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Original Research Article

Evaluation of Anti-Urolithiatic Activity of Hippophae Rhamnoides Linn. Fruits in Rats

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Abstract:

Background: These results suggested that Hippophae rhamnoides fruit extracts possessed nephroprotective properties, which could be beneficial in managing urolithiasis and other renal disorders. The study provided a foundation for further research into the pharmacological applications of this plant in herbal.

Methods: Adult Wistar albino rats were divided into six groups (n=6). Group I served as the normal control, receiving only the vehicle. Group II Ethylene glycol (0.75% v/v) dissolved in their drinking water to induce the formation of kidney stones. Group III was treated with standard drug cystone (100mg/kg) following Ethylene glycol (0.75% v/v) administration. Groups IV, V and VI were pre-treated with ethanolic extract of Hippophae rhamnoides at doses of 200mg/kg, 400mg/kg, and 600mg/kg respectively. Then the biochemical parameter such as Calcium, Oxalate, Magnesium, Phosphate Estimation in Urine And Blood Urea Nitrogen (BUN), Creatinine Estimation in Serum As well As Measurement of Body and Kidney Weight and Histopathological analysis of kidneys.

Result: In this study, the administration of 0.75% (v/v) ethylene glycol aqueous solution to male Wistar albino rats produced hyperoxaluria. Oxalate, calcium, and phosphate excretion were grossly increased in the calculi-induced animals. However, cystone and Hippophae rhamnoides significantly lowered the elevated level of oxalate, calcium, and phosphate in urine when compared to the model control group ($p < 0.005$).

Conclusion: The finding this study provide compelling evidence that Hippophae rhamnoides (HR) fruit extract exhibits significant antiurolithiatic activity in a well-established model of ethylene glycol-induced urolithiasis in rats.

Keywords: Hippophae rhamnoides, Pharmacognosy, Traditional medicine, Anti-Urolithiatic.

Introduction

Urolithiasis is a hard mass that develops from crystals within the urinary tract that separate from urine. Calcium and oxalate are the common part of human diet and are essential parts of the body, like muscles and bones. Along with calcium and phosphate are common component of urine is passes away through excreatary system. The natural presence of some chemicals inhibits the formation of crystals of calcium oxalate and calcium phosphate when these inhibitors do not work properly in any individual, crystal formation may occur. One of the important causes of stone formation is urinary infection. The small crystal particle can pass thru the renal tract without it being detected and travel through the urine out of the body. This form of stone is called a struvite or infection stone. Other form of stone, uric acid stonesis a little less common. Specific phytotherapy treatment has been attempted; furthermore, interest has resurged in recent years effective, safe and culturally acceptable medicinal plants. However few studies have been conducted using purpose methods to judge their useful effects in treatment of urolithiasis. [1,2,3,4]

Figure 1: Structure of Kidney

Hippophae rhamnoides L., commonly referred to as sea buckthorn, belongs to the family Elaeagnaceae.

This deciduous shrub is native to the cold-temperate regions of Europe and Asia and is well recognized for its resilience in harsh climates. The plant has garnered significant attention due to its nutritional and medicinal potential, widely explored in traditional and modern pharmacology. [5,6,7]

Common name: Sea berry, Sea buckthorn or Sallowthorn

Taxonomical classification of Hippophae rhamnoides Linn.[8]

Kingdom	Plantae
Sub kingdom	Phanerogamia
Division	Angiospermae
Class	Dicotyledons
Subclass	Monochalamydae
Series	Daphnales
Family	Elaeagnaceae
Genus	Hippophae
Species	rhamnoides Linn

Pharmacological effects: Sea buckthorn has many pharmacological effects, including:[9,10,11]

