International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR)
ISSN: 2582-7618 (Online), Volume-3 Issue-6, October 2023

Antiurolithiatic Activity of Desmodium Gangeticum L. and Heteropogon Contortus L

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Abstract: ***Over the past decade, there has been an increasing interest in investigating plant-based diuretic drugs. As the available medicinal efficacy is low and patients have to undergo painful surgeries in urolithiasis cases, they seek alternative medicine. This growing popularity necessitates a thorough investigation of plant extracts that can be used as antiurolithiatic agents. The main objective is to determine and confirm that the extracts of the selected weeds can be used as diuretic agents to treat urolithiasis conditions. The present study focuses on the extraction of weeds Desmodium gangeticum L. and Heteropogon contortus L. using a solvent and evaluates their in vivo antiurolithiatic potency. The plants Desmodium gangeticum L. and Heteropogon contortus L. were collected from around the Telangana region. Dr. K. Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh, India, authenticated the collected plants. Roots of Desmodium gangeticum L., and Heteropogon contortus L., were thoroughly washed under tap water, dried under shade, and powdered by using a mechanical grinder. The preparation of extracts was performed using Soxhlet extraction with methanol. The extracts were examined for their colour and consistency, and their percentage yield was calculated based on the quantity used for extraction. The preliminary phytochemical investigations were carried out using Desmodium gangeticum L. and Heteropogon contortus L. root extracts to determine the presence of carbohydrates, terpenes, tannins, flavonoids, steroids, phenols, and alkaloids. An acute oral toxicity study was conducted using male Wistar rats by the Organisation for Economic Co-operation and Development (OECD) Guideline 425. No toxicity was found up to a dose of 2000 mg/kg body weight. The higher dose of both plant root extracts showed good antiurolithiatic activity. Their potent in vitro and in vivo antiurolithiatic activity was dose-dependent and comparable to that of the standard drugs Cystone and Neeri. Based on the pattern of excretion of water and electrolytes, it appears that active principles are present in these extracts having a frusemide-like activity. In another set of experiments, the state of hyperoxaluria induced by oral administration of 0.75% ethylene glycol in rats was significantly normalised by the oral administration of methanolic root extracts of Desmodium gangeticum L. and Heteropogon contortus L. The preliminary phytochemical investigation of methanolic root extracts of Desmodium gangeticum L., and Heteropogon contortus L., showed the presence of alkaloids, triterpenes, saponins, steroids, and flavonoids. Desmodium gangeticum L., and steroids, phenols, flavonoids, and triterpenes in methanolic root extracts of Heteropogon contortus L. Phenolics, flavonoids, alkaloids, and tannins act individually or in synergy via multiple mechanisms to produce the observed effect. However, the specific compound is not yet determined. The findings from the present study support the traditional use of Desmodium gangeticum L. and Heteropogon contortus L. (roots) for their antiurolithiatic activity.***

Keywords: Desmodium Gangeticum L., Heteropogon contortus L., Antiurolithiatic, Extracts, etc.

Manuscript received on 12 July 2023 | Revised Manuscript received on 08 October 2023 | Manuscript Accepted on 15 October 2023 | Manuscript published on 30 October 2023.

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I. INTRODUCTION

Nephrolithiasis: Minerals in the urine form crystals (stones), which can develop large enough to obstruct urine flow, making it one of the most painful disorders. Most kidney stones pass on their own, but some are too large and require medical attention.

Lithiasis (stone formation) is a significant cause of both acute and chronic renal failure, encompassing nephrolithiasis (stone formation within the kidney) and urolithiasis (stone formation in the ureter, bladder, or both). Among the various kinds of stones identified, calcium stones occur mainly in Men, while phosphate stone formation is more prevalent in women. Despite extensive research aimed at establishing the mechanisms of calculus formation, dietary management, and the use of various medicinal plants and other agents in the treatment of urolithiasis, no drug is currently available that can produce a complete cure. The primary drawbacks in the development of standard medications may be due to the different chemical forms and various biochemical disorders that lead to the formation of renal stones. Calcium oxalate stones are a significant clinical problem, affecting approximately 12% of the population, with a high recurrence rate of 70-80% in males and 47-60% in females [1].

