wistar albino rats during the course of study.

Sex	Groups	Weeks				
		0	1	2	3	4
Males	Normal control	188.8 ± 8.460	207.1 ± 8.387	220.9 ± 7.228	234.1 ± 5.277	245.8 ± 5.346
	Disease control	183.2 ± 8.796	210.1 ± 16.20	216.9 ± 13.99	209.0 ± 15.36	211.8 ± 17.24
	Test	194.8 ± 8.542	210.0 ± 11.94	215.2 ± 15.28	215.2 ± 13.83	236.8 ± 14.32
	Standard I	202.8 ± 6.585	237.8 ± 9.261	249.6 ± 8.387	255.8 ± 8.472	255.0 ± 11.56
	Standard II	187.7 ± 10.09	211.2 ± 9.261	222.5 ± 10.67	229.8 ± 8.413	243.5 ± 5.683
Females	Normal control	189.5 ± 7.765	211.4 ± 9.713	223.8 ± 9.740	235.8 ± 9.637	248.9 ± 9.510
	Disease control	188.5 ± 8.264	205.9 ± 10.58	212.5 ± 9.772	208.0 ± 9.586	208.1 ± 10.11
	Test	192.4 ± 10.44	200.0 ± 11.22	204.8 ± 11.61	204.3 ± 9.873	207.7 ± 10.37
	Standard I	186.8 ± 14.99	193.2 ± 19.38	199.1 ± 16.71	201.2 ± 16.36	206.8 ± 15.84
	Standard II	181.3 ± 7.840	192.2 ± 8.472	205.5 ± 6.253	215.1 ± 5.783	221.2 ± 8.377

Test = Herbmed plus dose 90 mg/kg; Standard I = Cystone dose 750 mg/kg; Standard II = Alkastoninsta dose 210 mg/kg.

2.10. Determination of tissue proteins

Tissue protein was determined using SERI BSA - 3000 Chemistry analyser and Kit, Coral, CREST Biosystems.

2.11. Determination of liver and kidney lactate dehydrogenase (LDH), liver gamma glutamyl transpeptidase (GGT), and alkaline phosphatase (ALP)

The LDH was determined using the SERI BSA - 3000 Chemistry analyser and Kit, Coral, CREST Biosystems. The GGT was determined from liver homogenates using SERI BSA - 3000 Chemistry analyser and Kit, Autozyme GAMMA-GT Kit, Accurex Biochemical Pvt. Ltd., India. The ALP was determined from liver using SERI BSA - 3000 Chemistry analyser and Kit, Autozyme GAMMA-GT Kit, Accurex Biochemical Pvt. Ltd., India. The enzyme activity was determined as IU/gm of tissue protein.

2.12. Statistical analysis

All the results were expressed as mean ± SD. The statistical analysis was carried out by using Prism card software. The data were considered significant at $p < 0.05$. Overall, the data of treated group were compared with disease control group.

3. Results

All the animals appeared normal and showed no clinical signs of intoxication until the end of the study. There was no statistically significant change in the body weight as depicted in Table 1 and the amount of food consumed by all the animals. No mortality was observed in any of the groups. Table 2 shows the group mean haematological data in male and female rats across the group. There was a statistically significant increase ($p < 0.05$) in the haemoglobin levels and PCV in the test group and standard II group males but a decrease in standard II females when compared to disease control group ($p < 0.05$).

In males, the test drug and standard I significantly ($p < 0.001$) decreased the serum glutamic pyruvic transaminase (SGPT) values compared to disease control group. The serum glutamic oxaloacetic transaminase (SGOT) values were increased significantly ($p < 0.001$) in males of test and both standard drug groups. The SGPT, SGOT, and ALP values were decreased significantly ($p < 0.001$) in females of both the standard dose groups when compared to the disease control group indicating recovery (Table 3).

Table 4 shows group mean urine microscopic data for crystals. The reduction in the number of calcium oxalate crystals was maximum in the test drug (0.50) and both standard drug dose groups (standard I, 0.00 and standard II, 7.33) compared to the disease control group.