- Cardioprotective: Sea buckthorn has cardioprotective activity, including lowering blood pressure and cholesterol concentration, and inhibiting blood platelet activation.
- Anti-inflammatory: Sea buckthorn has anti-inflammatory properties. •Antibacterial and antiviral: Sea buckthorn has antibacterial and antiviral properties.
- Antioxidant: Sea buckthorn has antioxidant activity.
- Antiulcerogenic: Sea buckthorn has antiulcerogenic effects.
- Radioprotective: Sea buckthorn has radioprotective effects.
- Hepatoprotective: Sea buckthorn has hepatoprotective properties.
- Hypoglycemic and hypolipidemic: Sea buckthorn has hypoglycemic and hypolipidemic properties.
- Neuroprotective: Sea buckthorn has neuroprotective properties.
- Anti-tumor: Sea buckthorn has antitumor properties. •Anti-obesity: Sea buc

Methods and Materials:

Collection and Authentication of Plant Materials

The fruit of the Hippophae rhamnoides were collected from the Himachal Pradesh, India and authentication were obtained from the Sisco Research Laboratories Pvt. Ltd. Hippophae rhamnoides were acquired from outskirt area of Himachal Pradesh.

Drugs and Chemicals

The drug ethylene glycol were purchased from sigma-Aldrich. Cystone were obtained from the Sisco Research Laboratories Pvt. Ltd. Hippophae rhamnoides were acquired from outskirt area of Himachal Pradesh.

Evaluation of in vivo anti-urolithiatic activity:

Experimental Animals Thirty healthy adult male albino rats of Wistar strain (Rattus norvegicus) weighing 150–200gm were acquired from the animal house of Biogene lab, Bangalore. Rats were housed under controlled environmental conditions (Temperature 28±2°C; 12:12 h light: dark cycle; 50±10% humidity) in plastic cages with filter tops. All rats were fed with commercial rat pellets (SAI Animal Feed Ltd, Bangalore) and were given water ad libitum. Before experimental work, the rats were acclimated in the animal house for 10 days.[12]

Acute toxicity assay: The acute oral toxicity study were carried out as per Organization for Economic Co-operation and Development (OECD)

Guidelines 425. The Ethanolic Extract were administered at the dose level of 2000 mg/kg and observed mortality after 24 hr. One-tenth of the median lethal dose (LD50) were taken as an effective dose.[13]

Ethlene glycol - Induced Urolithiasis Model.[14]

The rats were organized into groups, each comprising six individuals for anti-urolithiatic activity.

- Group I Control group will receive water and food ad libitum
- Group II Ethylene glycol (0.75% v/v) dissolved in their drinking water
- Group III Ethylene glycol (0.75% v/v) and Cystone (100 mg/kg) p.o.
- Group IV Ethylene glycol (0.75% v/v) and low Dose of extract (200mg/kg) p.o.
- Group V Ethylene glycol (0.75% v/v) and medium Dose of extract (400mg/kg) p.o.
- Group VI Ethylene glycol (0.75% v/v) and large Dose of extract (600mg/kg) p.o

Urine samples will be collected from all the animals on both the 14th and 28th days. Metabolic cage will be used to collect urine sample. On the crucial 28th day of the study, blood samples (1 mL) will be obtained from each animal through the retro-orbital plexus under anesthesia.

Estimation of Biochemical Parameter:

- Estimation of Calcium[15], Oxalate[16], Mangnesium[17] and Phosphate[18] in Urine sample.
- Estimation of BUN[19], Creatinine[20] level in blood serum sample

Histopathological analysis of kidney;

On day 29 rats were anesthetized, sacrificed through an incision on their abdomen, and both kidneys were removed. A cold solution of normal saline were used to rinse the extraneous tissue of isolated kidneys to remove fat deposits, then it were fixed in a neutral formalin (pH 7.4) solution for 10%. Then, the kidney tissue portion were fixed in 10% neutral formalin (pH 7.4) solution. One of the two kidneys that were isolated was immersed in paraffin films using a traditional method, then sliced into 5 μm thin sections by a rotary vertical microtome. A hematoxylin and eosin solution were used to stain the sections to indicate changes in the histopathological analysis and crystal deposition. Hematological parameters like hemoglobin content, total erythrocyte count, and leukocyte count were analyzed in the blood.

