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A comprehensive evaluation of the diuretic and antihypertensive properties of *Lippia nodiflora* (L.) greene (Verbenaceae) in experimental models

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Abstract
Hypertension and fluid retention-related disorders, such as edema and congestive heart failure, require effective management strategies, often relying on diuretics and antihypertensive agents. The present study investigated the diuretic and antihypertensive potential of *Lippia nodiflora* (LN) aqueous and ethanolic extracts in Wistar albino rats over multiple time points (5 hours, 24 hours, 7 days, 14 days, and 21 days). The diuretic activity of LN extracts was dose-dependent, with the 500 mg/kg dose demonstrating the most significant effects. LN-treated groups exhibited increased urine output, particularly at 7 days (9.17 ± 0.85 mL compared to 4.17 ± 0.62 mL in the control group), along with enhanced sodium (319.97 ± 2.86 mmol/L) and chloride (170.03 ± 0.39 mmol/L) excretion. Strong natriuretic and saluretic activity was observed, with minimal impact on potassium and calcium levels, indicating a favourable safety profile for prolonged use. The antihypertensive effects of LN extracts were equally remarkable, showing substantial reductions in systolic, diastolic, and mean arterial blood pressures in DOCA-induced hypertensive rats. The highest dose (450 mg/kg) of LN reduced systolic blood pressure to 146 ± 6.27 mmHg, diastolic blood pressure to 101 ± 7.04 mmHg, and mean arterial pressure to 123 ± 2.42 mmHg, demonstrating its efficacy in managing hypertension. These findings highlight *Lippia nodiflora* as a promising natural alternative for managing hypertension and fluid retention disorders. Future research focusing on its active compounds and clinical efficacy in humans is essential to validate its therapeutic potential.

Keywords: Lippia nodiflora, diuretic activity, antihypertensive potential, cardiovascular health, experimental hypertension

Introduction

Hypertension, commonly referred to as high blood pressure, is a chronic condition characterized by elevated arterial pressure. It is a significant risk factor for cardiovascular diseases, including stroke, myocardial infarction, and heart failure, and is associated with substantial morbidity and mortality worldwide (Fuchs and Whelton, 2020; Mills *et al.*, 2020) [11, 18]. According to the World Health Organization (WHO), hypertension affects over 1 billion people globally, with its prevalence projected to rise due to aging populations, sedentary lifestyles, and unhealthy dietary habits (Mills *et al.*, 2020) [18]. Despite the availability of several classes of antihypertensive drugs, managing hypertension remains challenging due to issues such as drug resistance, side effects, and limited patient compliance (Hamrahian *et al.*, 2022) [13].

Conventional antihypertensive therapies, including angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers, diuretics, and beta-blockers, have proven effective in controlling blood pressure (Morgan *et al.*, 2001; Moser, 1997) [19, 20]. However, these medications are often associated with adverse effects such as electrolyte imbalances, fatigue, dizziness, and kidney dysfunction. Additionally, the long-term use of these drugs can lead to complications, highlighting the need for alternative approaches that are safe, effective, and affordable (Alderman, 1996; de Francisco *et al.*, 2012) [4, 10].

In recent years, there has been a growing interest in the use of medicinal plants and natural products as alternative or complementary therapies for managing hypertension. Plants contain a rich array of bioactive compounds, such as polyphenols, flavonoids, alkaloids, and saponins, which have demonstrated various pharmacological effects, including antihypertensive, diuretic, antioxidant, and anti-inflammatory properties. These natural compounds often exhibit fewer side effects and better patient tolerability, making them attractive candidates for therapeutic development (Cristani and Micale, 2024; Mhosva *et al.*, 2024; Riaz *et al.*, 2023; Roy *et al.*, 2022) [9, 17, 22, 23].

Lippia nodiflora (LN), commonly known as frogfruit or creeping phlox, is a perennial medicinal plant belonging to the Verbenaceae family. Traditionally, it has been used in various cultures to treat ailments such as inflammation, pain and urinary disorders (Sharma and Singh, 2013). Preclinical studies have suggested that LN possesses bioactive compounds that may exert antihypertensive and diuretic effects (Gadhvi *et al.*, 2012) [12]. However, its efficacy in scientifically validated models of hypertension, such as the DOCA salt-induced hypertensive rat model, remains underexplored. The DOCA salt model is a well-established experimental system for studying hypertension and its associated pathophysiological mechanisms. It mimics several features of human hypertension, including elevated blood pressure, fluid retention, and electrolyte imbalances, making it a valuable tool for evaluating potential antihypertensive and diuretic agents (Basting and Lazartigues, 2017).

This study aims to investigate the antihypertensive and diuretic potential of aqueous and 50% ethanolic extracts of *L. nodiflora* in a DOCA salt-induced hypertensive rat model. The primary objectives are to evaluate the effects of *L. nodiflora* extracts on blood pressure, urinary output and electrolyte excretion and to assess their dose-dependent efficacy. By elucidating the pharmacological properties of *L. nodiflora*, this study seeks to provide scientific evidence supporting its use as a natural therapy for hypertension and related conditions.

Materials and Methods

Plant material collection and identification

Lippia nodiflora (LN) plants were collected from the Veer Narmad South Gujarat University campus, India. The plant was authenticated by Prof. Minoo Parabia and a voucher specimen was deposited at the herbarium of Bapalal Vaidya Botanical Research Centre, VNSGU, Surat, Gujarat, India. Entire plants were washed thoroughly under running water to remove soil and debris, dried in oven and then powdered using a mechanical grinder (Adnan *et al.*, 2020) [1].

Preparation of plant extracts

Two types of extracts were prepared: aqueous extract and 50% ethanolic extract. Aqueous extract was prepared by taking fifty grams of the powdered plant material and mixed with 500 mL of distilled water and subjected to maceration at room temperature for 24 h. The mixture was then filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator under reduced pressure at 40 °C. The resultant concentrated extract was dried under vacuum to obtain a solid residue, which was stored at 4 °C until further use. Similarly, fifty grams of the powdered plant material was extracted with 500 mL of 50% ethanol (ethanol: water, 1:1) using the Soxhlet extraction method for 8 h. The extract was filtered and the filtrate was concentrated and dried under vacuum conditions as described for the aqueous extract. The dried extract was stored in an airtight container at 4 °C for subsequent pharmacological studies (Patel *et al.*, 2024) [22].