Table 5 presents the mean urine analysis data. The urine analysis showed a statistically significant decrease ($p < 0.001$) in

Table 2
Haematological data of Wistar albino rats during the course of study.

Sex	Groups	Differential count							
		Hb gm%	PCV %	Th Thousand/cmm	WBC Thousand/cmm	RBC Million/cmm	N	L	M
Males	Normal Control	10.4 ± 1.96	35.0 ± 5.76	535.5 ± 22.37	14.7 ± 3.49	6.00 ± 1.10	20.77 ± 7.517	75.45 ± 8.275	3.78 ± 1.33
	Disease Control	7.17 ± 0.23	25.0 ± 2.26	387.3 ± 23.0	6.18 ± 0.84	5.45 ± 1.43	25.00 ± 2.060	70.59 ± 2.083	4.42 ± 0.44
	Test	11.4$^{\$}$ ± 2.98↑	36.9$^{\#}$ ± 3.11↑	508.2$^{\#}$ ± 19.23↑	9.62 ± 2.79	6.54 ± 1.01	27.45 ± 3.266	65.83 ± 3.915	4.82 ± 0.32
	Standard I	7.28 ± 0.45	26.6 ± 1.48	317.0$^{\$}$ ± 13.70↓	7.00 ± 1.14	5.08 ± 0.41	25.08 ± 0.970	72.10 ± 0.853	2.85 ± 0.47
	Standard II	10.5* ± 1.35↑	33.5$^{\$}$ ± 4.80↑	422.5 ± 20.9	8.97 ± 3.75	7.07 ± 0.27	21.23 ± 2.077	75.80 ± 1.711	2.97 ± 0.54
Females	Normal Control	10.3 ± 1.30	35.5 ± 6.66	597.5 ± 25.03	13.0 ± 3.09	6.48 ± 1.18	18.30 ± 6.954	77.95 ± 7.525	3.75 ± 0.89
	Disease Control	13.4 ± 0.55	37.4 ± 2.90	5.32 ± 25.77	9.60 ± 1.81	7.24 ± 0.31	24.97 ± 1.111	70.72 ± 1.050	4.32 ± 0.33
	Test	7.35$^{\#}$ ± 3.29↓	28.8$^{\#}$ ± 5.99↓	333.5 ± 19.44	8.32 ± 3.47	4.60$^{\$}$ ± 1.97↓	23.25 ± 3.181	72.87 ± 3.972	3.88 ± 1.03
	Standard I	7.43$^{\#}$ ± 0.56↓	32.9$^{\#}$ ± 2.80↓	401.2 ± 18.97	7.52 ± 0.88	4.98$^{\$}$ ± 1.04↓	28.35 ± 4.433	67.57 ± 4.827	4.25 ± 0.60
	Standard II	11.8 ± 1.28	32.7$^{\#}$ ± 4.76↓	410.0 ± 23.95	8.45 ± 2.31	6.76 ± 0.40	23.68 ± 2.815	72.35 ± 2.696	3.42 ± 0.45

Hb = Haemoglobin, PCV - Packed Cell Volume, CT = Clotting Time, Th = Thrombocytes, RCB = Red Blood Cells, WBC = White Blood Cells, RT = Reticulocyte, N = Neutrophils, L = Lymphocyte, E = Eosinophil, M = Monocyte. Test = Herbmed plus dose 90 mg/kg; Standard I = Cystone; Standard II = Alkastoninsta.
Data presented as mean ± SD). Data compared to disease control group. $^{\#}$indicate statistical significance at $p < 0.001$; $^{\$}$indicate statistical significance at $p < 0.01$; *indicate statistical significance at $p < 0.05$. ↓indicates decrease in value and ↑indicates increase in value.

Table 3
Clinical blood chemistry data of Wistar albino rats during the course of study.

