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EVALUATION OF DIURETIC POTENTIAL OF METHANOLIC EXTRACT OF AMPELOCISSUS LATIFOLIA STEM IN RATS

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ABSTRACT: The present study investigated the diuretic activity of the hydro alcoholic extract of Ampelocissus latifolia in Wistar albino rats using the Lipschitz test, with Furosemide (10 mg/kg, p.o.) as the reference standard. Animals were divided into five groups (n=6) and urine was collected for 5 hours post-dosing in metabolic cages. Diuretic response was assessed by measuring total urine volume, urinary electrolyte excretion (Na^+, K^+, and Cl^-), diuretic index, saluretic index, natriuretic index, and ion quotient. The extract produced a significant, dose-dependent increase in urine output with diuretic indices of 1.61, 2.06, and 2.55 at doses of 250, 300, and 500 mg/kg, respectively, compared with control (1.0) and approaching the standard (2.9). Enhanced excretion of sodium, potassium, and chloride with mild reductions in their serum levels indicated marked saluretic and natriuretic effects. Serum total protein showed only a slight reduction within physiological limits, suggesting the absence of harmful protein depletion. Phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, phenols, saponins, tannins, steroids, and amino acids, which may contribute to the observed activity. The findings demonstrate that Ampelocissus latifolia possesses significant diuretic potential comparable to the standard drug and supports its traditional use as a natural diuretic agent.

Keywords: Ampelocissus latifolia, Diuretic activity, Lipschitz test, Furosemide, Saluretic index, Natriuretic index, Urinary electrolytes.

INTRODUCTION

The kidneys function as a major excretory organ for the elimination of metabolic wastes from the body. In most species, death occurs within a week after total cessation of renal function. Partial loss of renal function results in variable deviations from normal, depending on the quantity of functional tissue remaining. The term azotemia refers to the accumulation of nitrogenous wastes in the blood. Blood concentrations of creatinine and urea are measured as indices of azotemia, although neither imparts significant toxicity because of its accumulation. Animals with moderate to severe azotemia may have a constellation of clinical signs, including lethargy, anorexia, mucosal ulcers, vomiting, diarrhea, weight loss, anemia, and altered urine output. These signs are referred to as renal failure, uremia, or uremic syndrome, and reflect the development of abnormalities in a multitude of tissues secondary to subnormal renal functions. The role of the kidneys in maintaining life is a composite of several functions. Water and many electrolytes are conserved by the kidneys in times of negative body balance and are excreted in times of positive balance. During the processing of body fluids, the kidneys avidly conserve nutrients such as glucose and amino acids; consequently, urine is almost devoid of such materials. Hydrogen ions are excreted or conserved so that blood pH is kept within narrow limits. End products of nitrogen metabolism, such as urea, creatinine, and allantois, are removed

from the body via the urine so that blood levels remain low and relatively constant. The kidneys produce several hormones, including renin, erythropoietin, and prostaglandins, and perform a vital hydroxylation of vitamin D that is required for its activation. The kidneys also respond to several hormones, including antidiuretic hormone (ADH), parathyroid hormone (PTH), aldosterone, thyroid hormone, and others. The story of the evolution of the kidneys to full fill the changing needs of the organism has been told eloquently by [1-5], a pioneer in the study of the kidney. Single-celled and primitive organisms had little need for excretory specialization because simple diffusion of materials to and from their iso-osmotic aqueous environment was adequate both for intake and for excretory processes. As the complexity of organisms grew, an extracellular space developed that mimicked the osmolality and electrolyte makeup of the original aqueous environment. Simple excretory organs sufficed because extracellular water and wastes could be extruded, and needs could be replaced easily by ingestion. However, the threat of osmotic imbalance occurred as the seas increased in salinity. Some animals such as the shark solved this problem by retaining urea as a compound to balance the osmolality of the seas. Others depended on renal excretion of excess salt via renal tubular systems. The animals that eventually moved to freshwater from the ocean faced a different problem. The osmolality of their environment being nil, there was an influx of water from the environment into their tissues. A high glomerular filtration rate evolved to facilitate the removal of this water. But then there was a movement of some animals from freshwater to land and a need to conserve water rather than to excrete it. The high filtration kidney was then modified so that most of the filtered water was reabsorbed while solute wastes were still excreted. An understanding of the evolutionary processes previously outlined provides insight into an apparent paradox involving the mammalian kidney. In mammals, there is a continuous potential loss of sodium rich fluid equal to 4-6% of cardiac output via glomerular filtration. Energy is then expended by the tubules to reabsorb more than 99% of the filtered sodium, together with accompanying water. Certainly an engineer asked to design an excretory organ would be unlikely to choose this devious approach to the end result. The evolutionary development of the kidney has clinical relevance. In renal failure, one must deal not only with problems of inadequate excretion because of decreased glomerular filtration, but also with problems of excess losses arising from alterations in function of the tubular system reclaiming filtrate. Polyuria of renal failure is an example of such a problem [6-8].

