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RESEARCH ARTICLE

Herbal Diuresis: Investigating the Efficacy of *Wedelia chinensis* Leaf Extracts in Rats

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Abstract

Background: Herbal drugs are currently being explored for pharmacological efficacy. They are relatively safe and have fewer side effects. **Objective:** The objective of this research was to explore the diuretic effects of the extracts of the leaves of *Wedelia chinensis* (Osbeck) Merill, a member of the Asteraceae family, using an acute rat model. **Methods:** Dehydrated rats were administered single doses of the extracts, prepared in both aqueous and ethanol forms, at doses of 200 and 400 mg/kg orally. Additionally, rats were orally administered 25 mg/kg of frusemide and hydrochlorothiazide as standard diuretic drugs, for comparative analysis. A control group received oral administration of normal saline at a dose of 25 ml/kg. The metabolic cages were used to house the rats in pairs and urine output was measured at intervals of 5 and 24 hours. **Results:** Results indicated a significant increase in urine volume output with higher doses of both extracts. This diuretic effect was observed gradually within 5 hours and sustained throughout the 24-hour study period. Furthermore, the 400 mg/kg dose of aqueous extract exhibited a diuretic effect comparable to that of frusemide. Analysis of the urine samples revealed a significantly elevated Na^+ and K^+ with the aqueous extract of *W. chinensis* leaves. However, neither dose of the extracts significantly altered the pH nor specific gravity of urine during routine urinalysis. **Conclusion:** The findings suggest that extracts from *W. chinensis* leaves possess diuretic properties, supporting their traditional use in managing urinary problems. Further exploration is necessary to pinpoint the active phytoconstituents accountable for these observed effects.

Keywords: Diuretics, Frusemide, Hydrochlorothiazide, Urine, *Wedelia chinensis*

1. Introduction

Diuretics represent a class of medicinal agents aimed at augmenting urine output and modifying the composition and volume of body fluids, pivotal in managing various medical conditions such as hypercalciuria, liver cirrhosis and acute and chronic renal failure. Drug-induced diuresis plays a vital role in addressing life-threatening illnesses, notably including pregnancy toxaemia, nephritis, congestive cardiac failure and hypertension[1]. Commonly utilised diuretics in clinical practice encompass mannitol, thiazides, frusemide and spironolactone[2].

Despite their therapeutic efficacy, many diuretic drugs are associated with a spectrum of adverse effects. These may include metabolic alterations, shifts in electrolyte levels, the potential onset of diabetes, stimulation of the renin-angiotensin and neuroendocrine systems, as well as diminished sexual function. Such considerations underscore the importance of continued research into diuretic agents with improved safety profiles and efficacy to enhance patient outcomes[3].

In the current context, there is a growing demand for innovative diuretic treatments, particularly those derived from natural sources such as plants. These natural agents are perceived to offer safer alternatives with potentially fewer side effects. Traditional texts, notably those in Sanskrit associated with Ayurvedic

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medicine, have documented numerous native plants known for their urinary-stimulating properties.

Despite extensive research exploring the diuretic potential of medicinal plants, there remains a gap in the availability of truly satisfying, safe and efficacious diuretic drugs. While scientific studies have sought to establish the reliability of these plant-based diuretics, further development is still needed to meet the demands for effective and well-tolerated treatment options in this domain.

Wedelia chinensis (Osbeck) Merill, belonging to the Asteraceae family, is commonly known as '*Pilabhamgara*' or '*Bhringraj*' and holds a prominent place in various traditional medicine systems such as Ayurveda, Siddha, Chinese, and Unani[4]. Traditional wisdom suggests that this plant offers notable therapeutic benefits in managing ulcers, central nervous system disorders, cancer, inflammation and wound healing[5]. Its historical use spans across treating alopecia, colds, amenorrhea, hair colouring, skin diseases and renal failure. In the tribal communities of Kolli Hills, Namakkal, Tamil Nadu, India, the decoction of *W. chinensis* has been utilised to induce sleep, alleviate mental tension and reduce anxiety[6,7].