Sex	Groups	SGPT U/L	SGOT U/L	ALP U/L	Total Protein Gm %	Urea Mg %
Males	Normal control	45.8 ± 7.97	162.5 ± 17.58	190.3 ± 18.49	5.94 ± 1.16	25.34 ± 5.406
	Disease control	96.8 ± 9.87	202.3 ± 17.44	198.8 ± 11.70	6.29 ± 1.14	39.87 ± 5.631
	Test	69.2* ± 7.47↓	264.2* ± 16.13↑	392.8* ± 12.42↑	5.37 ± 0.73	38.79 ± 3.193
	Standard I	51.5* ± 1.87↓	276.3* ± 6.439↑	170.0 ± 11.17	6.70 ± 0.78	28.55 ± 6.035
	Standard II	89.8 ± 12.1	300.8* ± 11.48↑	297.0* ± 12.79↑	5.58 ± 1.30	38.51 ± 4.271
Females	Normal control	44.84 ± 7.79	216.5 ± 62.57	134.7 ± 15.04	6.52 ± 1.14	24.73 ± 9.090
	Disease control	100.5 ± 8.76	301.7 ± 14.17	248.8 ± 13.56	5.68 ± 1.89	45.34 ± 5.597
	Test	99.33 ± 10.39	308.2 ± 19.69	317.0* ± 19.44	4.88 ± 0.84	42.73 ± 2.381
	Standard I	75.67* ± 9.67↓	217.5* ± 7.740↓	182.8* ± 14.37↓	5.37 ± 1.23	43.92 ± 12.41
	Standard II	65.00* ± 15.27↓	209.8* ± 17.33↓	219.7* ± 14.96↓	4.86 ± 1.78	51.55 ± 6.558

Data presented as mean ± SD). Test = Herbmed plus dose 90 mg/kg; Standard I = Cystone; Standard II = Alkastoninsta.
Data compared to disease control group. *indicate statistical significance at $p < 0.001$. ↓indicates decrease in value and ↑indicates increase in value.

Table 4
Data on Urine volume and no. of crystals in urine of Wistar albino rats.

Sex	Groups	Urine Volume (mL)	No. of Crystals
Males	Normal control	4.850 ± 0.383	0.00 ± 0.00
	Disease control	3.467 ± 0.250	22.0 ± 4.86
	Test	5.433 ± 0.393	0.50 ± 0.84↓
	Standard I	5.300 ± 0.303	0.00 ± 0.00↓
	Standard II	4.933 ± 0.242	7.33 ± 9.35↓
Females	Normal control	4.867 ± 0.314	0.00 ± 0.00
	Disease control	3.233 ± 0.367	22.7 ± 2.34
	Test	5.333 ± 0.437	0.00 ± 0.00↓
	Standard I	5.200 ± 0.437	3.83 ± 4.31↓
	Standard II	5.033 ± 0.197	0.00 ± 0.00↓

Data presented as mean ± SD). Test = Herbmed plus dose 90 mg/kg; Standard I = Cystone; Standard II = Alkastoninsta.
Data compared to disease control group. ↓indicates decrease in value.

the urinary phosphorous in the test and standard dose male groups when compared to the disease control group. The citrate levels were significantly increased ($p < 0.001$) in the test and standard groups when compared to the disease control group. The sodium levels were significantly increased ($p < 0.001$) in the male test and standard dose groups when compared to the disease control group.

The LDH from liver and kidney and GGT, ALP from the liver was analysed in all the animals. In the urolithic conditions, the increase in the liver and kidney LDH level is reported. Treatment with test drug and standard I showed a significant decrease in the liver LDH in males. The kidney LDH was found to be decreased in the test and standard drug groups.

The liver GGT levels are reported to be increased in the diseased conditions. The liver GGT values were decreased significantly in standard I group males ($p < 0.01$) and females ($p < 0.05$) and standard II females ($p < 0.05$) when compared with the disease control group (Table 6). There was no statistically significant change in the absolute weights a shown in Table 7; of all the organs in all the groups in males when compared with the disease group animals analyzed by one-way analysis of variance method of prism card software.

Table 8 presents the group mean histopathology data of kidney. The histopathology of the kidney showed 76.19% inflammatory damage in males and 86.67% inflammatory damage in females of disease control group due to crystal deposition in the cells. The treatment with test drug "Herbmed Plus" showed a recovery of 69.70% in males and a 47.57% in females, standard I showed a recovery of inflammatory damage of 41.67% in males and 10.15% in females and standard II animals showed a recovery of 45.95% in males and 8.49% in females when compared to the disease control group.