Statistical Analysis:

The observations were statistically analyzed using GraphPad Prism 10.23. All results were expressed as Mean ± SEM. The results wer analyzed using One-Way Analysis of Variance (One Way ANOVA), followed by Tukey's multiple comparison test, with $p < 0.005$ considered statistically significance.

Result & Discussion:

Phytochemical Screening of Hippophae rhamnoides Fruit Extracts

Phytochemical screening revealed that H. rhamnoides fruits contain a wide spectrum of bioactive compounds, with strong representation of polyphenols, flavonoids, glycosides, saponins, and tannins.

These compounds are well-documented for their antioxidant, anti-inflammatory, and antiurolithiatic activities. The ethanol and ethyl acetate extracts were particularly rich in these constituents, whereas aqueous extracts showed a relatively modest profile. (Table 1)

Table 1: Phytochemical Screening of Hippophae rhamnoides Fruit Extracts

Phytoconstituents	Ethanol Extract (EE)	Ethyl Acetate Extract (EAE)	Aqueous Extract (AE)
Carbohydrates	+ve	+ve	+ve
Alkaloids	+ve	+ve	−ve
Phenolic Compounds	+ve	+ve	+ve
Flavonoids	+ve	+ve	+ve
Glycosides	+ve	+ve	+ve
Saponins	+ve	+ve	+ve
Tannins	+ve	+ve	+ve
Steroids	−ve	−ve	−ve
Proteins & Amino Acids	−ve	−ve	−ve
Fixed Oils & Fats	−ve	−ve	−ve

Physicochemical Parameters of Hippophae rhamnoides Fruit Extracts: The physicochemical characteristics of herbal materials serve as the foundational criteria for quality control and standardization. In the present study, the total ash content (3.25%) of H. rhamnoides fruit powder reflects the overall inorganic matter, while the acid-insoluble ash (0.68%) indicates a minimal presence of extraneous siliceous material like sand or soil. The low water-soluble ash (0.45%) further confirms the purity and appropriate post-harvest handling. Sulphated ash (2.81%), indicative of total mineral content, aligns well with the known mineral-rich nature of Sea buckthorn, suggesting the presence of beneficial inorganic constituents.(Table 2)

Table 2: Physicochemical parameters of Hippophae rhamnoides Fruit Extracts

Physicochemical Parameter	**Results (% w/w)**
Ash Value	
Total Ash	3.25
Acid Insoluble Ash	0.68
Water Soluble Ash	0.45
Sulphated Ash	2.81
Extractive Values	
Ethanolic Extract (EE)	28.45
Ethyl acetate Extract (EAE)	18.72
Aqueous Extract (AE)	15.89
Soxhelt etraction	
Moisture Content	6.85
Foreign Organic Matter	Less than 1%
Foaming Index	Nil
Swelling Index	Nil

Fluorescence Characteristics of Hippophae rhamnoides Fruit Extracts: The fluorescence characteristics under different wavelengths and chemical reagents provided additional insight into the nature of secondary metabolites present in the plant. The differential responses under UV and visible light suggest the presence of structurally diverse compounds such as phenolics, flavonoids, and glycosides. These observations are consistent with the phytochemical results and affirm the utility of fluorescence analysis in the preliminary identification of plant materials. (Table 3)

Table 3: Fluorescence characteristics of Hippophae rhamnoides Fruit Powder Extracts

Treatment/Reagent	**Visible Light**	**UV Light (Shortwave 254 nm)**	**UV Light (Longwave 365 nm)**
Powder fruits	Light brown	Dark brown	Greenish brown
Powder + 1N NaOH (aq.)	Yellowish brown	Dark green	Greenish yellow
Powder + 1N NaOH (alc.)	Yellow	Greenish brown	Bright green
Powder + 1N HCl	Pale yellow	Dark yellow	Yellowish green
Powder + Ammonia	Brownish yellow	Greenish brown	Bright yellow
Powder + Ferric chloride (5%)	Dark brown	Blackish green	Greenish black
Powder + Iodine solution	Brown	Black	Dark blue
Powder + Acetic acid	Yellow	Light green	Yellowish green
Powder + Ethanol	Yellowish brown	Dark brown	Greenish yellow
Powder + Chloroform	Light green	Greenish brown	Dark green