The leaves of *W. chinensis* contain a rich array of phytoconstituents, including flavonoids, saponins, triterpenoids, alkaloids, tannins, carotenes, phytosterols and coumestans, as evidenced by scientific studies[8,9]. Moreover, scientific literature attests to its diverse pharmacological activities, including anticancer, anxiolytic, anticonvulsant, antistress, antioxidant, hepatoprotective, wound healing, analgesic and anti-inflammatory properties[10].

However, despite the extensive exploration of its therapeutic potential, scientific research regarding the diuretic effects of *W. chinensis* remains scarce. Therefore, this study was undertaken to explore the diuretic action of *W. chinensis* in rats, unveiling a previously uninvestigated pharmacological aspect.

2. Materials and Methods

2.1 Collection of *Wedelia chinensis* Leaves

The leaves of *W. chinensis* were procured from the Solapur region of Maharashtra in February 2021. Dr D. L. Shirodkar, a research scientist at the Botanical Survey of India, Pune, Maharashtra, led the process of authentication (BSI/WRC/Iden. Cer./2021/0208210003912 dated August 2, 2021; Specimen No.: MSSSC2).

2.2 Preparation of Extracts

250 grams of coarsely powdered leaves of *W. chinensis* were extracted with absolute ethanol using a continuous hot extraction process to obtain the ethanolic extract. Another batch of 250 grams of coarsely powdered leaves underwent a cold maceration technique in water for seven days to prepare the aqueous extract.

The two extracts were concentrated individually under reduced pressure employing a rotary flash evaporator. Subsequently, the residue was dried over sodium sulfate using a desiccator. The percentage yield of the ethanolic and aqueous extracts was determined to be 13.83 and 11.75, respectively. The aqueous and ethanolic extracts were suspended in aqueous sodium carboxymethylcellulose (0.5% w/v) solution for oral administration in rats.

2.3 Qualitative Chemical Analysis

Each extract underwent phytochemical analysis to confirm the presence of primary and secondary metabolites in *W. chinensis* leaf.

2.4 Chemicals

Indoco Remedies Ltd. of Navi Mumbai, Maharashtra, India provided a complimentary sample of frusemide and hydrochlorothiazide. The urine test strips-UroColor 10 were procured from Abbott Diagnostics Korea Inc. and used for analysing the urine.

2.5 Animals

Healthy inbred male Wistar rats (150–200 g) from Crystal Biological Solutions, Pune, Maharashtra were used in the study. Rats were placed on a light-dark cycle of 12:12 hrs after being acclimated to laboratory conditions. They were supplied with a standard rat diet and free access to drinking water. The Ethics Committee of Biocyte Institute of Research and Development, Sangli, Maharashtra approved the research protocol (CPCSEA Registration no.: IAEC/Sangli.2020-21/19).

2.6 Acute Oral Toxicity Study

In accordance with the Organisation for Economic Cooperation and Development Guidelines No. 423, a

study on acute oral toxicity was done[11]. The effective therapeutic dose was calculated based on a threshold value of the median fatal dose (LD_{50}).

2.7 Diuretic Activity

An investigation of the diuretic activity was carried out using the methodology recommended by Lipschitz *et al.* Seven groups of rats, each consisting of six individuals, followed an 18-hour fasting period during which they were deprived of water before the experiment. The control group, designated as Group I, was administered the vehicle at a dose of 25 ml/kg. Group II and Group III administered frusemide and hydrochlorothiazide (each 25 mg/kg) in the form of vehicle as standard diuretic drugs[12,13]. Groups IV and V were, respectively given an aqueous extract of *W. chinensis* leaves at 200 and 400 mg/kg dose. Groups VI and VII received ethanolic extract of *W. chinensis* leaves (200 and 400 mg/kg, respectively).

2.8 Collection and Analysis of Urine

Urine samples were collected from a pair of rats housed in metabolic cages at 5-hour and 24-hour intervals upon administration. The UroColor test strips (Abbott Diagnostics Korea Inc.) were used to carry out routine urinalysis from urine samples in rats treated with extracts and control. The test comprised measurement of pH and specific gravity, in addition to the detection of proteins, glucose, bilirubin, urobilinogen, nitrite, occult blood, leucocytes and ketone bodies. The levels of Na^+, K^+ and Cl^- ions (measured in milliequivalents per litre per 100 grammes) in urine, as well as the volume of urine (measured in millilitres per 100 grammes), were determined[14], and various parameters for diuretic effect were calculated by using following equations[15,16].