4. Discussion

Urolithiasis or renal stones are formed from urinary crystals that are combined together which may lead to irritation and secondary infection causing significant pain and healthcare burden [9]. Studies including epidemiological data have demonstrated that

Table 5
Analysis on Urine parameters of Wistar albino rats.

Sex	Groups	Total Protein mg/dL	Uric Acid mg/dL	Ca mg/dL	P mg/dL	Mg mg/dL	Na mEq/L	K mEq/L	Citrate mg/24hr
Males	Normal control	0.75 ± 0.15	1.48 ± 0.70	1.98 ± 0.58	5.87 ± 2.01	1.39 ± 0.78	104.5 ± 10.67	25.30 ± 6.336	1.95 ± 0.136
	Disease control	0.80 ± 0.39	1.92 ± 0.27	2.65 ± 0.76	10.2 ± 1.54	0.77 ± 0.10	80.57 ± 8.482	7.27 ± 1.642	1.38 ± 0.159
	Test	0.33 ± 0.14	1.42 ± 0.30	1.74 ± 0.35	3.00# ± 2.80↓	1.25 ± 0.56	118.9# ± 4.815↑	10.88 ± 5.243	3.70# ± 0.286↑
	Standard I	0.75 ± 0.24	1.87 ± 0.44	2.00 ± 0.48	4.12# ± 0.91↓	1.27 ± 0.31	144.1# ± 20.43↑	10.00 ± 0.729	2.28# ± 0.131↑
	Standard II	1.45 ± 1.51	1.88 ± 0.23	3.31 ± 0.85	3.73# ± 1.06↓	1.48 ± 0.97	168.2# ± 12.23↑	10.47 ± 3.367	2.33# ± 0.142↑
Females	Normal control	0.69 ± 0.52	0.97 ± 0.21	2.53 ± 1.19	5.03 ± 1.23	1.37 ± 0.71	169.6 ± 11.69	23.32 ± 5.816	1.733 ± 0.096
	Disease control	0.74 ± 0.81	2.08 ± 2.57	3.10 ± 0.94)	8.12 ± 3.81	1.33 ± 0.74	136.1 ± 14.74	9.917 ± 3.590	1.288 ± 0.135
	Test	0.43 ± 0.41	2.39 ± 1.38	2.45 ± 1.34	4.08 ± 2.76	1.37 ± 0.93	1.24 ± 17.53	5.833 ± 2.487	2.655# ± 0.320↑
	Standard I	1.59 ± 1.28	2.43 ± 1.11	2.11 ± 0.31	2.64* ± 0.67↓	0.93 ± 0.82	134.4 ± 19.12	9.020 ± 2.477	1.857# ± 0.110↑
	Standard II	0.58 ± 0.19	2.23 ± 0.83	3.29 ± 1.37	4.26 ± 2.09	1.10 ± 0.45	161.5 ± 2.689	7.583 ± 3.294	2.775# ± 0.108↑

Ca = Calcium, P = Phosphorous, Mg = Magnesium, Na = Sodium, K = Potassium. Data presented as mean ± SD). Test = Herbmed plus dose 90 mg/kg; Standard I = Cystone; Standard II = Alkastoninsta.
Data compared to disease control group. #indicate statistical significance at $p < 0.001$. *indicate statistical significance at $p < 0.05$. ↓indicates decrease in value and ↑indicates increase in value.

Table 6
Tissue enzyme analysis of Wistar Albino rats upon treatment.