The complications or drawbacks of allopathic treatment:

a. Recurrence of stones even after precise and longer duration of treatment.
b. Most types of stones require both surgical and medical therapy for cure.
c. The success rate of treatment is much less in many cases than in the case of nephrectomy for removing struvite stones.
d. Longer periods of hospitalization in the case of nephrectomy may be uncomfortable for the patient.
e. High risk of probable damage to the ureters as seen in the case of Ureteroscopic stone removal.
f. Most patients complain that they have blood in their urine for a few days after treatment. Bruising and minor discomfort in the back or abdomen,

caused by the shock waves, is also common during ESWL treatment.

g. Generally, patients stay in the hospital for several days and may have a small tube called a nephrostomy tube left in the kidney during the healing process. This may cause discomfort and pain to the patient in case of percutaneous nephrolithotomy.

h. In ESWL, patients are exposed to various harmful radiations like X-rays and UV rays, which are not permissible in pregnant women, pediatric and geriatric patients.

i. In a few cases of medical therapy, the patient may achieve temporary relief but not a permanent cure and exhibit the chances of recurrence (as in the case of thiazide diuretics).

j. Due to many disadvantages, most patients with kidney stones prefer the indigenous system of treatment for kidney stones [2].

Despite the adverse effects of weeds, some plants that are commonly considered weeds may provide some benefits, such as soil stabilization and organic matter addition, wildlife habitat and food, bee nectar, aesthetic qualities, serving as a genetic reservoir for improved crops, providing products for human consumption and medicinal use, and creating employment opportunities [3]. Such weeds, which were traditionally used for dieresis and urolithiasis, are Desmodium gangeticum L. and Heteropogon contortus L. Desmodium, also known as Shalparni, contains gangetinin, gangetin, desmodin, and is used as an anthelmintic, anticatarrhal, carminative, diuretic, expectorant, febrifuge, nervine tonic, anti-diarrheal, and stomachic. Moreover, the use of this herb is quite effective in resolving complications such as enteric fever, respiratory complications, and piles. The plant contains calcium, phosphorus, magnesium, and vitamins A and C. Dasamula is known to pacify pain, arthritis, fever, cough, bronchitis, general weakness, neuropathy, and boost immune power. The whole plant decoction is used to treat digestive disorders, oedema, diarrhoea, intermittent fevers, malaria, and urinary tract infections. In folklore medicine, a decoction from D. gangeticum L. leaves is used to treat gallbladder and kidney stones [4, 5]. *Heteropogon contortus L., Grass contains myo-inositol, galactinol, and raffinose. It is reported to contain polysaccharides, steroids, and triterpenes (Blake et al, 170; Beveridge et al, 1972) [6, 7].*and is stimulant and diuretic. The plant is used to treat toothache, fever, atrophy, emaciation, haematuria, dysentery, muscular pain, scorpion sting, arthritis, rheumatism, and asthma. As there is no evidence to date to prove their activity, an attempt has been made to evaluate their antiurolithiatic activity.

II. METHODOLOGY

The plants Desmodium gangeticum L. and Heteropogon contortus L. were collected from around the Telangana region in January and authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh, India. The preparation of *Desmodium gangeticum* L., and *Heteropogon contortus L., was done by using Soxhlet extraction*. Approximately 200 g of root powder was placed in the Soxhlet device and extracted with (95%) methanol. The extraction procedure was carried out for 18 to 20 hours until a colourless solvent appeared in the side tube. The extract collected was dried by evaporating the solvent on a water bath maintained at <50°C.

