Phytochemical and Pharmacological Assessment of Anti-Diuretic Activity of Euphorbia Thymifolia in Male Sprague Dawley Rats.

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ABSTRACT :
The present study was designed to evaluate the acute oral toxicity and diuretic activity of Euphorbia thymifolia extracts in Wistar rats using the Lipschitz method. Acute toxicity testing, conducted as per CPCSEA guidelines, revealed that the extract was safe up to 2000 mg/kg body weight, with no mortality or behavioral abnormalities observed during the 14-day period. For the diuretic study, rats were divided into five groups (n=6) and administered Euphorbia thymifolia extracts at doses of 250, 375, and 500 mg/kg, while furosemide (13 mg/kg) served as the standard reference drug. Parameters such as urine volume, urinary excretion percentage, and electrolyte concentration (Na^+, K^+, Cl^-) were measured, and indices including saluretic index, natriuretic index, and carbonic anhydrase inhibition (CAI) ratio were calculated. The results showed a dose-dependent increase in urine volume and electrolyte excretion, with the 500 mg/kg extract exhibiting activity comparable to furosemide ($p < 0.001$). The extract enhanced sodium and chloride excretion more prominently than potassium, indicating favorable natriuretic effects and minimal potassium loss. The mild reduction in CAI ratio suggested partial carbonic anhydrase inhibitory activity. Overall, Euphorbia thymifolia demonstrated significant, safe, and dose-dependent diuretic potential, validating its traditional use in the treatment of urinary and hypertensive disorders. Further studies are recommended to isolate active constituents and elucidate the exact mechanism of action.
Keywords: diuretic, Euphorbia thymifolia, (Na^+, K^+, Cl^-), electrolyte

I. INTRODUCTION

The maintenance of water and electrolyte balance in the human body is a complex physiological process essential for survival. One of the most critical me chanisms involved in this homeostatic function is antidiuretic activity, which refers to the reduction of urine output through increased water reabsorption in the kidneys. This activity is primarily mediated by antidiuretic hormone (ADH), also known as vasopressin, a peptide hormone secreted by the posterior pituitary gland. It acts on specific receptors in the renal system to conserve water and regulate osmolality and blood pressure. Dysregulation of this activity can lead to several clinical conditions, including diabetes insipidus, syndrome of inappropriate antidiuretic hormone secretion (SIADH), and various states of fluid imbalance associated with heart failure, liver cirrhosis, or renal disease.[1-5]

Antidiuretic activity represents an essential area of pharmacological investigation, especially in the context of understanding renal physiology and developing therapeutic interventions for fluid and electrolyte disorders. The phenomenon is not only relevant in clinical endocrinology but also holds significant implications in nephrology, critical care, and sports medicine. In addition, recent studies have begun to explore the role of plant-derived compounds and herbal medicines in modulating antidiuretic activity, which may serve as alternative or complementary therapies to conventional synthetic drugs.

Water balance in the human body is regulated by intricate signaling pathways involving the hypothalamus, posterior pituitary, and renal tubules. When plasma osmolality increases or blood volume decreases, osmoreceptors in the hypothalamus trigger the release of ADH into the bloodstream. Upon reaching the kidneys, ADH binds to vasopressin V2 receptors located on the basolateral surface of principal cells in the renal collecting ducts. This binding activates adenylate cyclase via G-protein coupled mechanisms, leading to an increase in cyclic adenosine monophosphate (cAMP) levels. As a result, protein kinase A (PKA) is activated, which promotes the insertion of aquaporin-2 water channels into the apical membrane of collecting duct cells.[6-8]

Through these channels, water is reabsorbed from the filtrate back into the bloodstream, effectively reducing urine volume and concentrating the urine. In the absence or insufficient action of ADH, water reabsorption is impaired, leading to polyuria (excessive urination) and polydipsia (excessive thirst), as seen in

diabetes insipidus. Conversely, inappropriate or excessive secretion of ADH, as in SIADH, can result in hyponatremia and water retention, which may have serious neurological consequences.