Sex	Groups	Liver enzymes IU/gm of tissue protein		Kidney enzyme IU/gm of tissue protein	
		LDH	GGT	ALP	LDH
Males	Normal control	0.31 ± 0.053	0.31 ± 0.056	2.52 ± 0.224	0.63 ± 0.120
	Disease control	0.72 ± 0.145	0.79 ± 0.128	2.60 ± 0.226	1.84 ± 0.343
	Test	0.36$^{\#}$ ± 0.033↓	0.42 ± 0.067	1.38 ± 0.105	0.48$^{\#}$ ± 0.128↓
	Standard I	0.60 ± 0.066	0.42$^{\$}$ ± 0.059↓	2.21 ± 0.177	1.22$^{\$}$ ± 0.061↓
	Standard II	0.56 ± 0.142	0.62 ± 0.17	2.24 ± 0.36	1.13$^{\#}$ ± 0.181↓
Females	Normal control	0.340 ± 0.055	0.33 ± 0.067	1.50 ± 0.117	0.79 ± 0.144
	Disease control	0.630 ± 0.162	0.49 ± 0.03	2.43 ± 0.472	1.71 ± 0.128
	Test	0.550 ± 0.157	0.42 ± 0.065	1.39 ± 0.116	0.91$^{\#}$ ± 0.14↓
	Standard I	0.429* ± 0.135↓	0.24$^{\#}$ ± 0.042↓	1.21 ± 0.178	1.33$^{\#}$ ± 0.187↓
	Standard II	0.440 ± 0.065	0.32$^{\#}$ ± 0.07↓	1.24 ± 0.131	1.49* ± 0.068↓

Data presented as mean ± SD). Test = Herbmed plus dose 90 mg/kg; Standard I = Cystone; Standard II = Alkastoninsta.
Data compared to disease control group. $^{\#}$indicate statistical significance at $p < 0.001$; $^{\$}$indicate statistical significance at $p < 0.01$; *indicate statistical significance at $p < 0.05$.
↓indicates decrease in value.

majority of stones are composed of calcium oxalate as a predominant mineral [10]. Among many *in vivo* models developed to evaluate anti-urolithiatic effect, ethylene glycol induced calculi are widely used. In the present study rats are used to evaluate calcium oxalate deposition in kidney owing to their close resemblance to the human urinary system. Selection of male rats in the present study was based on a previous study showing a higher rate of crystal depositions in male as compared to female rats [11].

Ethylene glycol is a metabolic precursor of oxalate and is oxidized to glycolic acid which is, in turn, oxidized to oxalic acid. Administration of ethylene glycol may result in hyperoxaluria, calcium oxalate crystalluria, and deposition of calcium oxalate crystals in the kidney [12]. Therefore, we investigated the effect of the test sample on ethylene glycol-induced urolithiasis model in rats which is a well-established model and used by several investigators [13].

Usually, alteration in the body weight is considered as an important parameter for the assessment of the response of an individual to the drugs [14] and may also indicate its side effects [15]. In the present study, there was no statistically significant change in the body weight and amount of food consumed by all the animals. No mortality was observed in any of the groups. There was a statistically significant increase in the haemoglobin levels and RBC counts in the test group and standard II group males when compared to the disease control group. Increase in SGPT and SGOT values in urolithic rats is indicative of liver injury [16]. This study also reports an increase in SGPT and SGOT values in the test group after ethylene glycol administration.

However, the treatment with Herbmed Plus showed improvement in the SGPT levels, which indicates a reduction in the hepatotoxicity induced by 0.75% ethylene glycol. The SGPT and ALP values were comparatively lower in female rats. Whenever there is liver inflammation or injury there is rise in liver enzymes which may increase metabolites (e.g oxalates); however, the exact reason is not known. Thus increase in oxalate excretion causes its deposition and progression of urolithiasis. Hence decrease in liver enzymes indicates anti-urolithiatic activity of the formulation. The formulation does not have any adverse effect on liver, thus it can be proposed that the dose selected is optimum to manage urolithiasis effectively [17]. Microscopy of the urine samples showed a reduction in the number of crystals after treatment compared to the urolithic group. Citrate is an important inhibitor for calcium and oxalate nucleation. Increase in the citrate levels in urine in all the treatment groups indicated the anti urolithiatic activity of the drug [18]. Increase in their values suggest urolithiatic activities whereas decrease suggest antiurolithiatic function. Herbmed Plus showed a statistically significant reduction in the liver and kidneys LDH levels. Calcium oxalate crystal deposition produces cellular injury and inflammation.