$$\text{Diuretic Index} = \frac{\text{Volume of urine excreted by the test group}}{\text{Volume of urine excreted by the control group}}$$

$$\text{Saluretic Index} = \frac{\text{Electrolyte excretion in the test group}}{\text{Electrolyte excretion in the control group}}$$

$$\text{Natriuretic Index} = \frac{\text{Excretion of Na in the urine}}{\text{Excretion of K in the urine}}$$

$$\text{Ion Quotient} = \frac{\text{Excretion of Cl in the urine}}{\text{Excretion of Na and K in the urine}}$$

2.9 Statistics

The results are expressed in mean ± SEM, Oneway Annova followed by Tukey's post hock test. The $P< 0.05$ was considered statistically significant.

3. Results

The qualitative chemical investigation of *W. chinensis* leaf extracts showed the presence of tannins, anthraquinones, flavonoids, volatile oils, steroids, terpenoids and carbohydrates (Table 1). Urination and defecation increased in rats when aqueous and ethanolic extracts of *W. chinensis* were administered orally at an initial dose of 2000 mg/kg. No mortality was observed at the initial dose. Two rats per group, however, perished when the confirmatory dose of both extracts was administered. Additionally, it was observed that administering the aqueous and ethanolic extracts of *W. chinensis* at a lower dose (300 mg/kg, p.o.) resulted in no mortality rate and enhanced safety. Therefore, the therapeutic doses for both extracts were determined by dividing the LD 50 cut-off dose (2000 mg/kg) by $1/10^{th}$ and $1/5^{th}$, respectively.

When compared to the untreated control group, the reference diuretic drugs frusemide and hydrochlorothiazide caused water excretion levels of more than 100% (Table 2). The urinary output was markedly enhanced by both extracts in a dose-dependent way. Comparable to reference diuretic drugs, a gradual peak in diuretic action was seen during the first 5 hours. Urine excretion volume was considerably ($P < 0.001$) increased for up to 24 hours

Table 1. Qualitative chemical analysis of *W. chinensis* leaf extracts

Metabolites	AqE	EtE
Carbohydrates	+	-
Steroids	-	+
Terpenoids	-	+
Volatile oils	-	+
Anthraquinones	+	+
Flavonoids	+	+
Tannins	+	+

'+' Present; '-' Absent

(Figure 1). Aqueous extract at 400 mg/kg induced diuresis in 5 hours which was almost identical to hydrochlorothiazide and frusemide (Figure 1). The aqueous extract had a greater diuretic index than the ethanolic extract (Table 2).

The reference diuretic drugs exhibited a substantial ($P < 0.001$) increase in the urinary excretion of ions between 20% and 92% for 24 hours when compared to the control group (Table 4). Urinary Na^+ and Cl^- levels were significantly elevated by *W. chinensis* leaf extracts. The changes in kaliuretic action with aqueous extract were not significant, with the exception of ethanolic extract (400 mg/kg) which showed a substantial ($P < 0.01$) increase in K^+ excretion in the urine. Over the course of a day, the aqueous extract at 400 mg/kg indicated a substantial ($P < 0.001$) increase in Na^+ excretion compared to the control group. While administering ethanolic extract caused a significant amount of diuresis, the increase in urine electrolyte excretion was less than 10%, in contrast, when compared to the control group. Also, aqueous extract treatment resulted in an approximately 10 to 75% increase in urine electrolyte excretion (Tables 3 and 4). Additionally, an aqueous extract of 400 mg/kg showed an apparent saluretic action (Table 4). The aqueous extract revealed the presence of traces of protein in the urine at both doses. The remaining groups did not exhibit proteinuria or glucosuria (Table 5).

4. Discussion

An increase in the electrolyte excretion in the urine along with a net rise in urine volume are the two primary mechanisms responsible for diuresis[17]. These mechanisms are facilitated by the inhibition of tubular reabsorption, preventing the reuptake of electrolytes and water back into circulation. Frusemide increases urinary output and sodium excretion by obstructing the symporter of $Na^+/K^+/Cl^-$ thick ascending limb.