Experimental animals and ethical approval

The study was conducted on male and female Wistar albino rats (300–350 g, 8–9 weeks old). Ethical clearance was obtained from the Institutional Animal Ethics Committee of Trans-Genica Services Pvt. Ltd., Jalgaon, India (Protocol No.: TRS/PT/021/003).

Animal Preparation

Male and female Wistar albino rats (300–350 g, 8–9 weeks old) were housed under standard conditions in polypropylene cages (three per cage) with sterilized paddy husk bedding. A 12-hour light/dark cycle, temperature of 22 ± 3°C, and relative humidity of 30–70% were maintained throughout the study. Rats were acclimatized for seven days with ad libitum access to pellet feed (VRK Nutritional Solutions, Pune, India) and filtered water (reverse osmosis-treated).

Induction of Hypertension (DOCA Salt Model)

Hypertension was induced using the reported DOCA salt model with slight modifications (Iyer *et al.*, 2010) [14].

Uninephrectomy procedure

Rats were anesthetized using an intraperitoneal injection of Zoletil (ketamine: 74 mg/kg and xylazine: 8 mg/kg). A lateral abdominal incision was made to access the kidney. The left renal vessels and ureter were ligated and the left kidney was excised. The incision was sutured using sterile sutures and wound clips. Postoperative analgesia was provided using subcutaneous tramadol (1 mg/kg) for seven days to aid healing.

DOCA salt administration

Following uninephrectomy, rats received subcutaneous injections of DOCA (25 mg dissolved in 0.4 mL dimethylformamide) every fourth day. A 1% NaCl solution was provided as drinking water to all hypertensive groups to induce salt-sensitive hypertension.

Experimental design and treatment groups

The rats were randomized into 16 groups, with six rats in each group (Table - 1). Doses were calculated based on the body weight of the rats and administered orally via gavage once daily for 21 days.

Table 1: Grouping of experimental rats based on treatment with aqueous and 50% ethanolic extracts of *L. nodiflora* at different dosages

Group I	Control group, male uninephrectomy with no further treatment
Group II	Control group, female uninephrectomy with no further treatment
Group III	Male group receive the test materials from aq. extracts of LN at doses of 150 mg/kg, by weight
Group IV	Male group receive the test materials from aq. extracts of LN *at* doses of 300 mg/kg, by weight.
Group V	Male group receive the test materials from aq. extracts of LN *at* doses of 500 mg/kg, by weight.
Group VI	Male group receive the test materials from 50% ethanolic. extracts of LN at doses of 150 mg/kg, by weight.
Group VII	Male group receive the test materials from 50% ethanolic of LN *at* doses of 300 mg/kg, by weight.
Group VIII	Male group receive the test materials from 50% ethanolic of LN *at* doses of 500 mg/kg, by weight.
Group IX	Female group receive the test materials from aq. extracts of LN at doses of 150 mg/kg, by weight.
Group X	Female group receive the test materials from aq. extracts of LN at doses of 300 mg/kg, by weight.
Group XI	Female group receive the test materials from aq. extracts of LN at doses of 500 mg/kg, by weight.
Group XII	Female group receive the test materials from 50% ethanolic. extracts of LN at doses of 150 mg/kg, by weight.
Group XIII	Female group receive the test materials from 50% ethanolic of LN at doses of 300 mg/kg, by weight.
Group XIV	female group receive the test materials from 50% ethanolic of LN at doses of 500 mg/kg, by weight.

Measurement of blood pressure

Systolic, diastolic and mean arterial pressures were recorded using a non-invasive tail-cuff method on days 1, 7, 14, and 21. Rats were acclimated to the procedure during the week prior to measurement to minimize stress (Wang *et al.*, 2017) [28].

Urine collection and analysis

On the 21st day, rats were housed in metabolic cages for 24 h to collect urine samples. Urinary parameters analyzed included volume measured using graduated chambers, pH determined using a calibrated pH meter, electrolytes such as, Na^+, K^+ and Cl^- among sodium and potassium concentrations were measured by flame photometry, while chloride concentration was determined through titration (Scholz, 2010).

Statistical analysis

All data were expressed as mean ± SEM. Statistical comparisons were performed using one-way ANOVA followed by Dunnett's test to determine significance ($p < 0.05$).

Results

Diuretic activity of L. nodiflora

The diuretic potential of aqueous and 50% ethanolic extracts of LN was assessed over multiple time points (5 h, 24 h, 7 days, 14 days and 21 days) in Wistar albino rats. The results highlight significant diuretic, natriuretic and saluretic activity compared to control and standard groups (furosemide-treated rats).

Short-term diuretic effects (5 h)

At the 5 h time point, the aqueous extract of *L. nodiflora* demonstrated dose-dependent diuretic effects.

Urine Volume and Ph

The LN 500 mg/kg group showed the highest urine volume (2.17 ± 0.85 mL) compared to the control (1.17 ± 0.59 mL) and standard (1.97 ± 0.24 mL) groups. The pH was neutral (7.33 ± 0.94) in most groups, except for the LN 250 mg/kg group, which exhibited an alkaline pH of 8.00 ± 0.00 (Table – 2).

Electrolyte excretion

Sodium and chloride excretion were significantly higher in the LN-treated groups. Sodium excretion was 238.80 ± 7.90 mmol/L in the LN 500 mg/kg group and 231.67 ± 23.01 mmol/L in the LN 250 mg/kg group, compared to 224.48 ± 16.73 mmol/L in the control group. Chloride excretion was highest in the LN 500 mg/kg group (164.16 ± 17.17 mmol/L) and lowest in the LN 250 mg/kg group (135.26 ± 8.91 mmol/L). Potassium excretion was slightly lower in the LN-treated groups compared to the control, while calcium excretion remained minimal across all groups (Table – 2).

Table 2: Diuretic effect of aqueous extract of LN in Wistar albino rats at 5 h.

Time Point: 5 h						
	Urine vol.	**pH**	**Sodium**	**Potassium**	**Chloride**	**Calcium**
G1: CON	1.17 ± 0.59	7.33 ± 0.47	224.48 ± 16.73	47.39 ± 1.91	148.75 ± 7.70	4.67 ± 0.79
G2: STD	1.97 ± 0.24	7.33 ± 0.47	255.97 ± 15.25	48.16 ± 0.85	206.23 ± 9.17	12.09 ± 0.83
G3: LN 250mg	1.10 ± 0.29	8.00 ± 0.00	231.67 ± 23.01	42.23 ± 1.19	135.26 ± 8.91	4.03 ± 1.26
G4: LN 500mg	2.17 ±0.85	7.33 ± 0.94	238.80 ± 7.90	43.37 ± 1.92	164.16 ± 17.17	4.95 ± 0.74

Urinary excretion and diuretic parameters

The LN 500 mg/kg group exhibited the highest urinary excretion (121.72), diuretic action (2.003), and diuretic activity (2.733) compared to the control group (60.76, 1.000, and 1.364, respectively) and the standard group (105.35, 1.734, and 2.365, respectively) (Table – 3).