A. Evaluation of Antiurolithiatic Activity

The antiurolithiatic activity of *Desmodium gangeticum* L., and Heteropogon contortus L., in albino rats was studied in 0.75% EG mixture-induced urolithiasis. Healthy male albino rats, weighing between 140 and 200 g, were randomly divided into seven groups, each consisting of 6 animals treated with 30% lactose and 0.75% EG mixed in water continuously for 28 days.

Group-1: Normal rats fed with a standard rat chow diet and tap water *ad libitum* for 28 days.

Group-2: Rats fed with a lactose-rich lab diet. [Lactose-rich lab diet contains 3.68%sucrose, 30% lactose, 23.4% protein, 10% fat, 5.3% crude fiber, 6.9% ash minerals (calcium 0.95%, phosphorus 0.67%, magnesium 0.21%) Vitamin A-22 IU/g, Vitamin D3- 4.5 IU/g Vitamin E-49 IU/g] with 0.75% Ethylene glycol (in drinking water) for 28 days.

Group-3: Rats fed with lactose-rich lab diet +0.75% EG + Standard drug cystone (5ml/kg b.w) (Himalaya drug company, Bengaluru, India) for 28 days.

Group-4 (curative): Rats fed with lactose-rich lab diet + 0.75% EG + (400 mg/kg) of *Desmodium gangeticum* L., from 14-28 days.

Group-5 (curative): Rats fed with lactose-rich lab diet + 0.75% EG + (400 mg/kg) of *Heteropogon contortus* L., from 14- 28 days.

Group-6 (prophylactic): Rats fed with lactose-rich lab diet + 0.75% EG + (400 mg/kg) of *Desmodium gangeticum* L., for 28 days.

Group-7 (prophylactic): Rats fed with lactose-rich lab diet + 0.75% EG+(400 mg/kg) of *Heteropogon contortus* L., for 28 days.

On the 29th day, three rats from each group were placed in a single metabolic cage and urine was collected (pooled) for 24 hours. HCL was added to the urine before it was kept at 4 degrees Celsius. Urine volume was measured, and biochemical markers, including calcium, magnesium, and uric acid, were examined. On the 29th day, blood was taken through retro-orbital puncture under ether anaesthesia. The animals were sacrificed by cervical decapitation after their serum was separated by centrifugation at 10,000 rpm for 10 minutes and tested for calcium, magnesium, and creatinine.

Individually weighed rat kidneys were put in 10% formalin immediately after collection. Histopathological alterations were identified in thin slices of kidneys preserved in paraffin and stained with eosin and hematoxylin.

B. Collection and Analysis of Urine

After a 28-day experimental period, all animals were housed in individual metabolic cages with 15 mL of water provided for hydration. Urine samples were then collected over 24 hours. Animals had free access to drinking water during the urine collection period. A drop of

concentrated hydrochloric acid was added to the collected urine and stored at 4°C. After urine collection, the total urinary excretion of calcium, oxalate, phosphate, and uric acid was measured using various biochemical kits (Span Diagnostics, Surat, India) according to the manufacturer's instructions.

C. Serum Analysis

After urine collection on the 29th day, blood was collected retro-orbitally under mild anaesthetic conditions, and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000 rpm for 5-10 min and analysed for calcium, phosphate, urea, uric acid and creatinine by using various biochemical kits (Span Diagnostics, Surat, India) according to the manufacturer's instructions.

D. Preparation of Kidney Homogenate and Biochemical Estimation

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned of extraneous tissue, rinsed with ice-cold physiological saline, and dried at 80 °C in a hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 mL of 1 N hydrochloric acid for 30 minutes and then homogenised. The homogenate was centrifuged at 2000 rpm for 10 min, and the supernatant was separated. The supernatant was analysed for calcium, phosphate, oxalate, and uric acid using various biochemical kits (Span Diagnostics, Surat, India) according to the manufacturer's instructions [9].