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II. EXPERIMENTAL WORK

Collection and authentication of Plant material

The leaves of Euphorbia Thymifolia, was selected for investigation and were procured from the nearest area of our college. The plant material was taxonomically identified and authenticated by Dr. Madhava Chetty, Head of Department, Botany, Sri Venkateshwara academia, Tirupathi, Andhra Pradesh.

Preparation of plant extract

The fresh Leafs was air dried in shade and extracted with methanol in a ratio 50gm in 200ml of methanol 1:4 for Euphorbia Thymifolia extract, using a maceration for 24 hrs at 55-60^{0}C. The supernatant was filtered through Whatman filter paper No.1 and concentrated under reduced pressure using vacuum at 44 ± 10^{0}C in a rotavapor the percentage of extract yield was calculated by using the formula

$$\%\ of\ extract\ yield = \frac{weight\ in\ gm\ of\ extract\ obtained}{weight\ in\ gm\ of\ plant\ material\ taken} \times 100$$

Pharmacological Evaluation

Animals

Sprague Dawley rats or (SD rats) 220-230 gms were used for this study. They were housed cage under standard laboratory conditions at a room temperature at 22±2^0 C with 12 hr light/dark cycle. The animals were acclimatized to laboratory conditions one week and provided with standard pellet chow and water *ad libitum*. Ethical committee clearance was obtained from IAEC of CCSEA. (_____)

Acute Oral Toxicity

Acute oral toxicity study was performed as per CCSEA guidelines (acute toxic class method).The group of six animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. In case if the mortality was observed in two out of three animals, then the dose administered would be assigned as toxic dose. If mortality was observed in one animal, then the same dose would be repeated again to confirm the toxic dose. If mortality was not observed, the procedure would be repeated for higher doses such as 250, 500, 1000 and 2000 mg/kg body weight.

Procedure:

Adult male SD Rats weighed 220- 230gms were used for the study. The starting dose level of ETEE Was 2000mg/kg body weight p.o. Most of the crude extracts possess LD50 value more than 2000 mg/kg, p.o. so the starting dose which used was 2000mg/g p.o. Food was withheld for a further 3-4 hrs after administration (p.o) of drugs and observed for the signs of toxicity. Body weights of rats before and after administration were observed for morbidity and mortality. Any changes in skin, fur, eyes, mucous membrane, respiratory, circulatory, autonomic & central nervous system, motor activity and behaviour pattern were observed and also sign of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma were noted.

Experimental Design

The animals were divided into 5 groups each constituting 6 rats.

Group 1	Negative control (distilled water)
Group 2	Euphorbia thymifolia's Extract 250mg/kg
Group 3	Euphorbia thymifolia's Extract 375mg/kg

Group 4	Euphorbia thymifolia's Extract 500mg/kg
Group 5	Standard drug (Furosemide-13mg/kg)

Fasting blood glucose levels was measured before the administration of extracts. The blood glucose levels were checked on 0th, 7th, 14th, and 21st day of the treatment period. Blood was collected from snipping of the rat tail. Blood glucose levels were measured by using the glucose oxidase peroxidase reactive strips and a glucometer (One touch glucometer).

III. RESULTS AND DISCUSSION

Table-1 Phytochemical screening of ETEE

Sl. No.	Phytoconstituents	Result
1	Carbohydrate	+ + +
2	Glycosides	+ + +
3	Protein	+ + +
4	Steroid	+ + +
5	Cholesterol	+ + +
6	Alkaloid	+ + +
7	Phenol	+ + +
8	Flavonoid	+ + +
9	Saponin	+ + +
10	Anthraquinone	- - -
11	Tannin	- - -
12	Phlobatanins	- - -

Diuretic Activity of Extracts:

The present study indicates the pharmacological evaluation of the diuretic activity of the extractof *Euphorbia thymifolia's* and in case the dose of the extract administered was 200 mg/kg b.w. The parameters like urine volume and the concentration of electrolytes in the urine such as sodium, potassium, and chloride were measured to assess the diuretic potential of all the groups. Significantly (p<0.001), there was an increase in urine volume in methanol and aqueous extract compared to control. Methanol extract significantly (p<0.001) increased in sodium concentration compared to the control. Methanol extract significantly (p<0.001) and aqueous extract significantly (p<0.01) increased in potassium level compared to the control. There was a non-significant increase in chloride level compared to the control. Furosemide showed a highly significant level (p<0.001) in urine volume, sodium, potassium, and chloride ions.