Natriuretic and saluretic Effects

The Na+/K+ ratio and Na+ + Cl− levels were highest in the LN 500 mg/kg group (5.51 and 402.95, respectively), indicating strong natriuretic and saluretic activity compared to the control (4.74 and 373.23, respectively).

Overall, the LN 500 mg/kg dose exhibited superior diuretic effects, with increased urine volume, enhanced sodium and chloride excretion, and significant natriuretic and saluretic activity (Table – 3).

Table 3: Effect of aqueous extracts of LN on urinary excretion, diuretic action, diuretic activity, natriuretic effect, saliuretic effect and carbonic anhydrase inhibition at 5 h

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	Urinary excretion	**Diuretic action**	**Diuretic activity**	**Na+ + Cl-**	**Na+ /K+**	**Cl-/ (Na + K)**
G1: CON	60.76	1.000	1.364	373.23	4.74	0.55
G2: STD	105.35	1.734	2.365	462.21	5.31	0.68
G3: LN 250mg	62.50	1.029	1.403	366.94	5.49	0.49
G4: LN 500mg	121.72	2.003	2.733	402.95	5.51	0.58

Mid-Term Diuretic Effects (24 Hours and 7 Days)

Results at 24 h

At the 24 h time point, the aqueous extract of *L. nodiflora* exhibited significant diuretic activity in a dose-dependent manner compared to the control and standard groups.

Urine volume and pH

The *L. nodiflora* 500 mg/kg group recorded a urine volume of 4.07 ± 0.42 mL, close to the standard group (4.33 ± 0.24 mL) and higher than the control group (3.20 ± 0.50 mL). The urine pH in LN-treated groups was slightly acidic to neutral, measuring 6.33 ± 0.47 compared to the fixed pH of 6.00 in the control and standard groups (Table – 4).

Electrolyte excretion

Sodium excretion in the LN 500 mg/kg group (185.38 ± 7.32 mmol/L) was lower than the standard (407.27 ± 5.67 mmol/L) but higher than the LN 250 mg/kg group (106.52 ± 3.81 mmol/L). Chloride excretion followed a similar trend, with the LN 500 mg/kg group excreting 116.47 ± 17.05 mmol/L, which was intermediate between the standard (259.43 ± 9.02 mmol/L) and control (235.46 ± 19.61 mmol/L) groups. Potassium excretion in the LN 500 mg/kg group (50.40 ± 0.63 mmol/L) was comparable to the control group (55.61 ± 1.26 mmol/L) but lower than the standard (47.51 ± 0.48 mmol/L). Calcium excretion was negligible in LN-treated groups, with 4.47 ± 0.37 mmol/L observed in the 500 mg/kg group (Table – 4).

Table 4: Diuretic effect of aqueous extract of plants in Wistar albino rats at 24 h.

Time Point: 24 h						
	Urine vol.	**pH**	**Sodium**	**Potassium**	**Chloride**	**Calcium**
G1: CON	3.20 ± 0.50	6.00 ± 0.00	316.34 ± 16.90	55.61 ± 1.26	235.46 ± 19.61	13.32 ± 0.92
G2: STD	4.33 ± 0.24	6.00 ± 0.00	407.27 ± 5.67	47.51 ± 0.48	259.43 ± 9.02	12.89 ± 0.15
G3: LN 250mg	2.53 ± 0.53	6.33 ± 0.47	106.52 ± 3.81	43.91 ± 1.96	68.53 ± 2.41	2.55 ± 0.35
G4: LN 500mg	4.07 ± 0.42	6.33 ± 0.47	185.38 ± 7.32	50.40 ± 0.63	116.47 ± 17.05	4.47 ± 0.37

Urinary excretion and diuretic parameters

The LN 500 mg/kg group exhibited high urinary excretion (228.46) and diuretic activity (2.85), approaching the values recorded for the standard group (231.73 and 1.00, respectively). The LN 250 mg/kg group showed a reduced response (143.94 and 1.80) (Table – 5).

Natriuretic and saluretic effects

The Na^+/K^+ ratio and $Na^+ + Cl^-$ values for the LN 500 mg/kg group were 3.68 and 301.86, respectively, indicating moderate natriuretic and saluretic effects compared to the standard group (8.57 and 666.70) (Table – 5).

Table 5: Effect of aqueous extracts of the plants on Urinary excretion, Diuretic action, Diuretic activity, natriuretic effect, saliuretic effect and carbonic anhydrase inhibition at 24 h.

Time Point: 24 h						
	Urinary excretion	**Diuretic action**	**Diuretic activity**	**$Na^+ + Cl^-$**	**Na^+ /K^+**	**$Cl^-/ (Na + K)$**
G1: CON	166.67	1.000	2.08	551.80	5.69	0.63
G2: STD	231.73	0.481	1.00	666.70	8.57	0.57
G3: LN 250mg	143.94	0.864	1.80	175.05	2.43	0.46
G4: LN 500mg	228.46	1.371	2.85	301.86	3.68	0.49

Results at 7 Days

At the 7-day time point, the aqueous extract of *L. nodiflora* exhibited significant diuretic effects, particularly at the 500 mg/kg dose, with enhanced urine output and electrolyte excretion.

Urine volume and pH

The LN 500 mg/kg group showed a markedly increased urine volume (9.17 ± 0.85 mL), nearly double that of the standard group (4.83 ± 0.24 mL) and significantly higher than the control group (4.17 ± 0.62 mL). The urine pH in the LN 500 mg/kg group remained neutral (7.33 ± 0.47), while the LN 250 mg/kg group showed a slightly acidic pH (6.67 ± 0.47) (Table – 6).

Electrolyte excretion

Sodium

The LN 500 mg/kg group excreted 244.70 ± 25.41 mmol/L of sodium, lower than the control (322.32 ± 16.11 mmol/L) but still substantial.