III. RESULTS AND DISCUSSION

The urolithiatic treatment resulted in a loss of body weight in the animals of the stone-induced group ($P < 0.001$ vs. Group I). In contrast, the other group of animals showed a significant gain in body weight after the experiment. Water intake was not significantly different among the groups, except for the stone-induced group, which was considerably higher compared to the control group ($P < 0.001$ vs. Group I).

A. Urine Analysis

In the present experiment, administration of 0.75% ethylene glycol [10] in drinking water to male *Wistar* rats caused a significant ($P < 0.001$ vs. Group I) increase of phosphorus and calcium concentration and a decrease in the magnesium concentration in urine of the stone-induced group (Group II). However, treatment with DGMRE and HCMRE 400 mg/kg significantly ($P < 0.01$ vs. Group II) reduced the phosphorus and calcium excretion and increased the magnesium excretion in urine in both the prophylactic and curative groups (Group IV and V, respectively) and were comparable to the standard group (Group III, cystone-treated)

B. Serum Analysis

Renal function was evaluated by measuring serum phosphorus, calcium, urea, and creatinine in Group I–VII. The concentrations of phosphorus, calcium, urea, and creatinine in the serum were significantly ($P < 0.001$ vs. Group I) increased in the stone-induced group, indicating renal damage. However, treatment with DGMRE and HCMRE significantly ($P < 0.001$ vs. Group II) reduced the concentrations of phosphorus, calcium, urea, and creatinine in the serum in both the prophylactic and curative groups, with reductions comparable to those in the standard group.

C. Kidney Homogenate Analysis

The deposition of crystalline components in the renal tissue, specifically phosphorus and calcium, was significantly ($P < 0.001$ vs. Group I) increased in the stone-induced group, whereas the magnesium level was decreased. Treatment with DGMRE and HCMRE significantly ($P < 0.001$ vs. Group II) reduced the concentrations of phosphorus and calcium and increased the magnesium in both the prophylactic and curative groups as compared to the standard group.

IV. KIDNEY HISTOPATHOLOGY

Kidney histopathological analysis revealed no CaOx crystal deposit or other abnormalities in the kidney of the control group (Group I). On the other hand, many CaOx crystal deposits in the renal tubules and congestion and dilation of the parenchymal blood vessels were seen in the renal tissue of the stone-induced group (Group II) as shown in Fig. In the standard group (Group III), the kidney showed normal architecture with dilation of tubules in the cortico-medullary junction, minima interstitial inflammation, and occasional renal tubules showed CaOx crystal deposits In the prophylactic group of DGMRE and HCMRE (Group VI, VII), the kidney showed standard architecture and few renal tubules that revealed vacuolar degeneration with no CaOx crystal deposits However, in the curative group (Group IV, V), the kidney showed standard architecture and occasional renal tubules that revealed CaOx crystal deposit.

V. DISCUSSION

It has been reported earlier that ethylene glycol causes hyperphosphaturia, hypercalciuria, and hypomagnesemia leading to urolithiasis [8]. We also found an elevated concentration of phosphorus and calcium, and a lower concentration of magnesium in the urine of the stone-induced group. However, during the administration of DGMRE and HCMRE (400), the calcium and phosphorus levels decreased to near-normal levels, which suggests that DGMRE and HCMRE (400) are effective in inhibiting hypercalciuria and hyperphosphaturia. Magnesium is considered a potent inhibitor of CaOx crystals because it decreases supersaturation. 159,160 The magnesium level was significantly decreased in the stone-induced group compared to the control group, due to supersaturation and metabolic acidosis. Administration of DGMRE and HCMRE (400) restored magnesium excretion and was compared to the stone-induced group. These results agreed with previous reports.

VI. CONCLUSION

Based on the above results, both medicinal plants have been proven to be scientifically effective in treating urolithiasis.

Table I: Effect of Desmodium Gangeticum L., and Heteropogon Contortus L., Extracts on Change in Body Weight and Water Intake in Urolithiatic Male Wistar Rats.