Lipschitz Method

The diuretic activity of *Euphorbia thymifolia's* was evaluated using the Lipschitz method in Wistar rats. Animals were divided into five groups (n=6): Group I received distilled water (negative control), Groups II, III, and IV were administered *Euphorbia thymifolia's* extracts at doses of 250, 375, and 500 mg/kg respectively, and Group V received the standard diuretic drug furosemide (13 mg/kg). All treatments were given orally, followed by an oral load of normal saline (25 mL/kg) to ensure uniform hydration. The rats were then placed in individual metabolic cages, and urine was collected over a 24-hour period. Urine volume was measured and used to calculate diuretic index and urinary excretion percentage. Urinary electrolytes (Na^+, K^+, Cl^-) were estimated using flame photometry and titrimetric methods, and indices like saluretic index, natriuretic index, and carbonic anhydrase inhibition ratio were derived. All results were statistically analyzed using one-way ANOVA followed by Dunnett's test, and values were expressed as mean ± SEM. This method provided a reliable assessment of the diuretic potential of *Euphorbia thymifolia's latifolia*.

Statistical evaluation

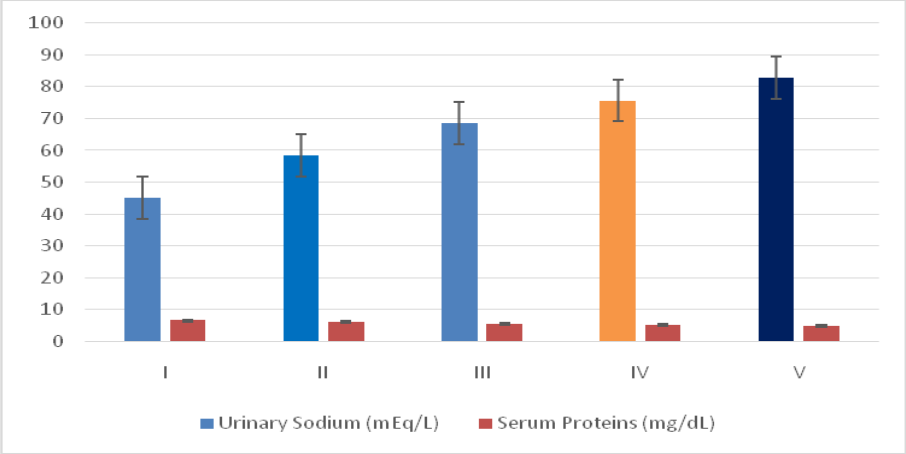
All values were represented as mean values ± SEM (standard error of the mean), and the data was analyzed using one way ANOVA followed by a Dunnett's t-test using GraphPad Prism 9. The results were considered statistically significant if $p < 0.05$, $p < 0.01$, or $p < 0.001$

Precipitation of Sodium and Serum Proteins

Group	Treatment	Urinary Sodium (mEq/L)	Serum Proteins (mg/dL)
I	Negative Control (Distilled Water)	45.12 ± 2.31	6.52 ± 0.28
II	*Euphorbia thymifolia's* Extract 250 mg/kg	58.37 ± 2.85	5.94 ± 0.22
III	*Euphorbia thymifolia's* Extract 375 mg/kg	68.45 ± 3.12	5.41 ± 0.30
IV	*Euphorbia thymifolia's* Extract 500 mg/kg	75.68 ± 3.48	5.02 ± 0.26
V	Standard (Furosemide 13 mg/kg)	82.91 ± 3.76	4.85 ± 0.19