Chloride

The LN 500 mg/kg group recorded the highest chloride excretion (207.16 ± 2.36 mmol/L), significantly surpassing the control (166.04 ± 7.09 mmol/L) and standard (165.87 ± 2.34 mmol/L) groups.

Potassium

Potassium excretion in the LN 500 mg/kg group (60.01 ± 2.34 mmol/L) was slightly higher than the control (54.85 ± 2.30 mmol/L) and standard (56.97 ± 1.58 mmol/L) groups.

Calcium

Calcium excretion was minimal, with the LN 500 mg/kg group excreting 9.69 ± 0.20 mmol/L, lower than the control (11.61 ± 1.09 mmol/L) and standard (10.26 ± 0.19 mmol/L) (Table – 6).

Table 6: Diuretic effect of aqueous extract of plants in Wistar albino rats at 7 days.

Time Point: 7 days						
	Urine vol.	**pH**	**Sodium**	**Potassium**	**Chloride**	**Calcium**
G1: CON	4.17 ± 0.62	7.00 ± 0.00	322.32 ± 16.11	54.85 ± 2.30	166.04 ± 7.09	11.61 ± 1.09
G2: STD	4.83 ± 0.24	7.00 ± 0.00	316.56 ± 15.49	56.97 ± 1.58	165.87 ± 2.34	10.26 ± 0.19
G3: LN 250mg	2.37 ± 0.69	6.67 ± 0.47	275.69 ± 33.22	52.08 ± 0.66	165.52 ± 3.23	12.30 ± 0.75
G4: LN 500mg	9.17 ± 0.85	7.33 ± 0.47	244.70 ± 25.41	60.01 ± 2.34	207.16 ± 2.36	9.69 ± 0.20

Urinary excretion and diuretic parameters

The LN 500 mg/kg group exhibited the highest urinary excretion (558.94) and diuretic activity (2.69), significantly exceeding the control (218.15 and 1.05, respectively) and standard (240.46 and 1.16, respectively) groups (Table – 7).

Natriuretic and saluretic effects

The LN 500 mg/kg group showed enhanced natriuretic and saluretic activity, with a $Na^+ + Cl^-$ value of 451.86 and a $Cl^-/ (Na^+ + K^+)$ ratio of 0.68, compared to 488.36 and 0.44, respectively, in the control group. The Na^+/K^+ ratio in the LN 500 mg/kg group was 4.08, lower than the control (5.88) and standard (5.56) groups (Table – 7).

Table 7: Effect of aqueous extracts of the plants on urinary excretion, diuretic action, diuretic activity, natriuretic effect, saliuretic effect and carbonic anhydrase inhibition at 7 days

Time Point: 7 days						
	Urinary excretion	**Diuretic action**	**Diuretic activity**	**$Na^+ + Cl^-$**	**Na^+ /K^+**	**$Cl^-/ (Na + K)$**
G1: CON	218.15	1.47	1.05	488.36	5.88	0.44
G2: STD	240.46	1.62	1.16	482.43	5.56	0.44
G3: LN 250mg	127.24	0.86	0.61	441.21	5.29	0.50
G4: LN 500mg	558.94	3.77	2.69	451.86	4.08	0.68

Long-Term Diuretic Effects (14 and 21 Days)

Results at 14 days

At the 14-day time point, the aqueous extract of *L. nodiflora* demonstrated significant diuretic activity, with dose-dependent increases in urine output and electrolyte excretion compared to the control and standard groups.

Urine volume and pH

The LN 250 mg/kg group exhibited the highest urine volume (6.00 ± 0.82 mL), followed by the LN 500 mg/kg group (4.17 ± 0.09 mL), which exceeded the standard group (5.50 ± 0.24 mL) and the control group (1.93 ± 0.12 mL). Urine pH was slightly alkaline in the LN 250 mg/kg group (7.33 ± 0.94) and neutral to acidic in the LN 500 mg/kg group (6.67 ± 0.94) (Table – 8).

Electrolyte excretion

Sodium

The LN 500 mg/kg group showed the highest sodium excretion (319.97 ± 2.86 mmol/L), surpassing the standard group (281.00 ± 16.67 mmol/L) and the control group (281.76 ± 16.66 mmol/L).

Chloride

Chloride excretion was highest in the LN 500 mg/kg group (170.03 ± 0.39 mmol/L) compared to the standard (165.34 ± 3.64 mmol/L) and control (105.01 ± 4.44 mmol/L) groups.

Potassium

Potassium excretion was consistent across all groups, with the LN 500 mg/kg group recording 53.52 ± 0.85 mmol/L.

Calcium

Calcium excretion was negligible, with the LN 500 mg/kg group showing 14.98 ± 0.36 mmol/L, comparable to the standard group (15.05 ± 0.04 mmol/L) (Table – 8).

Table 8: Diuretic Effect of aqueous extract of LN in Wistar albino rats at 14 days.

Time Point: 14 days						
	Urine vol.	**pH**	**Sodium**	**Potassium**	**Chloride**	**Calcium**
G1: CON	1.93 □ 0.12	6.00 □ 0.00	281.76 □ 16.66	55.27 □ 0.59	105.01 □ 4.44	12.40 □ 0.11
G2: STD	5.50 □ 0.24	6.00 □ 0.00	281.00 □ 16.67	57.97 □ 1.24	165.34 □ 3.64	15.05 □ 0.04
G3: LN 250mg	6.00 □ 0.82	7.33 □ 0.94	232.79 □ 24.61	56.17 □ 1.29	115.39 □ 3.30	11.08 □ 0.09
G4: LN 500mg	4.17 □ 0.09	6.67 □ 0.94	319.97 □ 2.86	53.52 □ 0.85	170.03 □ 0.39	14.98 □ 0.36

Urinary excretion and diuretic parameters

The LN 250 mg/kg group exhibited the highest urinary excretion (322.58), diuretic action (1.15), and diuretic activity (3.02), followed by the LN 500 mg/kg group (254.07, 0.90, and 2.38, respectively) (Table – 9).

Natriuretic and saluretic effects

The LN 500 mg/kg group showed significant natriuretic and saluretic effects, with a $Na^+ + Cl^-$ value of 490.00 and a $Cl^-/ (Na^+ + K^+)$ ratio of 0.46, compared to the standard group (446.34 and 0.49, respectively). The Na^+/K^+ ratio in the LN 500 mg/kg group was 5.98, higher than the control (5.10) and standard (4.85) groups (Table – 9).

Table 9: Effect of aqueous extracts of the LN on urinary excretion, diuretic action, diuretic activity, natriuretic effect, saliuretic effect and carbonic anhydrase inhibition at 14 days.