Table 2. Effect of leaf extracts of *W. chinensis* on urinary excretion of rats

Groups	Dose (mg/kg, p.o.)	5 hrs		24 hrs	
		Excretion of Urine (ml/100 gm)	Diuretic Index	Excretion of Urine (ml/100 gm)	Diuretic Index
Control	-	0.25 ± 0.03	1.00	1.20 ± 0.12	1.00
Frusemide	25	0.55 ± 0.04***	2.20	3.04 ± 0.29**	2.53
Hydrochlorothiazide	25	0.53 ± 0.06*	2.12	2.80 ± 0.36**	2.33
AqE	200	0.39 ± 0.08*	1.56	2.50 ± 0.28**	2.08
AqE	400	0.51 ± 0.06***	2.04	2.80 ± 0.22***	2.33
EtE	200	0.37 ± 0.13^{ns}	1.48	2.20 ±0.56^{ns}	1.83
EtE	400	0.43 ± 0.07*	1.72	2.50 ±0.31**	2.08

The results are given in mean ± S.E.M (n=6); **P<0.05, **P<0.01, ***P<0.001, ^{ns}P>0.05* vs control.

Table 3. Effect of leaf extracts of *Wedelia chinensis* on electrolyte excretion in urine over 5 hours

Groups	Dose (mg/kg, p.o.)	Concentration of Ions (mEq/l/100 g)			Saluretic Index			Na^+/K^+	Ion Quotient
		Na^+	K^+	Cl^-	Na^+	K^+	Cl^-		
Control	-	0.41 ± 0.05	0.17 ± 0.02	0.63 ± 0.04	1.00	1.00	1.00	2.41	1.08
Frusemide	25	0.76 ± 0.04**	0.30 ± 0.01**	1.07 ± 0.04***	1.85	1.76	1.70	2.53	1.01
Hydrochlorothiazide	25	0.83 ± 0.09**	0.32 ± 0.05*	1.02 ± 0.08**	2.02	1.88	1.62	2.59	0.88
AqE	200	0.62 ± 0.07*	0.21 ± 0.07^{ns}	0.79 ± 0.03*	1.51	1.23	1.25	2.95	0.95
AqE	400	0.71 ± 0.03**	0.28 ± 0.04*	0.76 ± 0.05*	1.73	1.65	1.21	2.53	0.77
EtE	200	0.42 ± 0.04^{ns}	0.21 ± 0.04^{ns}	0.71 ± 0.03^{ns}	1.02	1.23	1.13	2.00	1.12
EtE	400	0.51 ± 0.06^{ns}	0.25 ± 0.03^{ns}	0.80 ± 0.04*	1.24	1.47	1.27	2.04	1.05

The results are given in mean ± S.E.M (n=6); **P<0.05, **P<0.01, ***P<0.001, ^{ns}P>0.05* vs control.

Table 4. Effect of leaf extracts of *Wedelia chinensis* on electrolyte excretion in urine over 24 hours

Groups	Dose (mg/kg, p.o.)	Concentration of Ions (mEq/l/100 g)			Saluretic Index			Na^+/K^+	Ion Quotient
		Na^+	K^+	Cl^-	Na^+	K^+	Cl^-		
Control	-	2.83 ± 0.14	1.07 ± 0.03	3.70 ± 0.04	1.00	1.00	1.00	2.64	0.95
Frusemide	25	5.40 ± 0.22***	1.48 ± 0.08*	5.52 ± 0.22***	1.90	1.38	1.49	3.65	0.80
Hydrochlorothiazide	25	4.99 ± 0.17***	1.29 ± 0.05ns	4.99 ± 0.14***	1.76	1.21	1.34	3.88	0.79
AqE	200	3.91 ± 0.21**	1.05 ± 0.08ns	4.18 ± 0.13**	1.38	0.98	1.13	3.72	0.84
AqE	400	5.00 ± 0.31***	1.17 ± 0.05ns	4.96 ± 0.25**	1.76	1.09	1.34	4.27	0.80
EtE	200	3.14 ± 0.25ns	1.20 ± 0.06*	3.62 ± 0.19ns	1.11	1.12	0.98	2.62	0.83
EtE	400	3.31 ± 0.22ns	1.31 ± 0.05**	3.99 ± 0.07*	1.17	1.22	1.08	2.52	0.86

The results are given in mean ± S.E.M (n=6); **P<0.05, **P<0.01, ***P<0.001, nsP>0.05* vs control.