In Vivo Antiurolithiatic Evaluation: The oral administration of ethanolic extract of Hippophae rhamnoides fruits up to a dosage of 2000 mg/kg showed no signs of toxicity in rats, and for 14 days there is no death.

Therefore, for the anti-urolithiatic investigation in the present study, the therapeutic dosage is determined to be 200 mg/kg to 600 mg/ kg body weight.

In the current study, the effect of ethanolic extract of Hippophae rhamnoides (EEHR) on body weight is assessed in ethylene glycol-induced urolithiatic rats. All animals initially had comparable body weights, ranging between 169.6 g and 173.2 g across the six groups. The stone-induced group (Group II) exhibited a significant reduction in final body weight (179.3 ± 6.10 g) compared to the control group (192.9 ± 4.59 g), indicating the systemic stress and catabolic state associated with kidney stone formation ($p < 0.05$).

Measurement of Body Weight: Body weight changes serve as an indirect marker of the systemic effects of the disease and therapeutic intervention. The significant reduction in body weight observed in the stone-induced group indicates a catabolic state resulting from renal damage and metabolic distress. Treatment with EEHR led to a dose-dependent improvement in body weight, with the highest dose (600 mg/kg) nearly restoring normal weight, even surpassing the effect observed with the standard drug, Cystone. This finding underscores the potential of EEHR in ameliorating the systemic stress induced by urolithiasis.

Table 4: Body Weight of Experimental Groups

Group	Initial Body Weight (g)	Final Body Weight (g)
Control	169.6 ± 5.29	192.9 ± 4.59
Stone-induced	170.4 ± 4.87	179.3 ± 6.10
Cystone 100 mg/kg	171.5 ± 6.01	188.7 ± 5.74
EEHR 200 mg/kg	172.0 ± 5.45	184.3 ± 5.22
EEHR 400 mg/kg	173.2 ± 5.78	189.0 ± 5.88
EEHR 600 mg/kg	172.5 ± 5.31	193.4 ± 5.65