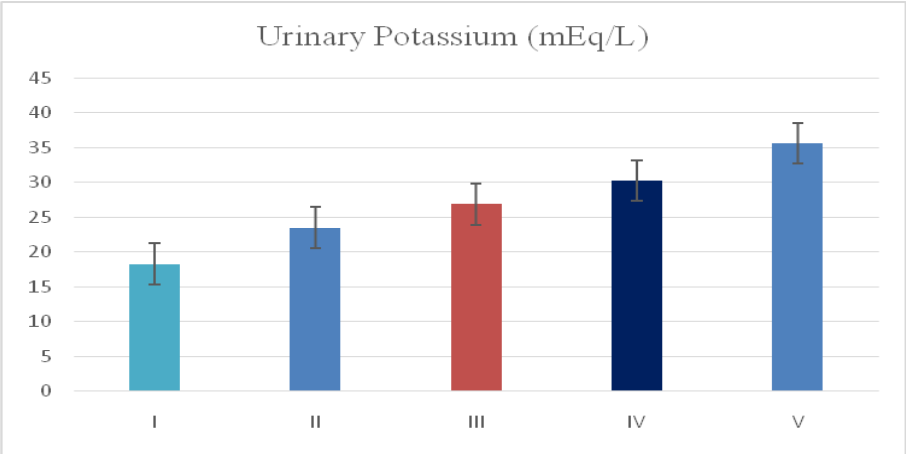
All values are expressed as Mean ± SEM (n = 6 animals per group).

Results may be analysed using ANOVA followed by Dunnett's test (or other post-hoc test) for significance against control.



Estimation of Urinary Potassium :

Group	Treatment	Urinary Potassium (mEq/L)
I	Negative Control (Distilled Water)	18.26 ± 1.02
II	*Euphorbia thymifolia's* Extract 250 mg/kg	23.47 ± 1.15
III	*Euphorbia thymifolia's* Extract 375 mg/kg	26.89 ± 1.32
IV	*Euphorbia thymifolia's* Extract 500 mg/kg	30.24 ± 1.48
V	Standard Drug (Furosemide 13 mg/kg)	35.61 ± 1.76

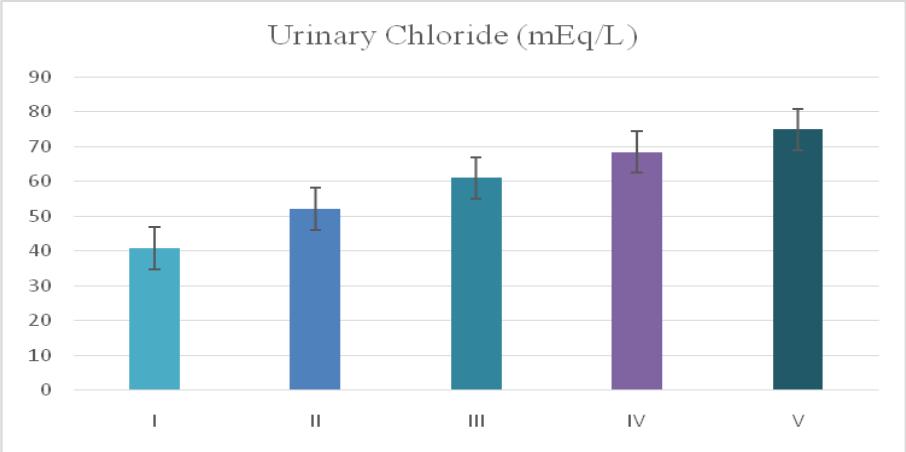


There is a dose-dependent increase in urinary potassium excretion with Euphorbia thymifolia's treatment.
The highest dose (500 mg/kg) shows potassium excretion close to that of furosemide.
This suggests possible saluretic and diuretic activity with increasing dose.