NORMAL RENAL FUNCTIONS

The Nephron as a Structural and Functional Unit

Most renal functions are the sum of the activities of thousands of nephrons. Each nephron consists of several anatomical divisions. Glomeruli are composed of a tuft of capillaries supplied by an afferent arteriole and drained by an efferent arteriole. Bowman's capsule surrounds the glomerular capillaries and channels filtrate into the proximal tubule. The convoluted portion of the proximal tubule assumes a meandering path within the cortex before the straight portion (pars recta) descends toward the medulla. A marked thinning of the straight portion occurs abruptly as it descends; this constitutes the beginning of the loop of Henle. This structure courses into the medulla, makes a hairpin turn, and ascends into the cortex. At some point in its ascent, the epithelium becomes thicker, which persists to the point of apposition of the loop of Henle with the glomerulus from which the tubule originates. The afferent and efferent arterioles of the glomerulus are adjacent to the ascending tubule. These structures, at the site of their confluence, are referred to as the juxtaglomerular apparatus. Modified tubular cells at this site are called the macula densa. The distal tubule is considered to be that segment of the nephron between the macula densa and the first union of two distal tubules into a collecting duct. Collecting ducts empty into the renal pelvis. The number of nephrons varies among species, with larger species having more of them. For example, the cat has about 190,000, the dog about 430,000, and the cow about 4 million per kidney [9-12]. Within the canine species, where there are large body size differences between breeds, nephron size rather than nephron number varies with body size . A simple view of nephron function can be summarized as follows. Perfusion of glomerular capillaries with blood under pressure results in the passage of material (filtrate) through the capillary walls into Bowman's space. Filtrate then passes through the tubular system of each nephron and is modified in composition during its passage. Modification occurs primarily by reabsorption of lumen

contents by tubular cells for return to the body. In addition, important secretory functions are performed by tubule cells, whereby materials delivered to the renal interstitium by the blood are transported from the interstitium to the tubule lumen for excretion. The end result of these tubular activities is a marked reduction in the volume of filtrate and a modification of its character.

Materials and methods

Collection and Authentication of plant material:

The plant Ampelocissus latifoliawas collected from natural habitat of Ranga Reddy District, Telangana, India. The taxonomic identification of the plant was confirmed by Dr. Madhavan Chetty, Assistant Professor, Sri Venkateshwara University, Tirupathi, and Andhra Pradesh.

The Ampelocissus latifoliawere cleaned and drying under sunlight.

Extraction procedure

Coarsely powdered Ampelocissus latifoliawere used for extraction with Ethanol by using Soxhlet method for 6-8 hours. 50g of dried powder was weighed and 250mL of solvent Ethanol is used for the extraction. The extracts were evaporated by using a rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4°C for further analysis.

Screening of Diuretic Activity in Rats

Animals Stock:

Wistar albino rats of either sex weighing between 150-200 g were obtained From Jeeva Life Sciences Animals were housed in groups of 6-8 per cage at a temperature of 25±1 0C and relative humidity of 45-55%. Animals had free access to food and water; however, food and water were withdrawn 18 hours before the experiment. Jeeva life sciences The Institutional Animal Ethics Committee approved the protocol of this study.