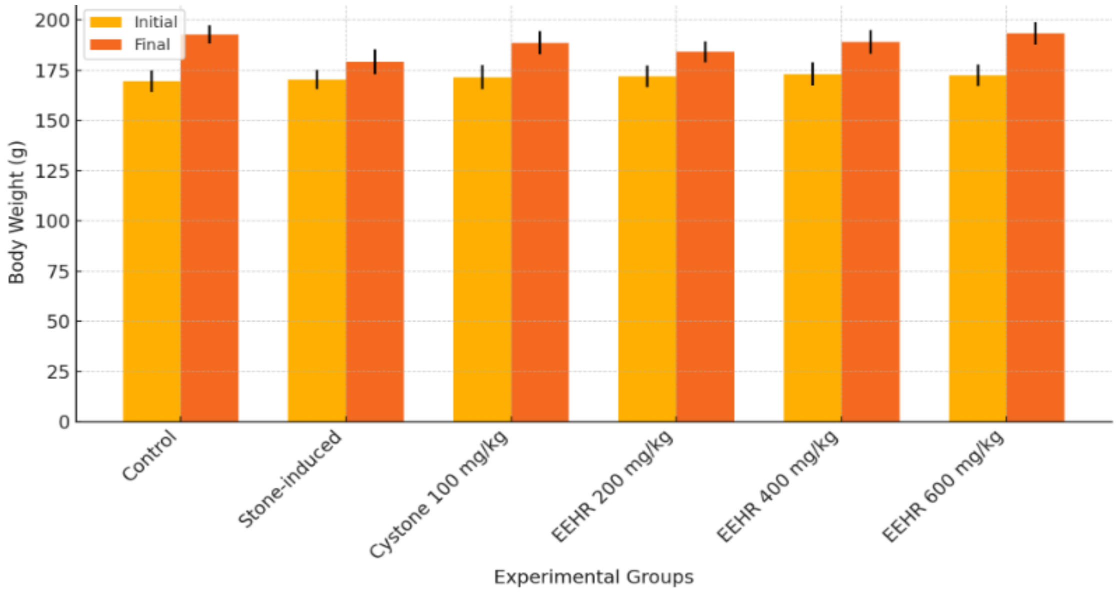

Figure 2: Initial and final body weight of experimental groups

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Treatment with EEHR led to a dose-dependent improvement in body weight, with the highest dose (600 mg/kg) nearly restoring normal weight, even surpassing the effect observed with the standard drug, Cystone. This finding underscores the potential of EEHR in ameliorating the systemic stress induced by urolithiasis.

Measurement of Kidney Weight: Kidney weight is an important biomarker of renal pathology. The significant increase in kidney weight in the disease group reflects inflammatory swelling, tissue hypertrophy, and crystal deposition. EEHR administration led to a dose-dependent normalization of kidney weight, supporting its ability to reduce renal inflammation and crystal load.

Table 5: Kidney Weight of Experimental Groups

Group	Kidney Weight (g)
Control	1.26 ± 0.07
Stone-induced	1.68 ± 0.10
Cystone 100 mg/kg	1.32 ± 0.08
EEHR 200 mg/kg	1.35 ± 0.09
EEHR 400 mg/kg	1.31 ± 0.06
EEHR 600 mg/kg	1.29 ± 0.05

