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SCREENING OF ANTIUROLITHIATIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *HIBISCUS ROSE-SINENSIS LINN* FLOWER PETALS IN WISTAR RATS

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ABSTRACT

Urolithiasis is the accumulation of solid, non-metallic minerals in the urinary system. After urinary tract infections and pathologic conditions of the prostate, this is the third most prevalent ailment of the urinary tract. An imbalance between the kidneys' promoters and inhibitors results in urolithiasis, a complicated illness. Kidney stones are the result of a variety of physicochemical processes, which begin received. Kidney stones can never be cured, hence the continued production and multiplication of calculi is a global health problem. Numerous plant extracts have been used to treat kidney stones, with positive results in both prevention and therapy. The rise of plant-based medicine has generated a lot of attention since it offers a richness of unique pharmacological compounds and may be used as a supplement or alternative for traditional treatment. Urolithiasis, a painful urologic disorder that affects around 12% of individuals globally, with a 70%–81% recurrence rate in males and a 47–60% recurrence rate in women. Numerous physical and chemical mechanisms, including as development, aggregation, and retention in the kidneys, contribute to the complex process of creation as the supersaturation. Currently, medical treatment for nephrolithiasis is costly and includes side effects. The

invasive treatments required for nephrolithiasis therapy carry a significant risk of major consequences and costly strain on the healthcare system. We are transitioning from utilizing pharmaceutical items for urolithiatic illness to employing herbal remedies instead in order to improve treatment outcomes and reduce side effects and risk factors.

KEYWORDS: Urinary tract infection, Kidney stones, Nephrolithiasis, Herbal remedies, Pharmacological compounds.

INTRODUCTION

Kidney stones, sometimes referred to as urolithiasis, are stones that occur in the urinary system. These stones come in different sizes and can form in the kidneys, ureters, bladder, or urethra. Urinary tract infections, severe flank discomfort, and blood in the urine are just a few of the symptoms that can accompany this unpleasant ailment. Kidney stones can develop due to a variety of factors, including genetic predisposition, nutrition, and dehydration. Adjusting one's lifestyle, staying hydrated, and occasionally undergoing surgery or medical intervention are all part of management.

Urolithiasis is a common disease leading to a high socioeconomic burden due to treatment costs and sickness leave. Nephrolithiasis is the term used to describe the formation of renal stones inside the kidneys. When these stones leave the renal pelvis and propagate into the bladder, urethra, and ureters, the resulting disorder is known as urolithiasis. Many urolithiasis patients can be treated with analgesics, antiemetic drugs, and expectant care; nevertheless, more and more intensive therapies are needed to treat stones linked to infection, renal failure, and obstruction.

According to reports, there is an average lifetime risk of 5–10% for stone formation. The population is dominated by men, with a peak incidence in the fourth and fifth decades of life. A solid, crystalline mineral called a kidney stone can form in the kidney or urinary tract. Kidney stones often cause blood in the urine and excruciating pain in the side, groin, or belly.

There is still no effective medication to use in clinical therapy,particularly for the prevention or recurrence of stones, despite a few recent reports of the positive effects of medical treatments in increasing clearance of stones in the distal ureters. In this sense, it has been demonstrated that a variety of plants can effectively treat kidney stones when taken traditionally.

MATERIALS AND METHODS

Plant Material

The flower petals of Hibiscus rosa-sinensis flowers was collected and authentified by taxonomist Dr. P Satyanarayana raju from Acharya Nagarjuna University, Guntur.

Experimental Animals

Wistar albino rats (160-180 g) were taken. The animals had free access to standard rat pellet, with water supplied ad libitum under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol (acclimatization) to minimise if any of nonspecific stress. The approval of the Institutional Animal Ethical Committee (IAEC) of BIOGENEI LABORATORIES, HYDERABAD (A.P) was taken prior to the experiments. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for Control and Supervision of Experiments on Animals (CCSEA).