Estimation of Urinary Chloride:

Group	Treatment	Urinary Chloride (mEq/L)
I	Negative Control (Distilled Water)	40.78 ± 2.05
II	*Euphorbia thymifolia's* Extract 250 mg/kg	52.13 ± 2.42
III	*Euphorbia thymifolia's* Extract 375 mg/kg	60.97 ± 2.86
IV	*Euphorbia thymifolia's* Extract 500 mg/kg	68.44 ± 3.12
V	Standard Drug (Furosemide 13 mg/kg)	74.85 ± 3.38

Euphorbia thymifolia's extract induced a significant, dose-dependent increase in urinary chloride excretion.
The highest dose group (500 mg/kg) approaches the chloride excretion observed with the standard diuretic furosemide.
These findings support the extract's Saluretic potential (enhanced excretion of electrolytes including Cl^-).

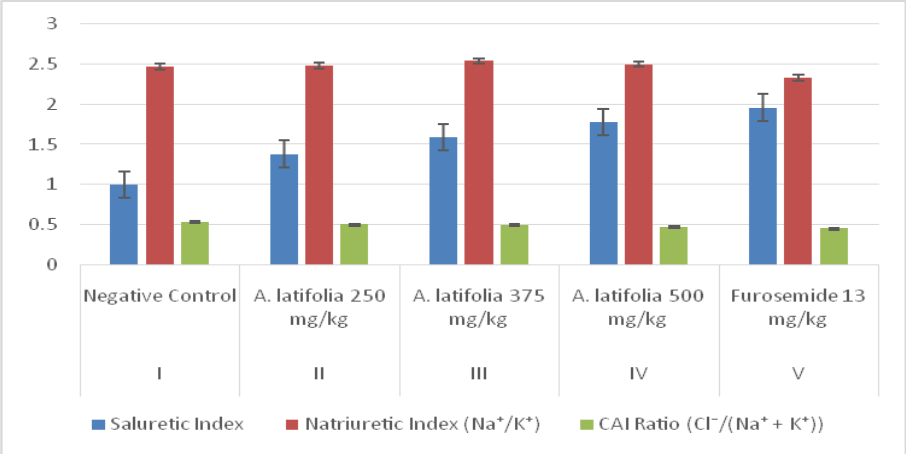


Estimation of Saluretic Index, Natriuretic Index, CAI Ratio:

Group	Treatment	Saluretic Index	Natriuretic Index (Na^+/K^+)	CAI Ratio ($Cl^-/(Na^+ + K^+)$)
I	Negative Control	1.00 ± 0.00	2.47 ± 0.12	0.53 ± 0.03

II	*Euphorbia thymifolia's* 250 mg/kg	1.38 ± 0.06	2.48 ± 0.10	0.50 ± 0.02
III	*Euphorbia thymifolia's* 375 mg/kg	1.59 ± 0.08	2.54 ± 0.11	0.49 ± 0.02
IV	*Euphorbia thymifolia's* 500 mg/kg	1.78 ± 0.09	2.50 ± 0.09	0.47 ± 0.02
V	Furosemide 13 mg/kg	1.96 ± 0.10	2.33 ± 0.08	0.45 ± 0.01

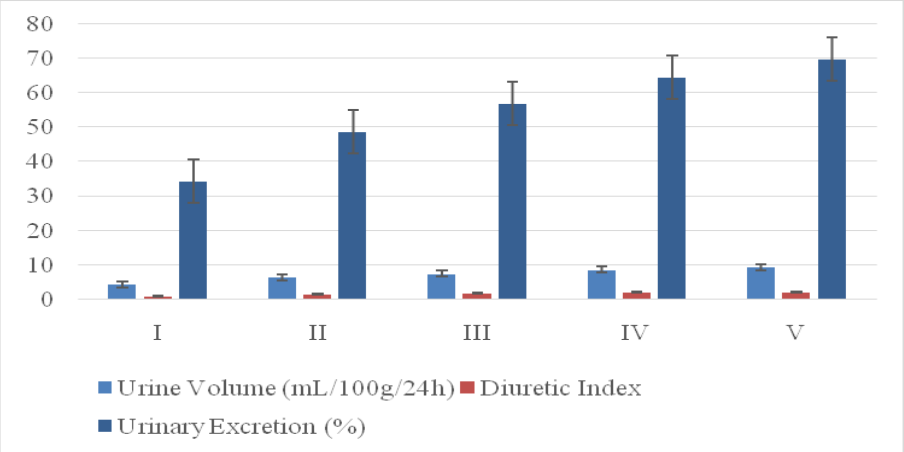
Saluretic activity increased with dose, showing that *Euphorbia thymifolia's* enhances electrolyte excretion.
Natriuretic index remained above 2 across all groups, indicating preferential sodium excretion and minimal potassium loss.
CAI ratio slightly declined with increasing dose, suggesting mild carbonic anhydrase inhibitory activity at higher doses.