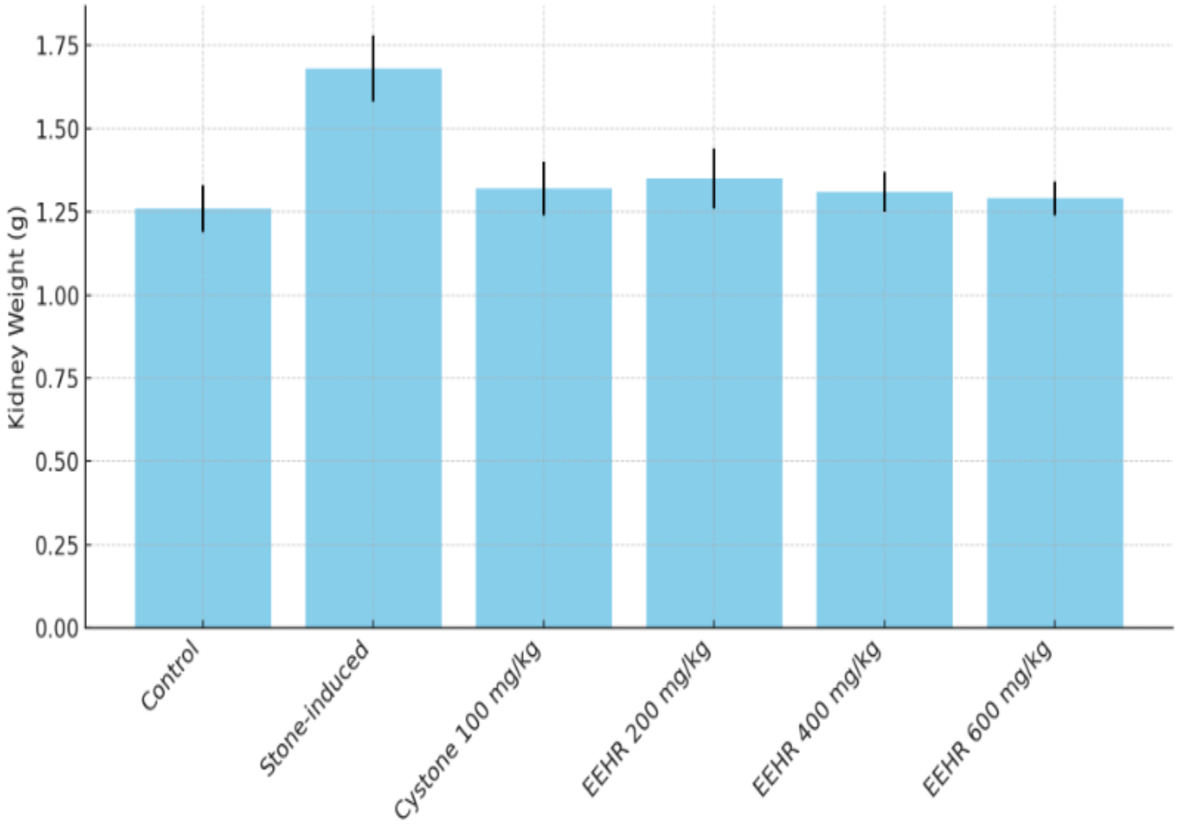

Figure 3: Kidney weight of Experimental Groups

Biochemical Analysis Interpretation

This biochemical analysis presented data on various parameters measured in different experimental groups. The groups included a Control group (Group I), a Disease Control group (Group II), a standard treatment group with Cystone (Group III), and three experimental treatment groups (Groups IV, V, and VI) that received different doses (200 mg/kg, 400 mg/kg, and 600 mg/kg, respectively).

Table 6: Biochemical Parameters in Different Experimental Groups

Parameter	Group I (Control)	Group II (Disease Control)	Group III (Cystone 100 mg/kg)	Group IV (200 mg/kg)	Group V (400 mg/kg)	Group VI (600 mg/kg)
Calcium (mg/dL)	4.5 ± 0.3	9.9 ± 0.5	5.2 ± 0.4	6.8 ± 0.5	5.9 ± 0.3	4.8 ± 0.2
Oxalate (mg/dL)	0.9 ± 0.1	3.7 ± 0.2	1.5 ± 0.1	2.5 ± 0.2	1.8 ± 0.1	1.2 ± 0.1
Magnesium (mg/dL)	2.1 ± 0.1	0.9 ± 0.1	1.8 ± 0.1	1.5 ± 0.1	1.7 ± 0.1	2.0 ± 0.1
Phosphate (mg/dL)	2.5 ± 0.2	5.8 ± 0.3	3.1 ± 0.2	4.4 ± 0.3	3.5 ± 0.2	2.9 ± 0.2
BUN (mg/dL)	18.4 ± 0.9	42.3 ± 1.5	24.2 ± 1.2	34.5 ± 1.3	27.2 ± 1.1	20.9 ± 1.0
Creatinine (mg/dL)	0.7 ± 0.05	2.3 ± 0.1	1.1 ± 0.06	1.8 ± 0.08	1.3 ± 0.07	0.9 ± 0.05