Histopathological findings showed a recovery of 69.70% in males and 47.57% in females rat kidneys when compared to the disease control group. This demonstrates a possible renoprotective activity and could be an interesting area to explore in future. Reduction in inflammation will be helpful in further reduction in growth of calculus thus preventing recurrence. Absence of mortality, adverse

Table 7
Data on weight of various organs of wistar albino rats upon treatment (G) (absolute value).

Sex	Groups	Adrenals	Heart	Kidneys	Liver	Spleen	Lungs	Brain	Testes
Males	Normal control	0.061 ± 0.01	0.826 ± 0.09	1.757 ± 0.23	7.649 ± 0.79	0.836 ± 0.04	1.35 ± 0.15	1.310 ± 0.37	3.042 ± 0.52
	Disease control	0.057 ± 0.009	0.789 ± 0.08	1.799 ± 0.28	7.562 ± 0.64	0.800 ± 0.07	1.359 ± 0.16	1.407 ± 0.57	3.045 ± 0.53
	Test	0.062 ± 0.10	0.778 ± 0.11	1.575 ± 0.27	8.847 ± 0.92	0.766 ± 0.06	1.546 ± 0.17	1.325 ± 0.41	2.519 ± 0.30
	Standard I	0.054 ± 0.02	0.860 ± 0.14	0.153 ± 0.32	6.876 ± 1.27	0.795 ± 0.11	1.350 ± 0.19	1.590 ± 0.25	2.637 ± 0.34
	Standard II	0.054 ± 0.004	0.867 ± 0.08	1.540 ± 0.15	8.286 ± 1.05	0.793 ± 0.14	1.526 ± 0.16	1.654 ± 0.13	2.500 ± 0.46
Females	Normal control	0.065 ± 0.01	0.742 ± 0.08	1.325 ± 0.16	5.874 ± 0.21	0.891 ± 0.06	1.710 ± 0.26	1.563 ± 0.22	0.160 ± 0.02
	Disease control	0.063 ± 0.01	0.740 ± 0.08	1.327 ± 0.17	5.787 ± 0.37	0.882 ± 0.06	1.710 ± 0.26	1.563 ± 0.22	0.158 ± 0.02
	Test	0.052 ± 0.005	0.695 ± 0.06	1.336 ± 0.08	6.088 ± 0.70	0.792 ± 0.11	1.664 ± 0.12	1.559 ± 0.15	0.113 ± 0.01
	Standard I	0.071 ± 0.01	0.761 ± 0.08	1.392 ± 0.17	7.735 ± 0.84	0.869 ± 0.42	1.714 ± 0.29	1.537 ± 0.17	0.137 ± 0.02
	Standard II	0.066 ± 0.007	0.724 ± 0.06	1.412 ± 0.13	7.007 ± 0.80	0.854 ± 0.11	1.598 ± 0.28	1.605 ± 0.12	0.137 ± 0.04

Data presented as mean ± SD). Test = Herbmed plus dose 90 mg/kg; Standard I = Cystone; Standard II = Alkastoninsta.
Data compared to disease control group.

Table 8
Histopathology analysis of Kidney tissue of wistar albino rats.

Sex	Groups	Grade of kidney damage	% of kidney damage compared to control group	% recovery compared to disease control group
Males	Normal control	5.0	0.00	–
	Disease control	21.0	76.19	–
	Test	6.5	23.08	69.70
	Standard I	9.0	44.44	41.67
	Standard II	8.5	41.18	45.95
Females	Normal control	3.0	0.00	–
	Disease control	22.5	86.67	–
	Test	5.0	45.45	47.57
	Standard I	12.5	77.78	10.15
	Standard II	14.5	79.31	8.49

Test = Herbmed plus dose 90 mg/kg; Standard I = Cystone; Standard II = Alkastoninsta.

effect on body weight and food consumption in all the study groups were reported.

5. Conclusion

Herbmed Plus showed a significant reduction in oxalate synthesizing enzymes, anti-urolithiatic activity and anti-inflammatory property in cellular injury caused by crystal deposits.