Preparation of hydroalcoholic extract

The dried powder of Hibiscus rosa-sinensis flowers were collected. The dried powder is subjected to seived to get uniform coarse powder which was further subjected to extraction. The 500 grams of powder was taken and subjected for hydroalcoholic extraction. It is prepared by mixing of distilled water and ethanol (Ethanol: Water in 50:50 ratio). The extraction process continued for four days. A thick extract is obtained, which is evaporated over night in an oven at a temperature of 60˚c. It is then subjected to lyophilisation to get dried powder.

EXTRACTION PROCEDURE

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over night in an oven at a temperature of 60˚c. It is then subjected to lyophilisation to get dried powder.

EVALUATION OF ANTI UROLITHIATIC ACTIVITY

Ethylene Glycol Induced Urolithiatisis Model

Twenty male Wistar rats (weighing 160-180 g) are divided with matched body weights into 5 groups of 6 animals each, which will then randomly be selected to receive various treatments depending on their groups. Group I rats treated as normal control, will receive normal saline 2.5 mL once in 24 hrs and water daily for 21 days. Group II rats treated as positive control, will receive stone induction treatment for 21 days which comprises 0.75%(w/v) EG with 1%(w/v) ammonium chloride for 5 days; following this the water supply will be switched to 0.75%(w/v) EG alone in water, along with saline treatment. Group III rats include treatment with low dose of extract, will receive extract (50mg/kg) through gastric gavages and simultaneously receives stone-inducing treatment similar to the positive control daily for 21 days. Group IV rats will receive high dose (100 mg/Kg of extract) through gastric gavages and simultaneously receives stone-inducing treatment similar to the positive control for 21 days. Group V rats will receives standard drugCystone, (100mg/kg) through gastric gavages and simultaneously received stone-inducing treatment similar to the positive control daily for 21 days (treatment group, standard drug).

Immediately after 21 day, 24 hrs of urine sample was collected in presence of few thymol crystals, by housing animals individually in metabolic and diuretic cages, for measurement of urine parameters. Blood is collected from each animal of each group by retro-orbital method,for measurement of serum parameters. Kidneys are isolated and stored in neutral formalin buffer solution for histopathology studies.

Table: Induced Urolithiasis Model.

GROUPS	TREATMENT METHOD
I (control group)	Normal saline 2.5 ml/24 hrs and water for 21 days.
II (disease control)	0.75% (w/v) EG+1%(w/v)ammonium chloride for 5 days and ethylene glycol alone in water for 21 days.
III (HEHR 50mg/kg)	Extract (50mg/kg) and stone induction treatment similar to positive control group for 21 days.
IV (HEHR 100mg/kg)	Extract (100mg/kg) and stone induction treatment similar to positive control group for 21 days.
V (standard cystone drug100mg/kg)	Standard drug – cystone (100 mg/kg) and stone induction treatment similar to positive control group for 21 days.

RESULTS

1. Preliminary phytochemical Screening

The revealed results of the preliminary phytochemical screening of the hydroalcoholic flower petals extract of Hibiscus rosa-sinensis were shown below.

Table 0.1: Preliminary Phytochemicals of The Hydroalcoholic Flower Petals Extract of Hibiscus Rosa-Sinensis.

S. no	Phytochemicals	Results
1.	Carbohydrates	_
2.	Volatile oils	+
3.	Flavanoids	+
4.	Steroids	+
5.	Terpenoids	+
6.	Tannins	+
7.	Alkaloids	+
8.	Proteins	+
9.	Reducing sugars	_
10.	Cardiac glycosides	_
11.	Anthraquinone	_

+ ⟶ indicates presence of compound

_ ⟶ indicates absence of compound

Table no 0.2: Serum Parameters of Ethylene Glycol Induced Urolithiasis Model.