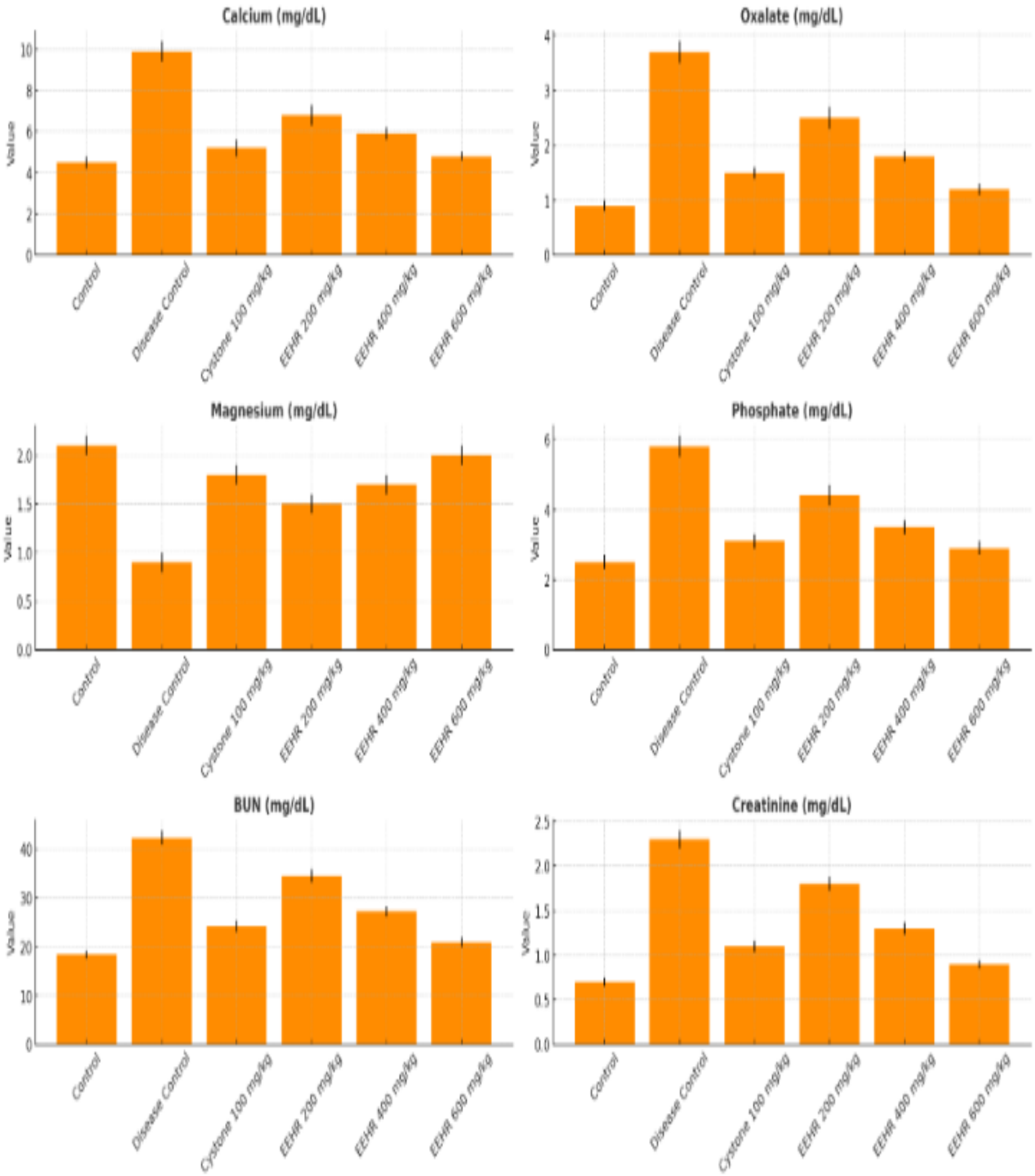

Figure 4: Biochemical parameters in Experimental groups

The biochemical profile of urolithiatic rats showed profound derangements, particularly elevated levels of calcium, oxalate, phosphate, creatinine, and blood urea nitrogen (BUN), along with reduced magnesium levels.

These markers are consistent with impaired renal filtration, tubular damage, and crystal accumulation.

Histopathological Studies

Histological examination of renal tissues provided conclusive visual evidence of the protective effects of EEHR. The disease control group exhibited pronounced histopathological damage, including glomerular congestion, tubular dilation, epithelial disruption, and inflammatory infiltration, all hallmarks of nephrolithiasis.