Table 5. Effect of *W. chinensis* leaf extracts on routine urine analysis

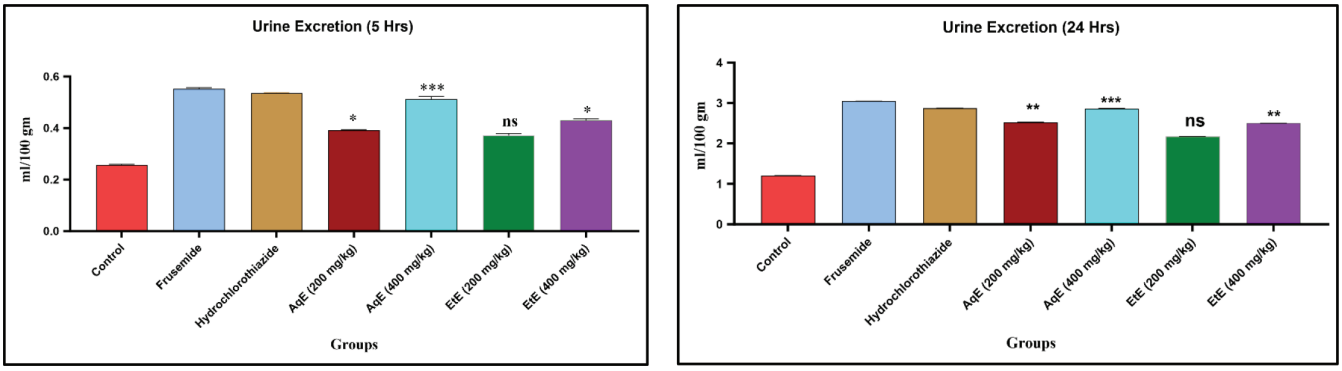
Groups	Dose (mg/kg)	pH	Specific gravity	Glucose	Protein
Control	--	6.89 ± 0.13	1.01 ± 0.001	-	-
AqE	200	7.15 ± 0.21	1.019 ± 0.003	-	+
	400	7.01 ± 0.23	1.016 ± 0.004	-	+
EtE	200	7.03 ± 0.23	1.028 ± 0.002	-	-
	400	6.92 ± 0.20	1.024 ± 0.003	-	-

The results are given in mean ± S.E.M (n=6); '-' Absent, '+' Present

Thiazides enhance sodium and chloride excretion by competing for the chloride binding site in the distal convoluted tubule[17].