Time Point: 14 days						
	Urinary excretion	**Diuretic action**	**Diuretic activity**	**$Na^+ + Cl^-$**	**Na^+ /K^+**	**$Cl^-/ (Na + K)$**
G1: CON	595.11	2.12	5.57	386.77	5.10	0.31
G2: STD	273.63	0.97	2.56	446.34	4.85	0.49
G3: LN 250mg	322.58	1.15	3.02	348.18	4.14	0.40
G4: LN 500mg	254.07	0.90	2.38	490.00	5.98	0.46

Results at 21 days

Urine volume and pH

At 21 days time point, the male control group (G1-M-Control) had a urine volume of 4.69 ± 2.5 mL, while the female control group (G2-F-Control) had 4.52 ± 2.2 mL. The urine pH for both control groups was around 7.15 ± 0.24 and 7.12 ± 0.31, respectively. In the DOCA-induced groups, the male group (G3-M-DOCA) showed a higher urine volume of 7.57 ± 1.8 mL and a pH of 8.73 ± 0.42, indicating more alkaline urine. Similarly, the female DOCA-treated group (G4-F-DOCA) showed 7.38 ± 1.3 mL of urine with a pH of 8.62 ± 0.18. For *L. nodiflora* treated groups (G5-Aq-M-LN150, G6-Aq-M-LN300, G7-Aq-M-LN450, G8-Et-M-LN150, G9-Et-M-LN300, G10-Et-M-LN450, G11-Aq-F-LN150, G12-Aq-F-LN300, G13-Aq-F-LN450, G14-Et-F-LN150, G15-Et-F-LN300, G16-Et-F-LN450), the urine volume ranged from 4.73 ± 1.8 mL (G8-Et-M-LN150) to 7.05 ± 2.8 mL (G9-Et-M-LN300), with pH values showing slight alkalinity to neutral (around 7.83 ± 0.34 for G8-Et-M-LN150 to 8.24 ± 0.34 for G10-Et-M-LN450) (Table – 10).

Diuretic action and activity

The *L. nodiflora* groups demonstrated a dose-dependent increase in diuretic action and activity. For instance, the male 450 mg/kg dose group (G7-Aq-M-LN450) had the highest diuretic action (7.57) and diuretic activity (1.16). Similarly, the female 450 mg/kg group (G13-Aq-F-LN450) exhibited diuretic action (0.76) and activity (1.23). In comparison, the control and standard groups showed diuretic action and activity values of 1.00 and 0.59 (G1-M-Control) and 1.00 and 0.57 (G2-F-Control), respectively, indicating a baseline for comparison (Table – 10).

Table 10: Effect of aqueous & 50% Ethanolic extracts of the LN on Urinary excretion, Diuretic action, Diuretic activity after 21 days

Group	Urine Volume (ml)	Urine pH	Diuretic Action	Diuretic activity
G0-M-STD-DOCA	-	-	-	1
G0-F-STD-DOCA	-	-	-	1

G1-M-Control	4.69 ±2.5	7.15 ±0.24	1	0.59
G2-F-Control	4.52 ±2.2	7.12 ±0.31	1	0.57
G3-M-DOCA	7.57 ±1.8#	8.73 ±0.42#	1.61	0.95
G4-F-DOCA	7.38 ±1.3@	8.62± 0.18@	1.63	0.94
G5-Aq-M-LN150	5.68 ±2.7*	8.12 ±0.35*	4.69	0.71
G6-Aq-M-LN300	5.47 ±3.1*	8.15 ±0.47*	4.52	0.70
G7-Aq-M-LN450	5.42 ±2.4*	7.89 ±0.19*	7.57	1.16
G8-Et-M-LN150	4.73 ±1.8*	7.83 ±0.34*	7.38	1.05
G9-Et-M-LN300	7.05 ±2.8*	8.08 ±0.27*	5.68	0.93
G10-Et-M-LN450	6.79 ±3.1*	8.24 ±0.34*	5.47	0.92
G11-Aq-F-LN150	5.78 ±2.5*	8.14 ±0.17*	1.28	0.73
G12-Aq-F-LN300	4.74 ±1.9*	8.08 ±0.34*	0.63	1.01
G13-Aq-F-LN450	5.58 ±2.2*	8.17 ±0.94*	0.76	1.23
G14-Et-F-LN150	6.07 ±3.2 *	8.07 ±0.31*	1.07	0.80
G15-Et-F-LN300	5.74 ±1.9*	7.89 ±0.27*	1.05	0.78
G16-Et-F-LN450	6.24 ±2.4*	7.92 ±0.41*	1.15	1.10

Electrolyte excretion

Sodium (Na^+)

Sodium excretion varied across the groups, with the highest excretion observed in the standard groups (255 mmol/L in G0-M-STD-DOCA, 243 mmol/L in G0-F-STD-DOCA). Among the *L. nodiflora* treated groups, the male 450 mg/kg dose group (G7-Aq-M-LN450) exhibited sodium excretion of 221 mmol/L and the female 450 mg/kg group (G13-Aq-F-LN450) showed 236 mmol/L.

Potassium (K^+)

Potassium excretion was similar across most groups, with no significant differences. The control groups showed potassium excretion around 44 mmol/L, while the *L. nodiflora* treated groups had potassium values ranging from 44.53 mmol/L (G10-Et-M-LN450) to 45.47 mmol/L (G16-Et-F-LN450).

Chloride (Cl^-)

Chloride excretion was notably higher in the standard groups, with male (206 mmol/L) and female (189 mmol/L) DOCA-treated groups showing elevated chloride levels compared to the *L. nodiflora* treated groups. The highest chloride excretion in the LN-treated groups was seen in the male 300 mg/kg dose group (G9-Et-M-LN300) with 110 mmol/L and the female 450 mg/kg dose group (G13-Aq-F-LN450) with 100 mmol/L (Table – 11).

Natriuretic and Saluretic ratioss

Na^+/K^+ Ratio

The Na^+ /K^+ ratio in the *L. nodiflora* treated groups was consistent across doses, with the male 450 mg/kg group (G7-Aq-M-LN450) showing a ratio of 4.93 and the female 450 mg/kg group (G13-Aq-F-LN450) at 5.29. This was lower than the standard group (5.0) but consistent with the goal of balancing sodium and potassium excretion.