Group	Change in B. W	Water Intake(ml/24hr)
Control	15.01±3.20	7.00±1.42
Stone induced	-3.67±5.23***	14±3.12
Standard	6.89±2.52**	8.50±3.26
Curative regimen		
DGMRE (400mg/kg)	8.89±2.45**	10.47±2.50
DGMRE (400mg/kg)	7.65±1.89**	9.80±1.32
Prophylactic regimen		
HCMRE (400mg/kg)	6.98±2.13**	9.53±.089
HCMRE (400mg/kg)	5.67±1.19**	8.76±1.56

Table II: Effect of Desmodium Gangeticum L., and Heteropogon Contortus L., Extracts on Change in Urine(mg/dl) Parameters in Urolithiatic Male Wistar Rats.

Group	Phosphorus	Calcium	Magnesium
Control	2.003±0.05	8.90±0.15	5.75±0.23
Stone induced	4.010±0.08	20.89±16.26	1.26±1.10**
Standard	2.720±0.01**	12.37±0.26***	2.35±0.57**
Curative regimen			
DGMRE (400mg/kg)	2. 853±0.02**	15.48±0.12**	2.86±0.88*
HCMRE (400mg/kg)	3.126±0.15**	13.26±0.12***	3.01±1.17*
Prophylactic regimen			
DGMRE (400mg/kg)	2.756±0.34***	14.12±0.13**	2.42±3.21
HCMRE (400mg/kg)	2.528±0.12***	12.63±0.18**	1.99±0.76*

Values expressed as mean ± S.E.M.,n=6. Significance at p<0.05*, p<0.01**, p<0.001***. Compared with control group (One Way ANOVA followed by Dunnett t-test).

Table III: Effect of Desmodium Gangeticum L., and Heteropogon Contortus L., Extracts on Change in Serum (mg/dl) Parameters in Urolithiatic Male Wistar Rats.

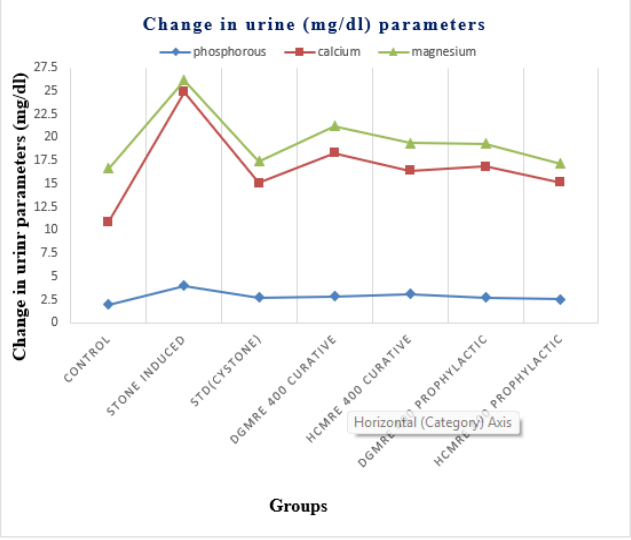
Group	Phosphorus	Calcium	Creatinine	Uric acid
Control	7.90±1.01	8.12±0.74	0.35±0.06	4.183±0.13
Stone induced	15.03±0.90**	14.96±0.37***	2.34±0.11	6.012±0.19
Standard	10.76±0.78**	11.34±2.90**	0.88±0.14**	4.897±0.12***
Curative regimen				
DGMRE (400mg/kg)	11.92±0.57**	12.32±1.13**	1.42±0.24**	5.315±0.10**
HCMRE (400mg/kg)	11.53±0.20**	11.97±0.56**	1.19±0.87*	5.168±0.09
Prophylactic regimen				
DGMRE (400mg/kg)	11.22±2.24**	11.26±3.42**	0.94±0.73***	4.890±0.17**
HCMRE (400mg/kg)	10.98±1.34**	11.40±2.22**	0.80±0.65***	4.732±0.15**

Values expressed as mean ± S.E.M.,n=6. Significance at p<0.05*, p<0.01**, p<0.001***. Compared with control group (One Way ANOVA followed by Dunnett t-test).