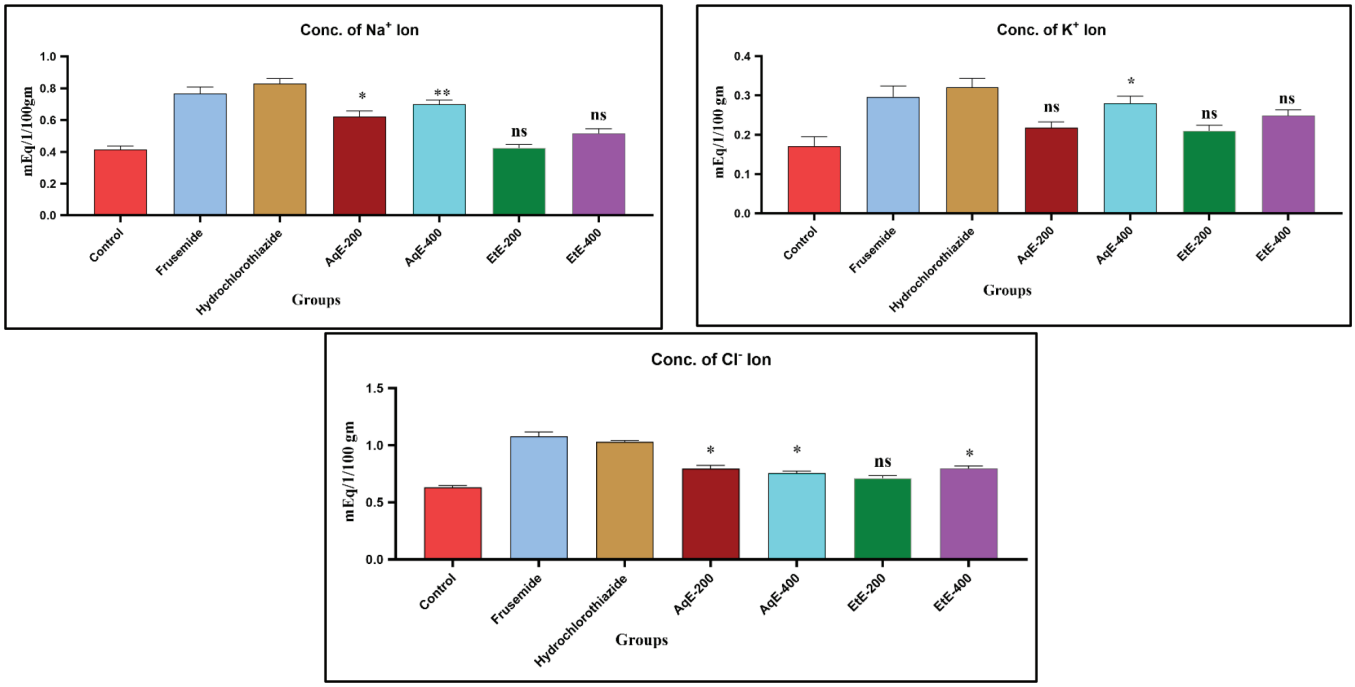
In this study, rats administered with frusemide and hydrochlorothiazide at a dose of 25 mg/kg had significant diuresis over 24 hours. In comparison, the aqueous extract and ethanolic extract of *W. chinensis* showed a similar increase in urine output when given orally in dehydrated rats (Table 2, Figure 1). However, there may be a variation in the relative diuretic profiles of both extracts of *W. chinensis* based on different findings on electrolytic excretion. The gradual and dose-dependent excretion of Na^+, K^+, and Cl^- was observed in both the aqueous and ethanolic extracts (Tables 3 and 4).

In particular, the ethanolic extract showed a small increase in K^+ levels in the urine (Figures 2 and 3). Further, no alkalisation of urine was observed (Table 5). According to the natriuretic index, these findings collectively imply that the aqueous extract acts as potassium-sparing diuretics[18–20]. While thiazide diuretics, such as hydrochlorothiazide, solely elevate urinary K^+ levels, they do modify urinary Na^+/K^+ ratio[18,19], the aqueous extract caused elevated concentrations of urinary Na^+, K^+, and Cl^-, with no substantial change found in a ratio of Na^+/K^+ (Table 3 and 4). The activity of aqueous extract indicates its mode of action, such as that of thiazides or loop diuretics, is not likely to predict. Moreover, it is evident from the results that aqueous extract, especially when administered at 400 mg/kg dose, in addition to its saluretic activity possibly has a kaliuretic action. Moreover, the aqueous extract of *W. chinensis* leaves induced significantly increased Na^+ and K^+ levels in urine together with an intensity of aquaresis identical to that of furosemide and hydrochlorothiazide. Furthermore, there were no significant variations in urine pH. These observations suggest that the aqueous extract exhibits characteristics resembling a loop diuretic rather than a thiazide diuretic, primarily due to its potent capacity to enhance sodium, potassium, and chloride excretion. Loop diuretics predominantly



The results are given in mean ± S.E.M (n=6); **P<0.05, **P<0.01, ***P<0.001, ^{ns}P>0.05* vs control.

Figure 1. Total excretion of urine (ml/100 gm) in the control, standard, and experimental groups over a period of 5 and 24 hours.