Serum parameters			
groups	**Serum creatinine**	**Creatinine Clearence**	**Blood Urea Nitrogen**
control	0.84±0.008	0.83±0.023	19.51±0.356
disease control	1.55±0.038	0.62±0.026	58.02±0.064
HERS 50mg/kg	1.26±0.023	0.64±0.028	46.53±0.757
HERS 100mg/kg	0.87±0.020[b]	0.84±0.015 [b]	20.63±0.215
Std cystone(100mg/kg)	1.08±0.059[a]	0.78±0.021[a]	35.14±0.160

N=6 all values are mean ± SD ‘a’ significant $p<0.01$ compared to disease control and’b’ significant $p<0.001$ compared to disease control

2. URINE PARAMETERS

The results reveal that HERS showed antiurolithiatic activity in a dose dependent manner. HERS at a dose of 100mg/kg showed significant results at $p<0.0001$ compared to disease control in view of urine oxalate and calcium levels. HERS at dose of 100mg/kg showed better results compared to 50mg/kg dose of HERS. Compared to standard HERS at 100mg/kg showed significant results.

Tabel no 2.1: Urine Parameters of Ethylene Glycol Induced Urolithiasis Model.

Urine parameters						
Groups	**Urine pH**	**Urine Oxalate**	**Calcium**	**Magnesium**	**Inorganic Phosphate**	**Uric acid**
control	6.38± 0.02	0.36± 0.01	1.73± 0.16	2.65± 0.03	6.29± 0.03	0.55± 0.03
disease control	5.08± 0.07	2.12± 0.03	3.57± 0.13	3.48± 0.02	7.87± 0.03	1.28± 0.02
HERS 50mg/kg	6.22± 0.03	1.31± 0.02	2.61± 0.04	3.12± 0.02	7.42± 0.05	1.07± 0.02
HERS 100mg/kg	6.41± 0.06	0.43± 0.03[b]	1.82± 0.03 [b]	2.77± 0.03	6.91± 0.23	0.71± 0.03
Std cystone 100mg/kg	6.35± 0.02	1.71± 0.03[a]	2.88± 0.03 [a]	2.89± 0.03	7.25± 0.08	0.90± 0.03

N=6 all values are mean± SD ‘a’ significant at p<0.001 compared to disease control and ‘b’ significant at p<0.0001 compared to disease.

3. KIDNEY PARAMETERS

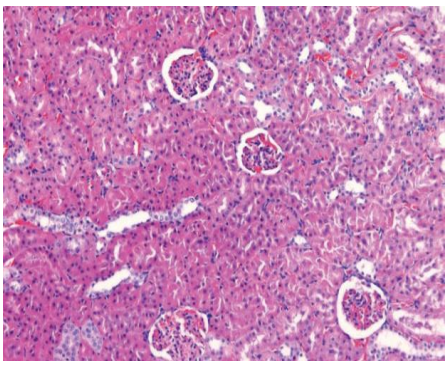
The results reveal that in the case of disease control group there is increase in the weight of the kidney indicating stone deposition. HERS treatment for 21days at a dose of 100mg/kg showed significant results at p<0.001 compared to disease control. Crystal deposition is not significant at a dose of HERS 100mg/kg. Compared to HERS 50mg/kg, 100mg/kg showed better results.

Tabel no 3.1: Kidney Parameters of Ethylene Glycol Induced Urolithiasis Model.

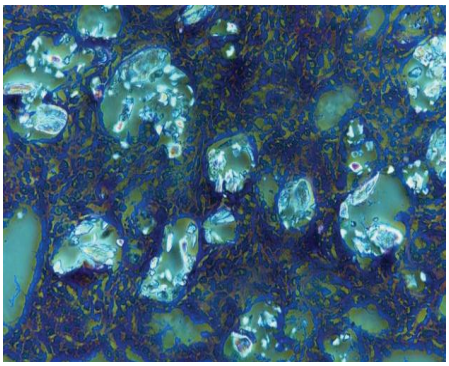
Kidney parameters		
groups	**weight(gm)**	**Crystal deposition**
control	0.75	Absent
disease control	1.27	Significant
HERS 50mg/kg	1.07	Moderate
HERS 100mg/kg	0.85[b]	not significant
Std cystone(100mg/kg)	0.93[a]	Moderate

N=6 all values are mean± SD ‘a’ significant at p<0.01 compared to disease control and ‘b’ significant at p<0.001 compared to disease control.