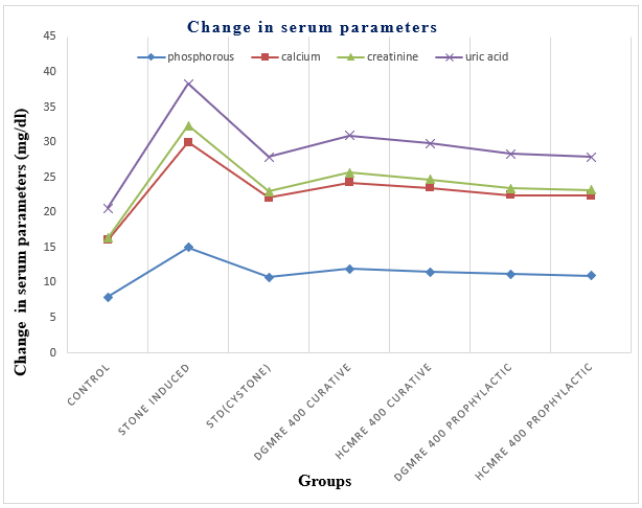
Table IV: Effect of Desmodium Gangeticum L., and Heteropogon Contortus L., Extracts on Change in Kidney Homogenate (mg/g) Parameters in Urolithiatic Male Wistar Rats.

Group	Phosphorus	Calcium	Magnesium
Control	2.52±0.46	9.80±2.01	3.73±1.70
Stone induced	8.44±0.58***	17.78±0.97**	0.80±2.38
Standard	3.15±1.15**	12.32±1.36**	2.41±0.67
Curative regimen			
DGMRE (400mg/kg)	3.56±0.09**	12.75±1.60**	2.67±1.35
HCMRE (400mg/kg)	3.43±0.13**	12.56±2.12**	2.33±0.23
Prophylactic regimen			
DGMRE (400mg/kg)	3.18±1.20**	12.10±2.46**	1.97±0.78
HCMRE (400mg/kg)	3.20±1.28**	12.44±3.56**	1.82±0.53

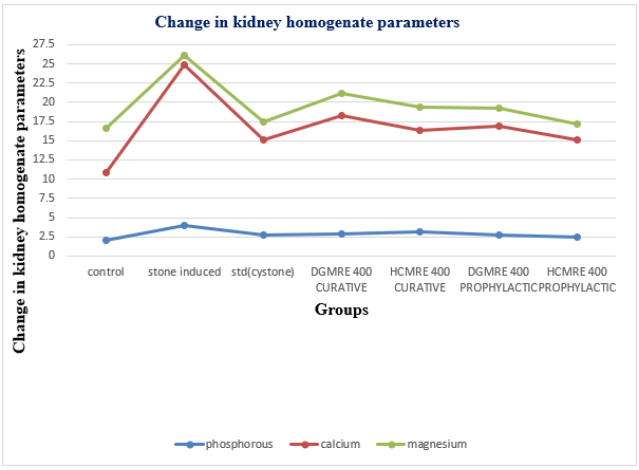
Values expressed as mean ± S.E.M.,n=6. Significance at p<0.05*, p<0.01**, p<0.001***. Compared with control group (One Way ANOVA followed by Dunnett t-test).