Diuretic activity:

Animals were divided into a total of five groups (n = 6 in each group). All animals were deprived of food and water 18 hours before the experiment. On the day of the experiment, the dosing was scheduled as follows:

Animal Grouping

Group I: Normal saline.

Group II: Furosemide 10 mg/kg p.o (standard drug)

Group III: Ampelocissus Latifolia extracts 250 mg/kg p.o

Group IV: Ampelocissus Latifolia 300 mg/kg p.o

Group V: Ampelocissus Latifolia 500 mg/kg p.o

Immediately after the dosing, animals were placed in metabolic cages and urine was collected up to 5 h after dosing. Room temperature was maintained up to 25± 0.5 0C.

During this period no water or food was made available to the animals. Diuretic activity was assessed by measuring the following parameters:

- ✓ Total urine volume.
- ✓ Urine concentration of Na+, K+ and Cl-.

The effect of different extracts of Ipomoea hederifoliaexhibiting diuretic activity.

Biochemical Estimation [13]

Sodium:

Principle:

The sodium and the protein in the serum are precipitated with magnesium uranyl acetate. After separation by centrifugation, the excess of uranyl ions in the supernatant reacts with potassium ferrocyanide forming a colored complex whose absorbance varies inversely to the concentration of sodium in the sample.

Sodium Assay

Step-1: Precipitation of Sodium and Serum Proteins:

Pipette into clean dry test tubes labelled as Standard (S) and Test (T)

	Standard (S)	Test (T)
R1 Precipitating Reagent	1000 µl	1000 µl
R4 Standard	10 µl	-
Serum Sample	-	10 µl

Shake vigorously and incubate at room temperature for 5 minutes. Then Centrifuge at 2000-3000 rpm for 2 minutes to obtain a clear supernatant.

Step 2: Colour development

Pipette into clean dry test tubes labeled as Blank (B), Standard (S), and Test (T):

	Blank (B)	Standard (S)	Test (T)
R2 Colour Reagent	1000 µl	1000 µl	1000 µl
Supernatant from Step 1		20 µl	20 µl
R1 Precipitating Reagent	20 µl		

Mix well and allow it to stand at room temperature for 5 minutes. Then measure the absorbance of Blank (B), standard (S), and Test (T) on a photo colorimeter with green filter or on a spectrophotometer at 530nm (505 - 530 nm) within 10 minutes.

Calculations:

$$Sodium\ in\ mmole/L = \frac{Abs\ of\ B - Abs\ of\ T}{Abs\ of\ B - Abs\ of\ S} \times 150$$

Potassium:

Principle:

Potassium ions in a protein-free alkaline medium react with sodium tetraphenyl boron to produce a finely dispersed colloidal suspension of potassium tetraphenyl boron. The turbidity produced is proportional to the potassium concentration in the sample.

Potassium Assay

Pipette into two clean dry test tubes labeled Standard (S) and Test (T)

	Standard (S)	Test (T)
R3 Boron Reagent	1000 µl	1000 µl
R4 Standard	50 µl	-
Serum Sample	-	50 µl

Mix well and incubate for 5 minutes at RT. Read absorbance of the Standard (Abs.S) and Test Sample (Abs.T) against Distilled water at 620 nm.

Calculations

$$Potassium\ in\ mmol/L = \frac{Abs\ of\ T}{Abs\ of\ S} \times 5.0$$

Chloride:

Principle: When chloride is mixed with a solution of undissociated mercuric thiocyanates, the chloride preferentially combines with mercury, forming mercuric chloride. The thiocyanates that are released then combines with ferric ions present in the solution forming strongly colored ferric thiocyanates with absorption maxima at 480 nm.

Assay procedure

Wavelength 492 (470 - 630) nm

Cuvette 1 cm

	Reagent blank	Standard (Cal.)	Sample
Reagent 1	1000 µl	1000 µl	1000 µl
Sample	-	-	10 µl
Standard (Cal.)	-	10 µl	-
Distilled water	10 µl	-	-

Mix and incubate for 5 min. at 37 0C, measure absorbance at the sample Asam and standard Ast against reagent blank.