Cl^-/ (Na + K) Ratio

The Cl^-/ (Na + K) ratio showed a slight increase in the *L. nodiflora* treated groups compared to the control and standard, with the male 450 mg/kg group (G7-Aq-M-LN450) showing a ratio of 0.32, and the female 450 mg/kg group (G13-Aq-F-LN450) showing 0.30.

Overall, the results indicate that *L. nodiflora* treatment led to a significant increase in urine volume and enhanced diuretic activity, especially at higher doses (450 mg/kg). Sodium and chloride excretion were elevated in the *L. nodiflora* treated groups, indicating strong natriuretic and saluretic effects. The data also suggest that *L. nodiflora* can regulate electrolyte balance effectively, making it a promising natural diuretic (Table – 11).

Table 11: Effect of aqueous extracts of the LN on natriuretic effect, saliuretic effect and carbonic anhydrase inhibition at 21 days

Group	Na^+	K^+	Cl^-	$Na^+ + Cl^-$	Na^+/K^+	$Cl^-/(Na + K)$
G0-M-STD-DOCA	255	48.16	206	461	5	1
G0-F-STD-DOCA	243	42.1	189	432	6	1
G1-M-Control	241	44.27	103	344	5.44	0.30
G2-F-Control	240	44.58	101	341	5.38	0.30
G3-M-DOCA	213	45.65	102	315	4.67	0.32
G4-F-DOCA	209	45.9	105	314	4.55	0.33
G5-Aq-M-LN150	217	45.24	99	316	4.80	0.31
G6-Aq-M-LN300	223	45.31	107	330	4.92	0.32
G7-Aq-M-LN450	221	44.84	105	326	4.93	0.32
G8-Et-M-LN150	232	45.32	104	336	5.12	0.31
G9-Et-M-LN300	238	44.67	110	348	5.33	0.32
G10-Et-M-LN450	229	44.53	98	327	5.14	0.30
G11-Aq-F-LN150	231	45.34	103	334	5.09	0.31
G12-Aq-F-LN300	230	45.81	101	331	5.02	0.31
G13-Aq-F-LN450	236	44.64	100	336	5.29	0.30
G14-Et-F-LN150	241	44.72	102	343	5.39	0.30
G15-Et-F-LN300	234	45.21	107	341	5.18	0.31
G16-Et-F-LN450	228	45.47	105	333	5.01	0.32

Effect on Blood pressure

The effects of *L. nodiflora* aqueous and ethanolic extracts on systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) were evaluated in male and female rats at different dosages over a 21-day period. The control and standard groups (G1-M-Control and G2-F-Control) showed normal blood pressure levels with systolic pressures of 124 ± 1.42 mmHg and 121 ± 1.00 mmHg, diastolic pressures of 82 ± 2.24 mmHg and 80 ± 3.84 mmHg, and MAPs of 101 ± 3.25 mmHg and 99 ± 1.42 mmHg, respectively. The positive control groups, G3-M-DOCA and G4-F-DOCA, exhibited significantly elevated blood pressures, with systolic pressures of 184 ± 1.42 mmHg and 176 ± 3.65 mmHg, diastolic pressures of 143 ± 4.16 mmHg and 136 ± 1.09 mmHg, and MAPs of 161 ± 3.56 mmHg and 152 ± 1.42 mmHg, respectively. The aqueous and ethanolic extracts of *L. nodiflora* significantly reduced systolic, diastolic and mean arterial blood pressures in a dose-dependent manner. Aqueous extracts at the 150 mg/kg dose (G5-Aq-M-LN150), systolic pressure was 173 ± 4.15 mmHg, diastolic pressure was 137 ± 6.04 mmHg and MAP was 155 ± 3.51 mmHg. At the 300 mg/kg dose (G6-Aq-M-LN300), systolic pressure decreased to 164 ± 6.21 mmHg, diastolic pressure to 126 ± 5.17 mmHg, and MAP to 145 ± 4.08 mmHg. The highest dose, 450 mg/kg (G7-Aq-M-LN450), resulted in further reductions, with systolic pressure at 160 ± 4.08 mmHg, diastolic pressure at 124 ± 5.64 mmHg, and MAP at 143 ± 3.34 mmHg. Ethanolic extracts at 150 mg/kg (G8-Et-M-LN150), systolic pressure was reduced to 166 ± 3.58 mmHg, diastolic pressure to 128 ± 4.07 mmHg, and MAP to 147 ± 5.42 mmHg. At the 300 mg/kg dose (G9-Et-M-LN300), systolic pressure decreased to 159 ± 5.87 mmHg, diastolic pressure to 119 ± 4.06 mmHg, and MAP to 137 ± 3.62 mmHg. The 450 mg/kg dose (G10-Et-M-LN450) led to the most significant reduction in blood pressure, with systolic pressure at 146 ± 6.27 mmHg, diastolic pressure at 101 ± 7.04 mmHg, and MAP at 123 ± 2.42 mmHg. The aqueous and ethanolic extracts also showed similar effects in female rats. At the 150 mg/kg dose (G11-Aq-F-LN150), systolic pressure was 174 ± 5.07 mmHg, diastolic pressure was 138 ± 8.04 mmHg and MAP was 156 ± 3.74 mmHg. At 300 mg/kg (G12-Aq-F-LN300), systolic pressure decreased to 161 ± 6.09 mmHg, diastolic pressure to 124 ± 3.34 mmHg, and MAP to 142 ± 3.28 mmHg. At 450 mg/kg (G13-Aq-F-LN450), systolic pressure was reduced to 152 ± 5.27 mmHg, diastolic pressure to 114 ± 2.62 mmHg, and MAP to 132 ± 3.61 mmHg. For the ethanolic extract, at 150 mg/kg (G14-Et-F-LN150), systolic pressure was 172 ± 6.17 mmHg, diastolic pressure was 134 ± 4.02 mmHg, and MAP was 155 ± 2.71 mmHg. At 300 mg/kg (G15-Et-F-LN300), systolic pressure was 148 ± 5.98 mmHg, diastolic pressure was 108 ± 4.16 mmHg, and MAP was 130 ± 6.07 mmHg. At the highest dose, 450 mg/kg (G16-Et-F-LN450), systolic pressure decreased to 132 ± 7.18 mmHg, diastolic pressure to 95 ± 3.42 mmHg, and MAP to 113 ± 5.32 mmHg (Table – 12).