4. HISTOLOGICAL STUDIES



[A]



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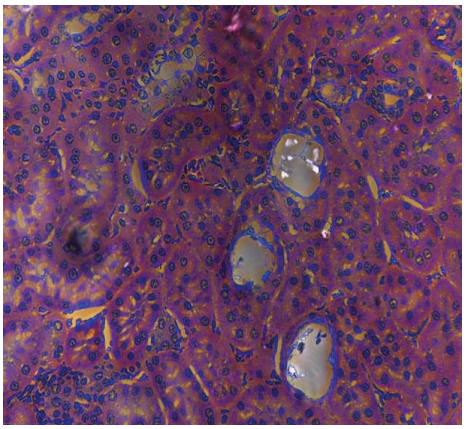

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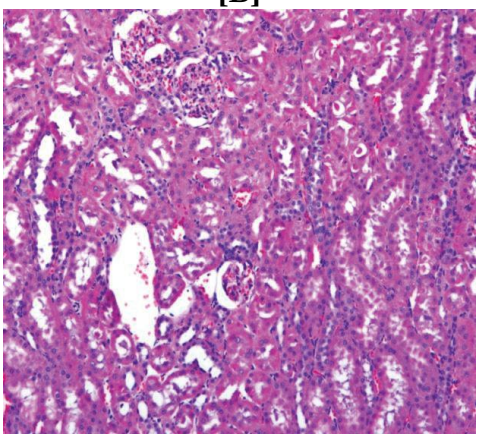

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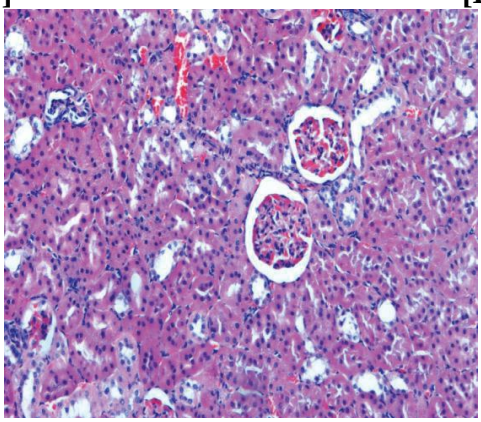

[E]

(a) Normal cellular structure of control rats kidney (Gr I).

(b) Ethylene-glycol-induced urolithic rats kidney showing irregular crystals dilation and inflammation under polarized microscope (Gr II). **(c)** Kidneys of urolithic rats treated with HERS (50mg/kg) showing near normal cellular structure under polarized microscope (Gr III).

(d) Kidneys of urolithic rats treated with HERS (100mg/kg) showing normal cellular Structure (Gr IV).

(e) Kidneys of urolithic rats treated with Cystone (100 mg/kg) showing normal cellular Structure (Gr.V).

CONCLUSION

The current study found that Hibiscus rosa-sinensis flower petals exhibited strong, dose-dependent antiurolithiatic efficacy. Hibiscus rosa-sinensis flower petals could be a novel natural source for the treatment of urolithiasis.

Where patients are waiting for more effective curative therapy is the better place to find them.

For the majority of patients, it is safe and reasonably priced. The presence of flavanoids suggests that it has anti-oxidant action, which may also effectively interfere with the occurrence of free radicals; as a result, oxidative stress conditions can be efficiently treated with a shorter healing period than traditional therapies.

It is necessary to use Hibiscus rosa-sinensis in a methodical manner to treat the underlying issues preventing the best kidney stone formation. Hibiscus rosasinensis flower petals can be used to effectively manage kidney stones by integrating appropriate care and nutritional measures, which will boost their healing properties and reduce their dangers.

As a result, Hibiscus rosa-sinensis may be crucial to the therapeutic management of urolitiatic formulations in the future.

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