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Conflicts of interest

None.

References

[1] Pearle MS, Lingeman JE, Leveillee R, Kuo R, Preminger GM, Nadler RB, et al. Prospective, randomized trial comparing shock wave lithotripsy and ureteroscopy for lower pole caliceal calculi 1 cm or less. J Urol 2005;173:2005–9.
[2] Shafi H, Moazzami B, Pourghasem M, Kasaeian A. An overview of treatment options for urinary stones. Caspian J Intern Med 2016 Winter;7:1–6.
[3] Trinchieri A. Epidemiology of urolithiasis: an update. Clin Cases Miner Bone Metab 2008;5:101–6.
[4] Singh PP, Hussain F, Ghosh R. Effect of simultaneous sodium oxalate and methionine feeding with and without varuna (Crataevanurvala Hook and Frost) therapy on urolithiogenesis in Guinea pigs. Indian J Clin Biochem 1992;7:23–6.
[5] Patankar S, Dobhada S, Bhansali M, Khaladkar S, Modi J. A prospective, randomized, controlled study to evaluate the efficacy and tolerability of Ayurvedic formulation “varuna and banana stem” in the management of urinary stones. J Altern Complement Med 2008;14:1287–90.
[6] Patankar S, Mujumdar A. Acute and sub acute safety studies of Herbmed Plus - a herbal formulation in laboratory animals. Int JPharm Pharmaceutical Sci 2014;6:288–91.
[7] Ananymous. Shushrut Samhita vol 2, commentary by anant Ram Sharma Choukhamba Surbharati Prakashan. Varanasi Chikitsasthan 2004;7(22):236.
[8] Soundararajan P, Mahesh R, Ramesh T, Begum VH. Effect of Aervalanata on calcium oxalate urolithiasis in rats. Indian J Exp Biol 2006;44:981–6.
[9] Hosseinzadeh H, Khooei AR, Khashayarmanesh Z, Motamed-Shariaty V. Antiurolithiatic activity of Pinus eldarica medw. fruits aqueous extract in rats. Urol J 2010;7:232–7.
[10] Butterweck V, Khan SR. Herbal medicines in the management of urolithiasis: alternative or complementary? Planta Med 2009;75:1095–103.
[11] Shukla AB, Mandavia DR, Barvaliya MJ, Baxi SN, Tripathi CB. Anti urolithiatic effect of Cow urine ark on ethylene glycol induced renal calculi. Int Braz J Urol 2013;39:565–71.
[12] Muthukumar A, Selvam R. Renal injury mediated calcium oxalate nephrolithiasis: role of lipid peroxidation. Ren Fail 1997;19:401–8.
[13] Saeidi J, Bozorgi H, Zendehdel A, Mehrzad J. Therapeutic effects of aqueous extracts of *Petroselinum sativum* on ethylene glycol-induced kidney calculi in rats. Urol J 2012;9:361–6.
[14] Winder CV, Lembke LA, Stephens MD. Comparative bioassay of drugs in Adjuvant-induced arthritis in rats: Flufenamic acid, mefenamic acid and phenylbutazone. Arthritis Rheum 1969;12(Suppl 5):472–82.
[15] Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90 day oral gavage toxicity studies of D-methyl penidate and DL methyl penidate in Sprague Dawley Rats. Toxicology 2002;179(Suppl 3):183–96.
[16] El Menyiy N, Al Waili N, Bakour M, Al-Waili H, Lyoussi B. Protective Effect of Propolis in Proteinuria, Crystaluria, Nephrotoxicity and Hepatotoxicity Induced by Ethylene Glycol Ingestion. Arch Med Res 2016;47:526–34.
[17] Sharma I, Khan W, Parveen R, Alam MJ, Ahmad I, Ansari MH, et al. Anti-urolithiasis activity of bioactivity guided Fraction of bergenia ligulate against ethylene glycol induced renal calculi in rat. BioMed Res Int 2017;2017:1969525.
[18] Barnela SR, Soni SS, Saboo SS, Bhansali AS. Medical management of renal stone. Indian J Endocrinol Metab 2012;16:236–9.