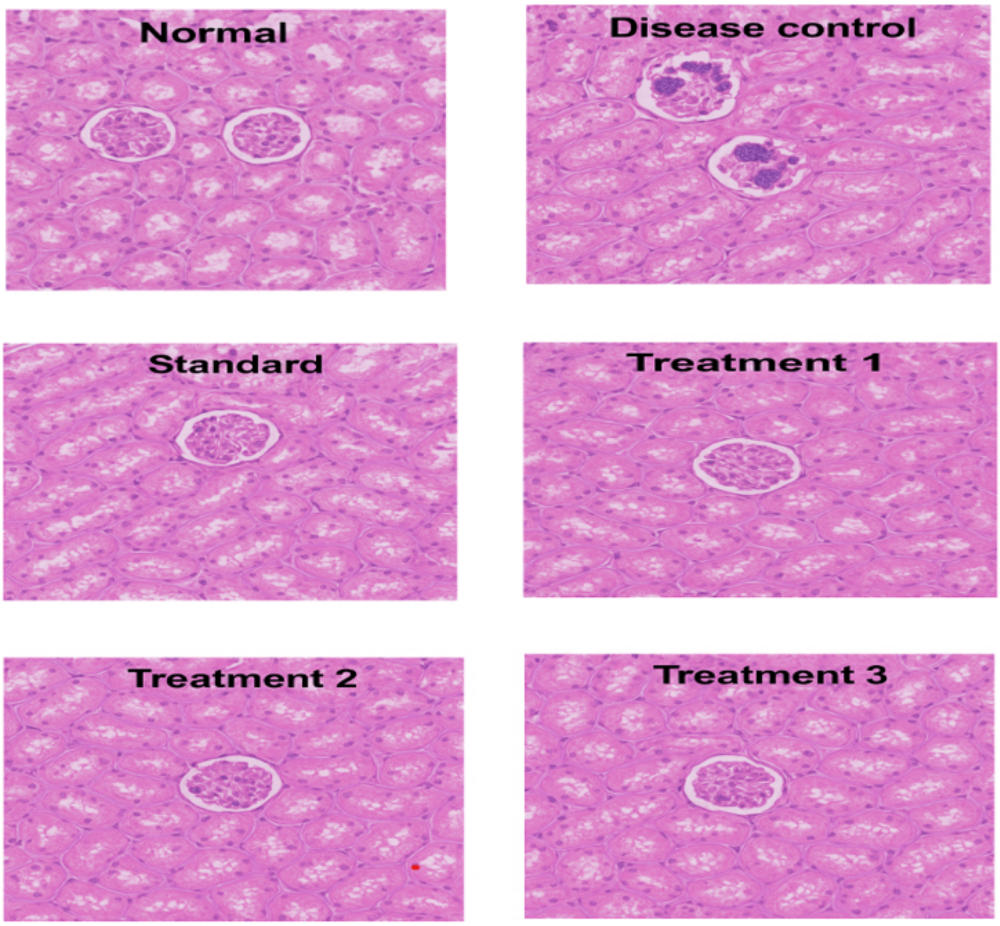

Figure 5: Histopathological analysis of kidney at 400HX

At the highest dose, renal tissue showed a nearly normal appearance, comparable to the control group, with intact glomeruli and well-structured tubules. The absence of crystal deposits and inflammatory cells indicates that EEHR not only prevents stone formation but also facilitates tissue healing and repair.

Conclusion:

The findings of this study provide compelling evidence that Hippophae rhamnoides (HR) fruit extract exhibits significant antiurolithiatic activity in a well-established model of ethylene glycol-induced urolithiasis in rats.

The study demonstrated that the HR extract effectively mitigated various pathological consequences of urolithiasis, including abnormal urinary biochemistry, elevated serum markers of renal dysfunction, and excessive deposition of calcium and oxalate crystals in the kidneys. These effects were observed to be dose-dependent, with higher doses (400 mg/kg and 600 mg/kg) showing greater efficacy comparable to that of the standard drug Cystone.

This study provides a pharmacological basis for the traditional use of Hippophae rhamnoides in urinary disorders and supports its development as a promising natural remedy for nephrolithiasis. Future studies focusing on isolation of active constituents, mechanism of action, and clinical validation in human models will further strengthen its potential as an evidence-based phytotherapeutic agent in the management of kidney stone.

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