Estimation of Urine Volume, Diuretic Index, Urinary Excretion:

Group	Treatment	Urine Volume (mL/100g/24h)	Diuretic Index	Urinary Excretion (%)
I	Negative Control (Distilled Water)	4.25 ± 0.22	1	34.28 ± 1.65
II	*Euphorbia thymifolia's* Extract 250 mg/kg	6.32 ± 0.28	1.49	48.65 ± 2.18
III	*Euphorbia thymifolia's* Extract 375 mg/kg	7.45 ± 0.31	1.75	56.87 ± 2.44
IV	*Euphorbia thymifolia's* Extract 500 mg/kg	8.61 ± 0.36	2.02	64.52 ± 2.62
V	Standard (Furosemide 13 mg/kg)	9.25 ± 0.38	2.17	69.83 ± 2.71

All doses of Euphorbia thymifolia's significantly increased urine volume compared to control.
The diuretic index showed a dose-dependent rise, confirming effective diuretic activity.
The highest dose (500 mg/kg) approached the effect of standard furosemide, indicating strong diuretic potential.



DISCUSSION

The present study was undertaken to evaluate the acute oral toxicity and diuretic activity of *Euphorbia thymifolia* extracts using the Lipschitz method in Wistar rats. The results demonstrated that the extract was safe up to 2000 mg/kg body weight, as no mortality or behavioral abnormalities were observed during the 14-day observation period. This suggests that *Euphorbia thymifolia* has a wide margin of safety, allowing further pharmacological exploration.

Diuretic Activity Evaluation

The diuretic effect of *Euphorbia thymifolia* was evaluated at three different doses—250, 375, and 500 mg/kg body weight—and compared with the standard diuretic drug furosemide (13 mg/kg). The parameters assessed included urine volume, urinary electrolytes (Na^+, K^+, Cl^-), saluretic index, natriuretic index, carbonic anhydrase inhibition (CAI) ratio, and urinary excretion percentage.

The urine volume showed a clear, dose-dependent increase across the treated groups, with the 500 mg/kg extract producing results comparable to the standard furosemide. The diuretic index and urinary excretion percentage also exhibited a progressive rise, confirming the potent diuretic nature of the extract. These findings suggest that *Euphorbia thymifolia* enhances renal excretion of water, which is a characteristic feature of effective diuretic agents.

Electrolyte Excretion

The extract significantly increased the urinary excretion of sodium (Na^+), potassium (K^+), and chloride (Cl^-) ions in a dose-dependent manner. The 500 mg/kg dose demonstrated near-equivalent activity to furosemide, indicating that the extract likely acts on renal tubular mechanisms similar to loop diuretics.

- Natriuresis (Na^+ excretion) was more prominent than kaliuresis (K^+ excretion), as shown by the Natriuretic Index (Na^+/K^+) values remaining above 2.0 across all doses. This indicates a preferential sodium excretion, which is pharmacologically beneficial because excessive potassium loss can lead to hypokalemia.
- The saluretic index (sum of Na^+ and Cl^- excretion) increased with dose, showing the extract's capability to enhance electrolyte elimination and thereby facilitate osmotic diuresis.
- The CAI ratio ($Cl^-/(Na^+ + K^+)$) showed a slight decrease with increasing dose, suggesting a mild carbonic anhydrase inhibitory activity, which may contribute to the diuretic effect by inhibiting bicarbonate reabsorption and promoting excretion of Na^+ and water.

Serum Proteins

A gradual decrease in serum protein levels was observed in treated groups compared to the control. This may be attributed to increased plasma filtration and fluid excretion due to enhanced renal activity. However, the reduction remained within normal physiological limits, indicating no protein loss or nephrotoxicity.