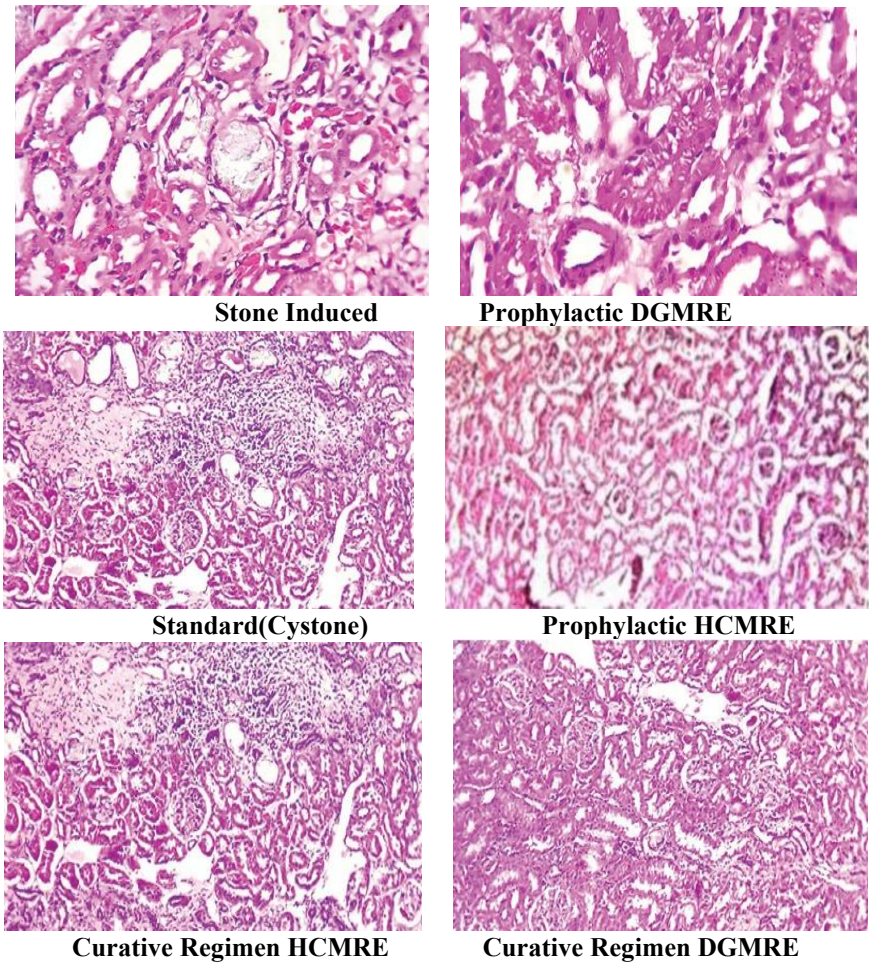
[Fig.1: Effect of Desmodium Gangeticum L., and Heteropogon Contortus L., Extracts on Change in Urine(mg/dl) Parameters in Urolithiatic Male Wistar Rats]



[Fig.2: Effect of Desmodium Gangeticum L., and Heteropogon Contortus L., Extracts on Change in Serum (mg/dl) Parameters in Urolithiatic Male Wistar Rats]



[Fig.3: Effect of Desmodium Gangeticum L., and Heteropogon Contortus L., Extracts on Change in Kidney Homogenate (mg/g) Parameters in Urolithiatic Male Wistar Rats]



[Fig.4: Effect of Desmodium gangeticum L., and Heteropogon contortus L., extracts on Histopathological sections of Kidney]

DECLARATION STATEMENT

After aggregating input from all authors, I must verify the accuracy of the following information as the article's author.

- **Conflicts of Interest/ Competing Interests:** Based on my understanding, this article has no conflicts of interest.
- **Funding Support:** This article has not been funded by any organizations or agencies. This independence ensures that the research is conducted with objectivity and without any external influence.
- **Ethical Approval and Consent to Participate:** The content of this article does not necessitate ethical approval or consent to participate.

participate with supporting documentation.

- **Data Access Statement and Material Availability:** The adequate resources of this article are publicly accessible.
- **Author's Contributions:** The authorship of this article is contributed equally to all participating individuals.

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