Table 12: Effect of Aq. & 50% Ethanolic LN extract on Systolic BP, Diastolic BP, and Mean Arterial BP in SD rats (all values are expressed as mean ± SEM (n=2) $^{\#}$ $p<0.05$ as compared with the Male Positive control group & $^{@}$ $p<0.05$ as compared with the Female Positive control group. All data are analyzed by one-way ANOVA followed by Dunnett's test)

Group	Systolic BP (mmHg)	Diastolic BP (mmHg)	Mean Arterial BP (mmHg)
G0-M-STD-DOCA	120 ± 1.32	84 ± 1.24	109 ± 2.35
G0-F-STD-DOCA	121 ± 1.02	82 ± 2.84	105 ± 2.42
G1-M-Control	124 ± 1.42	82 ± 2.24	101 ± 3.25
G2-F-Control	121 ± 1.00	80 ± 3.84	99 ± 1.42
G3-M-DOCA	184 ± 1.42	143 ± 4.16	161 ± 3.56
G4-F-DOCA	176 ± 3.65	136 ± 1.09	152 ± 1.42
G5-Aq-M-LN150	173 ± 4.15	137 ± 6.04	155 ± 3.51
G6-Aq-M-LN300	164 ± 6.21	126 ± 5.17	145 ± 4.08
G7-Aq-M-LN450	160 ± 4.08	124 ± 5.64	143 ± 3.34
G8-Et-M-LN150	166 ± 3.58	128 ± 4.07	147 ± 5.42
G9-Et-M-LN300	159 ± 5.87	119 ± 4.06	137 ± 3.62
G10-Et-M-LN450	146 ± 6.27	101 ± 7.04	123 ± 2.42
G11-Aq-F-LN150	174 ± 5.07	138 ± 8.04	156 ± 3.74
G12-Aq-F-LN300	161 ± 6.09	124 ± 3.34	142 ± 3.28
G13-Aq-F-LN450	152 ± 5.27	114 ± 2.62	132 ± 3.61
G14-Et-F-LN150	172 ± 6.17	134 ± 4.02	155 ± 2.71
G15-Et-F-LN300	148± 5.98	108 ± 4.16	130 ± 6.07
G16-Et-F-LN450	132 ± 7.18	95 ± 3.42	113 ± 5.32

Discussion

Hypertension, or high blood pressure, is a chronic medical condition characterized by persistently elevated blood pressure levels, which poses a significant risk factor for cardiovascular diseases, stroke, kidney failure, and other severe complications. It is one of the most prevalent and pressing global health challenges, affecting millions of people worldwide. The global rise in hypertension cases is driven by several factors, including an aging population, sedentary lifestyles, poor dietary habits, and increased stress levels (Burnier and Damianaki, 2023; Mills *et al.*, 2020) [7, 18]. Managing hypertension is crucial to preventing long-term cardiovascular damage, and it often requires a combination of lifestyle modifications and pharmacological interventions. However, current antihypertensive drugs can cause significant side effects, such as electrolyte

imbalances, kidney damage, and increased risk of cardiovascular events over prolonged use. This underscores the importance of developing safer, more effective treatment options (Khalil and Zeltser, 2023; Nguyen *et al.*, 2010) [21].

In this context, *L. nodiflora*, a traditional medicinal plant, emerges as a promising candidate for hypertension management. Known for its diuretic, anti-inflammatory, and antioxidant properties, LN has been utilized in various cultures for treating a range of ailments, including hypertension, edema, and urinary disorders (Cheng *et al.*, 2015; Gadhvi *et al.*, 2012; Sharma, 2018) [8, 12, 17]. This plant's ability to enhance urine output (diuresis), promote sodium excretion (natriuresis), and regulate electrolyte balance positions it as an ideal alternative or adjunct to conventional antihypertensive drugs (Gadhvi *et al.*, 2012). The significant reduction in blood pressure observed in the present study further validates the therapeutic potential of *L. nodiflora* in controlling hypertension, particularly given its mild side effect profile compared to synthetic diuretics.

Moreover, *L. nodiflora* offers several advantages in managing hypertension. Its natural origin makes it a more acceptable and safer alternative, especially for long-term use (Al-Snai, 2019) [2]. By promoting diuresis and enhancing electrolyte regulation, it helps reduce blood volume, thus lowering blood pressure, without the adverse effects commonly associated with many pharmaceutical diuretics (Gadhvi *et al.*, 2012) [12]. As the search for natural remedies to treat chronic conditions like hypertension grows, *L. nodiflora* stands out as a viable candidate that may provide an effective, sustainable, and side-effect-free solution for patients struggling with hypertension (Kamyab *et al.*, 2021) [15]. Further pharmacological studies and clinical trials are necessary to fully understand its mechanisms and establish its role in modern hypertension management.