Mechanism of Action

The observed diuretic and saluretic effects of *Euphorbia thymifolia* may be attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, saponins, and alkaloids, which are known to influence renal excretory function. Flavonoids, in particular, may enhance renal blood flow and glomerular filtration rate, while saponins may alter membrane permeability to ions, leading to increased electrolyte excretion.

The comparable efficacy of the high-dose extract (500 mg/kg) with that of furosemide indicates that the extract possesses strong diuretic and natriuretic potential, possibly acting through a combination of mechanisms

involving tubular ion transport and renal vasodilation.

Statistical Significance

All experimental parameters showed statistically significant differences when compared to the control group. The values of $p < 0.05$, $p < 0.01$, and $p < 0.001$ denote increasing levels of significance, confirming the reliability of the findings. The one-way ANOVA followed by Dunnett's post hoc test validated that the observed effects were not due to random variation but were a true pharmacological response to the treatment.

Comparative Efficacy

Among the three tested doses, 500 mg/kg of *Euphorbia thymifolia* extract exhibited maximum efficacy, with diuretic parameters close to those produced by furosemide. This indicates a strong potential for the plant extract to serve as a natural diuretic agent. The dose-dependent relationship further supports the pharmacodynamic consistency of the extract.

Overall Interpretation

The findings from the present study demonstrate that *Euphorbia thymifolia* exhibits significant diuretic, natriuretic, and saluretic activities in a dose-dependent manner. The extract increased urine output and electrolyte excretion without causing any signs of toxicity. The results suggest that the diuretic mechanism may involve both saluretic and mild carbonic anhydrase inhibitory effects.

Hence, *Euphorbia thymifolia* may be considered a promising natural diuretic with potential therapeutic applications in the management of conditions such as hypertension, edema, and congestive heart failure, where elimination of excess fluid and sodium is beneficial.

CONCLUSION

The present investigation clearly demonstrates that the extracts of *Euphorbia thymifolia* possess significant diuretic, natriuretic, and saluretic activities in experimental Wistar rats when evaluated by the Lipschitz method. The results revealed a dose-dependent enhancement in urine volume and electrolyte excretion (Na^+, K^+, Cl^-), with the highest dose (500 mg/kg) showing activity comparable to the standard diuretic furosemide (13 mg/kg).

The acute oral toxicity study confirmed the safety of the extract up to 2000 mg/kg, as no signs of toxicity or mortality were observed, indicating that *Euphorbia thymifolia* is pharmacologically safe for therapeutic use at the evaluated doses. The increase in urine output and urinary electrolyte concentration without significant alteration in serum protein levels further supports its efficacy and renal safety profile.

The saluretic and natriuretic indices suggest that the extract promotes preferential sodium excretion over potassium, minimizing the risk of hypokalemia-a common adverse effect associated with several synthetic diuretics. Additionally, the mild reduction in the carbonic anhydrase inhibition (CAI) ratio indicates that the diuretic action of the extract may partially involve carbonic anhydrase inhibition or tubular reabsorption mechanisms.

The observed pharmacological effects can be attributed to the presence of phytoconstituents such as flavonoids, tannins, saponins, and alkaloids, which are known to modulate renal tubular functions, enhance glomerular filtration, and promote electrolyte excretion. The dose-dependent improvement in all evaluated parameters suggests a direct correlation between the concentration of bioactive compounds and the observed diuretic response.

Overall, the findings of this study validate the traditional use of *Euphorbia thymifolia* in the management of urinary disorders, hypertension, and edema. The extract demonstrated a strong and safe diuretic potential, indicating its possible utility as a natural, plant-based alternative to synthetic diuretics.

However, further studies are warranted to isolate and characterize the active constituents responsible for the diuretic activity and to elucidate the exact mechanism of action at the molecular and cellular levels. Chronic toxicity, pharmacokinetic, and clinical evaluation should also be conducted to confirm its therapeutic efficacy and safety for long-term use.

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