Calculation

$$Chloride\ mmol/L = \frac{\Delta A_{sam}}{\Delta A_{st}} C_{st}$$

Cst = standard (calibrator) concentration

Diuretic activity [14]

Diuretic activities of normal and treated animals were estimated using an estimation of the difference between the output of urine and consumption of liquids. The percentage of variance between saline-administered and test animals was calculated.

$$Diureticaction = \frac{\text{Urinary excretion volume of the test group}}{\text{Urinary excretion volume of the control group}}$$

$$Diureticactivity = \frac{diureticactionoftest}{diureticactionofstandard}$$

Saluretic and natriuretic activity and carbonic anhydrase inhibitor [15]

Saluretic index, natriuretic index, and ion quotient were determined using the following formulae.

$$Salureticindex = \frac{\text{Urinary excretion of electrolytes of the test group}}{\text{Urinary excretion of electrolytes in the control group}}$$

$$\text{Natriuretic index} = \frac{\text{Urinary excretion of } Na^+}{\text{Urinary excretion of } K^+}$$

$$\text{Carbonic anhydrase inhibition} = \frac{\text{Urinary excretion of } Cl^-}{\text{Urinary excretion of } Na^+ \text{ and } K^+}$$

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 $p < 0.001$.

RESULTS

Percentage of yield

$$\textbf{\textit{Percentage of yield}} = \frac{\textbf{\textit{Weight of crude extract}}}{\textbf{\textit{Weight of dried material}}} \times \mathbf{100}$$

$$Percentage\ of\ yield = \frac{50g}{250g} \times 100 = 20\%$$

50gms of drug dissolved in 250ml of solvent in 1:10 Ratio

Qualitative Chemical Analysis:

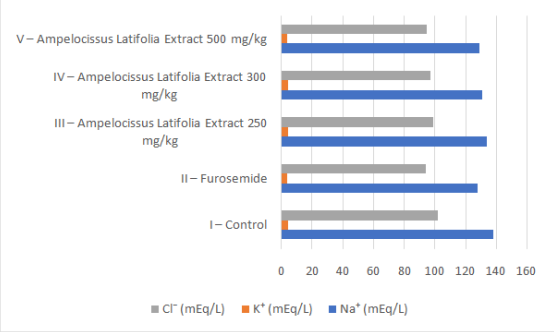
Sl. No.	Phytoconstituents	Test result
1	Alkaloid	+ve
2	Glycosides	+ve
3	Carbohydrate	+ve
4	Protein	-ve
5	Amino acid	+ve
6	Steroids	+ve
7	Flavonoids	+ve
8	Trepeniods	-ve
9	Phenols	+ve
10	Saponins	+ve
11	Tannin	+ve

+: Present; -: Absent

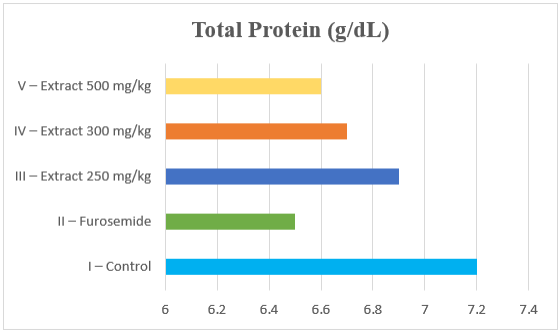
Electrolytes and Serum Proteins

Estimation of electrolytes and serum proteins after treatment showed marked changes compared to control. The standard diuretic Furosemide produced a significant increase in urinary sodium, potassium, and chloride excretion with a mild reduction in serum protein levels. The extract of Ampelocissus latifolia demonstrated a dose-dependent effect on electrolyte elimination.