The present study investigated the diuretic and antihypertensive effects of *L. nodiflora* in Wistar albino rats, using aqueous and ethanolic extracts at varying doses. The results from this study demonstrate significant diuretic, natriuretic and saluretic effects, along with a dose-dependent reduction in blood pressure, which suggests the potential therapeutic use of *L. nodiflora* in managing conditions like hypertension and fluid retention. The diuretic activity of *L. nodiflora* was assessed over multiple time points (5 h, 24 h, 7 days, 14 days, and 21 days), with the highest diuretic effects observed at 5 h and 7 days. In the short-term (5 h) study, the LN-treated groups, particularly the 500 mg/kg dose, demonstrated a significant increase in urine volume (2.17 ± 0.85 mL), which was nearly double that of the control group (1.17 ± 0.59 mL). This increase in urine volume was accompanied by a shift to an alkaline pH in the LN 250 mg/kg group, further supporting the diuretic potential of LN. The enhanced electrolyte excretion, specifically sodium (238.80 ± 7.90 mmol/L) and chloride (164.16 ± 17.17 mmol/L) in the LN 500 mg/kg group, also indicates a strong natriuretic and saluretic effect, while potassium excretion remained relatively stable across the groups. At the 24 h and 7-day time points, the diuretic effects persisted, with the 500 mg/kg dose showing significant increases in urine volume and electrolyte excretion. Notably, the sodium excretion (185.38 ± 7.32 mmol/L) and chloride excretion (116.47 ± 17.05 mmol/L) were higher than the control group and the Na^+/K^+ ratio was elevated, indicating that *L. nodiflora* extracts exhibit strong natriuretic and saluretic activities. These results are consistent with previous studies on natural diuretics that have demonstrated similar sodium and chloride excretion patterns, suggesting that LN could be beneficial for conditions associated with fluid retention, such as edema or heart failure. The long-term diuretic effects observed at the 14-day and 21-day time points further highlight the sustainability of *L. nodiflora* effects. At 14 days, the urine volume increased significantly in the 250 mg/kg and 500 mg/kg groups. The sodium and chloride excretion were notably higher in the LN-treated groups, with the 500 mg/kg dose (319.97 ± 2.86 mmol/L for sodium and 170.03 ± 0.39 mmol/L for chloride) surpassing both the control and standard groups. Furthermore, the Na^+/K^+ ratio and $Cl^-/(Na + K)$ ratio indicated that *L. nodiflora* also enhances the balance of electrolytes, promoting the excretion of sodium and chloride while maintaining potassium levels within physiological limits. This consistent diuretic activity over 21 days reinforces the therapeutic potential of *L. nodiflora* in chronic conditions requiring prolonged diuretic action. Moreover, the antihypertensive effects of *L. nodiflora* were assessed by measuring systolic blood pressure, diastolic blood pressure and mean arterial pressure over a 21-day period. The control and standard groups (furosemide-treated) showed normal blood pressure levels, while the DOCA-induced hypertensive rats exhibited significantly elevated blood pressure, with SBP reaching up to 184 ± 1.42 mmHg in males and 176 ± 3.65 mmHg in females. However, treatment with *L. nodiflora* extracts significantly reduced blood pressure in a dose-dependent manner. In male rats, the 450 mg/kg dose of *L. nodiflora* resulted in the most significant reduction in SBP (160 ± 4.08 mmHg), DBP (124 ± 5.64 mmHg), and MAP (143 ± 3.34 mmHg). Similarly, the female rats treated with the 450 mg/kg dose showed a marked decrease in SBP (152 ± 5.27 mmHg), DBP (114 ± 2.62 mmHg), and MAP (132 ± 3.61 mmHg). These results suggest that *L. nodiflora* possesses strong antihypertensive properties, possibly due to its diuretic effects, as the reduction in blood pressure was associated with increased urinary excretion of sodium and chloride.

The antihypertensive and diuretic effects of *L. nodiflora* may be attributed to its active compounds, which likely act on the kidneys to promote natriuresis and diuresis (Al-Snai, 2019; Alafnan *et al.*, 2023; Amir, 2011) [2, 5]. The increased sodium excretion leads to a reduction in blood volume, thereby lowering blood pressure. Additionally, the saluretic effects further contribute to maintaining fluid balance and reducing edema, which are common concerns in hypertensive patients. The ability of *L. nodiflora* to regulate electrolyte balance, particularly its mild effect on potassium and calcium excretion, further supports its potential as a safe and effective diuretic for long-term use. Moreover, the minimal changes in calcium excretion suggest that *L. nodiflora* may be safer than other diuretics that can lead to significant electrolyte imbalances.

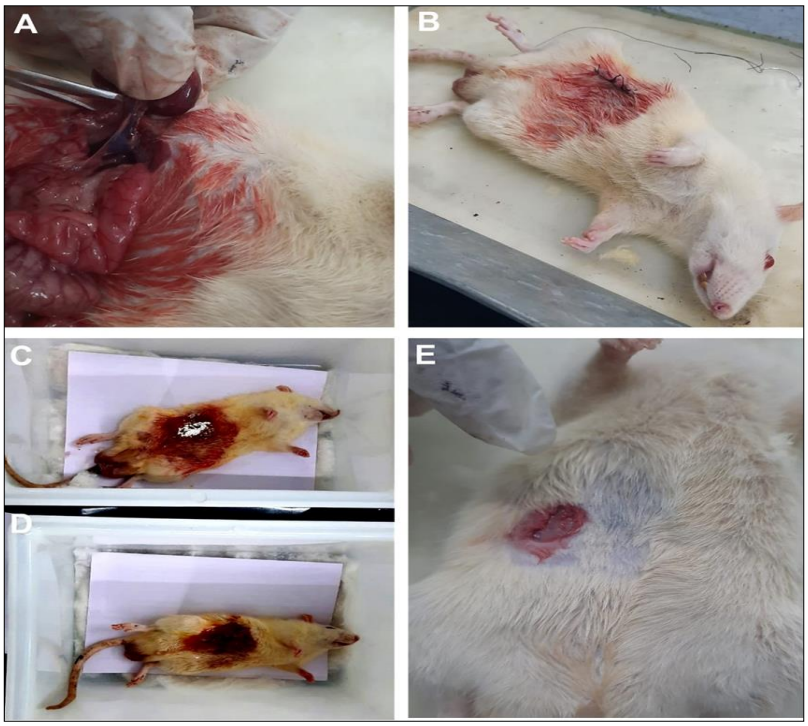

Fig 1: Different stages of the DOCA-salt method to induce hypertension in Wistar albino rats. **A:** Excise the kidney carefully, ensuring no remaining attachments. **B:** Close the muscle layer with absorbable sutures, then close the skin with non-absorbable sutures. Apply a topical antibiotic ointment to the incision site. **C, D:** Gently retract the muscles to expose the peritoneal cavity. **E:** Make a small (1–2 cm) incision in the skin and muscle layers along the flank, parallel to the spine and below the rib cage. Retract the muscles gently to expose the peritoneal cavity.

Conclusion

The present study demonstrates the significant diuretic and antihypertensive potential of *L. nodiflora* aqueous and ethanolic extracts in Wistar albino rats. The results revealed that LN extracts exhibit dose-dependent diuretic activity, characterized by increased urine volume, enhanced sodium and chloride excretion and strong natriuretic and saluretic effects, with minimal impact on potassium and calcium levels. These findings highlight the ability of LN to regulate electrolyte balance effectively, making it a safe option for long-term use. The antihypertensive effects of LN were equally promising, as evidenced by significant reductions in systolic, diastolic and mean arterial blood pressures in hypertensive rats. The extracts were particularly effective at higher doses (450 mg/kg), demonstrating sustained blood pressure control over a 21-day period. These effects are likely linked to the diuretic action of LN, which facilitates sodium and fluid excretion, reducing blood volume and pressure. Overall, *L. nodiflora* presents itself as a potential natural alternative for the management of hypertension and conditions associated with fluid retention, such as edema and congestive heart failure. Future studies focusing on the isolation and characterization of its active compounds, as well as clinical trials, are essential to establish its efficacy and safety in humans. The findings of this study providing the way for the development of LN-based therapeutic interventions for cardiovascular and renal disorders.

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