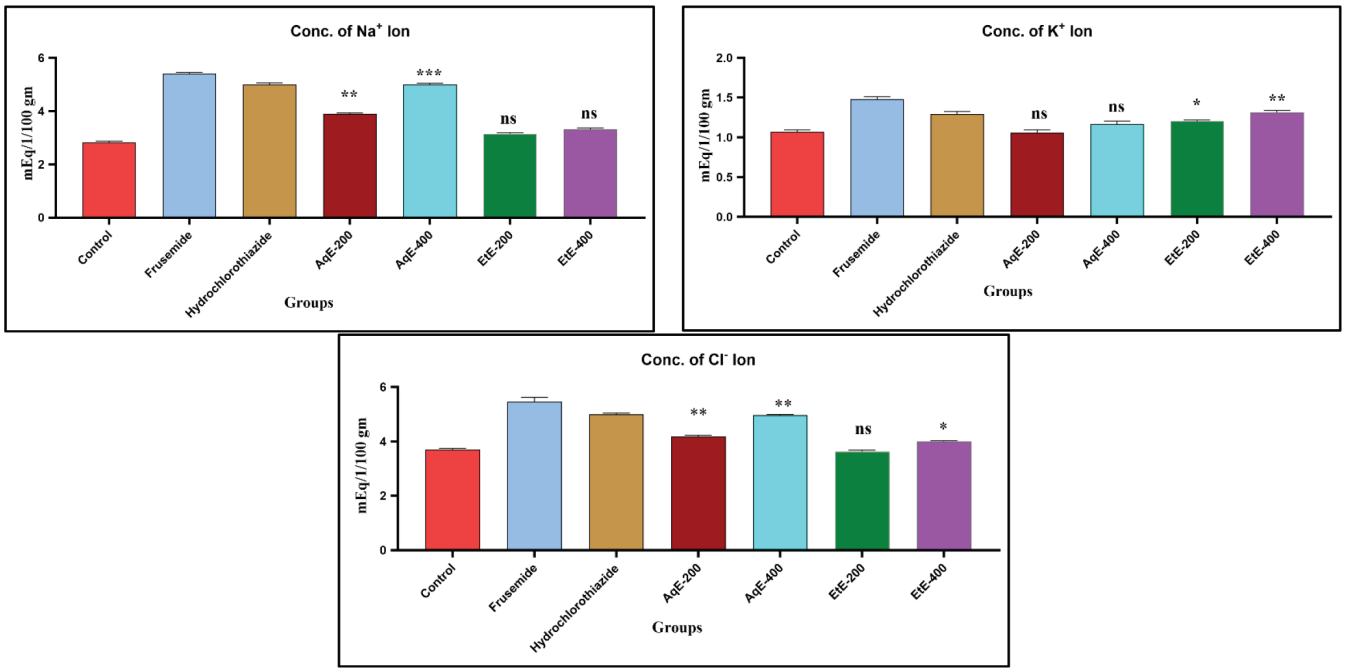


The results are given in mean ± S.E.M (n=6); **P<0.05, **P<0.01, ***P<0.001, ^{ns}P>0.05* vs control.

Figure 2. Effect of *W. chinensis* leaf extracts on urinary excretion of electrolytes in 5 hours urine.

inhibit $Na^+/K^+/Cl^-$ symporter in the thick ascending limb of the Loop of Henle, leading to increased excretion of sodium and potassium[18–20]. However, considerable urine acidification is also seen when using these diuretics[18,19,21]. The ion quotient, observed in the experimental groups and ranging from 0.8 to 1.0, presents convincing evidence directly linking them to the inhibition of carbonic anhydrase[12].

According to clinically used loop diuretics, the effect of the extracts was gradually initiated until the first 5 hrs after dose administration[18,19]. It's interesting to point out that rats treated with aqueous and ethanolic extracts showed significantly lower urine osmolarity despite having considerable losses in urinary Na^+ and K^+. Considering this, it can be noted that polyurea with low osmolarity is caused by ADH inhibition[21,22]. It is likely that the diuretic effects induced by both extracts in this study were also triggered by decreased ADH levels and/or sensitivity of the uriniferous tubules.



The results are given in mean ± S.E.M (n=6); **P<0.05, **P<0.01, ***P<0.001, nsP>0.05* vs control.

Figure 3. Effect of *W. chinensis* leaf extracts on urinary excretion of electrolytes in 24-hour urine.

5. Conclusion

The study findings demonstrate that both aqueous and ethanolic extracts of *W. chinensis* leaves possess diuretic properties in treated rats, as evidenced by increased excretion of electrolytes and urine volume. The observed diuretic activity might be due to the presence of tannins, flavonoids and triterpenoids. These results support the traditional use of *W. chinensis* for its diuretic effects. Further research is needed to pinpoint the active phytoconstituents responsible for these observed effects.

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