Group	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)
I – Control	138 ± 4	4.5 ± 0.3	102 ± 3
II – Furosemide	128 ± 3	3.8 ± 0.2	94 ± 3
III –Ampelocissus Latifolia Extract 250 mg/kg	134 ± 3	4.2 ± 0.2	99 ± 2
IV – Ampelocissus Latifolia Extract 300 mg/kg	131 ± 3	4.0 ± 0.2	97 ± 2
V – Ampelocissus Latifolia Extract 500 mg/kg	129 ± 2	3.9 ± 0.2	95 ± 2



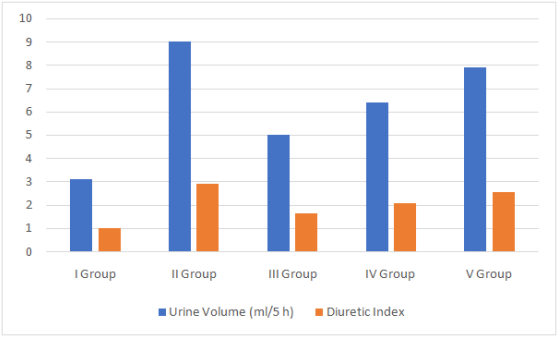
Group	Total Protein (g/dL)
Treatment	7.2 ± 0.3
Normal saline	6.5 ± 0.2
Furosemide 10 mg/kg	6.9 ± 0.2
Ampelocissus latifolia 250 mg/kg	6.7 ± 0.2
Ampelocissus latifolia 300 mg/kg	6.6 ± 0.2



Serum total protein levels showed a mild reduction in the treated groups compared with control. The standard diuretic Furosemide produced a noticeable decrease in serum protein concentration, which is commonly observed due to increased fluid loss and haemodilution during diuresis. The extract of Ampelocissus latifolia also showed a dose-dependent, slight reduction in serum protein levels. However, the values remained within the normal physiological range, indicating that the extract did not cause harmful protein depletion. This mild change supports the diuretic effect without producing significant protein imbalance in the body.

Diuretic index

Group	Treatment	Urine Volume (ml/5 h)	Diuretic Index
I Group	Normal saline	3.1 ± 0.3	1
II Group	Furosemide 10 mg/kg	9.0 ± 0.5	2.9
III Group	Ampelocissus latifolia 250 mg/kg	5.0 ± 0.4	1.61
IV Group	Ampelocissus latifolia 300 mg/kg	6.4 ± 0.5	2.06
V Group	Ampelocissus latifolia 500 mg/kg	7.9 ± 0.6	2.55



DISCUSSIONS

The present study evaluated the diuretic potential of the hydro alcoholic extract of Ampelocissus latifolia in Wistar albino rats using Lipschitz test, with Furosemide as the standard. The extract exhibited a clear dose-dependent increase in urine output over 5 hours, reflected by rising diuretic indices (1.61,

2.06, and 2.55 at 250, 300, and 500 mg/kg, respectively) compared with control (1.0) and approaching the standard (2.9). Treated groups showed enhanced urinary electrolyte excretion with reductions in serum Na^+, K^+, and Cl^- relative to control, indicating effective saluretic and natriuretic activity. The natriuretic pattern (Na^+/K^+ ratio) suggested favourable sodium elimination without excessive potassium loss, while the ion quotient supported mild carbonic anhydrase inhibitory involvement. A slight, dose-related reduction in serum total protein remained within physiological limits, consistent with fluid loss during diuresis rather than protein depletion. Phytochemical screening revealed alkaloids, glycosides, flavonoids, phenols, saponins, tannins, steroids, and amino acids, which may underlie the observed diuretic action.

Conclusion

the hydroalcoholic extract of Ampelocissus latifolia demonstrated significant, dose-dependent diuretic activity in Wistar rats as assessed by the Lipschitz test, with effects approaching those of the standard diuretic Furosemide. The extract increased urine output and promoted marked excretion of Na^+, K^+, and Cl^-, indicating pronounced saluretic and natriuretic actions without causing harmful protein depletion. The pattern of electrolyte elimination suggests a mechanism comparable to loop diuretics rather than primary carbonic anhydrase inhibition. The presence of bioactive phytoconstituents such as flavonoids, glycosides, saponins, tannins, phenols, and steroids may contribute to the observed activity. Overall, the study supports the potential of Ampelocissus latifolia as a promising natural diuretic agent.

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