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Diuretic activity of ethanolic extract and fraction enriched in saponins from *Solanum sisymbriifolium* Lam. root in rats

W.J. ARRÚA, J.G. DUARTE, M.C. HELLIÓN-IBARROLA, D.A. IBARROLA

Department of Pharmacology, Faculty of Chemical Sciences, National University of Asuncion, UNA Campus, San Lorenzo, Paraguay

Abstract. – OBJECTIVE: *Solanum sisymbriifolium* Lam. is a native perennial plant with chemical characteristics of therapeutic importance. In Paraguayan traditional medicine, it is attributed to antihypertensive and diuretic activities. For this reason, the objective of the study was to evaluate the effect of acute oral administration of the ethanolic extract and fraction enriched in saponins obtained from the root of *S. sisymbriifolium* on the diuresis profile of rats.

MATERIALS AND METHODS: Male Wistar rats were used, randomly distributed in 6 groups to evaluate the diuretic activity. The control group received distilled water; the diuretic group was treated with 20 mg/kg of furosemide. Two groups were treated with 50 and 100 mg/kg of the ethanolic extract of *S. sisymbriifolium*, and two other groups were treated with 1 and 10 mg/kg with the fraction enriched in saponins. The animals were placed in individual metabolic cages for a period of 24 h. Urine volume was determined at 5 and 24 h, and urinary electrolytes, pH, and glomerular filtration rate at 24 h.

RESULTS: The findings indicated that both doses of the ethanolic extract and the saponin-enriched fraction significantly increased diuresis after 24 hours of treatment. Urinary pH was not affected. A significant increase in the urinary excretion of Na^+ and Cl^- was observed without affecting the elimination of K^+ with both doses of the extracts. In addition, a significant increase in GFR was evidenced.

CONCLUSIONS: Both ethanolic extract and saponins enriched fraction, presented natriuretic and saluretic effects with a possible mechanism of action mediated, at least partially, by the inhibition of carbonic anhydrase. Furthermore, it was possible to demonstrate the participation of the COX/PG pathway in the diuretic mechanism of the extracts in male rats.

Key Words:

Solanum sisymbriifolium Lam., Diuresis, Furosemide, Saponin, Urinary electrolytes.

Introduction

Diuretic agents are first-line therapy for generalized cardiovascular and non-cardiovascular disease. Traditional diuretics are commonly prescribed for the treatment of hypertension, edema, and heart failure, as well as a number of kidney problems[1]. These pathologies present high mortality globally and regionally, and their incidence is increasing year after year. Some diuretics currently available for clinical use exhibit an overall favorable risk/benefit balance. However, they are not free of side effects. Diuretics induce a loss of electrolytes and fluids, thus stimulating various compensatory mechanisms, such as the renin-angiotensin-aldosterone system (RAAS), which results in increased renal sodium retention by all segments of the nephron, a phenomenon known as diuretic resistance[2]. The main side effect of loop and thiazide diuretics is deficiency of major electrolytes, particularly sodium and potassium[3]. Hypokalemia can increase the risk of arrhythmia and cardiac arrest, including cramps, polyuria, and muscle weakness. Most diuretics also decrease urate excretion and increase blood uric acid, leading to gout in predisposed patients[4]. The use of traditional diuretics that directly block the reabsorption of salts is limited by the hydro-electrolyte imbalance that it produces[5]. This electrolyte imbalance, together with resistance to diuretics and other problems in their clinical use, has been the focus of the search for new alternatives for the development of drugs with a more favorable and safe pharmacological profile.

Medicinal plants with diuretic properties are promising agents for the search for new drugs since they can be subjected to pre-clinical and clinical studies that support this biological effect[6].

Corresponding Author: Wilfrido Javier Arrúa Báez, MD; e-mail: warrua@qui.una.py

Solanum sisymbriifolium Lam. is a perennial subshrub native to Paraguay, popularly known as Ñuatĩ pytã (in Guarani), red spine, or horse burst. The root decoction is used in traditional Paraguayan medicine for its diuretic and antihypertensive properties. Several studies[7-9] reported the antihypertensive and cardioprotective ability of extracts from the root of *S. sisymbriifolium*. The plant contains saponins, such as nuatigenin-3-O-β-chacotriose, which are the most abundant group of biologically active compounds present in the plant[10]. Recent research[11] has demonstrated the antihypertensive and diuretic effect of saponin-enriched extracts of *S. sisymbriifolium* in hypertensive rat models induced experimentally by L-NAME.

The study examines the diuretic properties of *S. sisymbriifolium* Lam., a plant commonly used in Paraguayan medicine, which bridges the gap between traditional phytotherapy and modern pharmacology. Thus, the purpose of this research was to evaluate the acute oral diuretic effect of ethanolic extract and fraction enriched in saponins from *S. sisymbriifolium* Lam. root in male rats by determining its urinary volume and pH, urinary electrolyte concentration, and the glomerular filtration rate.

Materials and Methods

Chemicals

For the biological assay, furosemide, indomethacin, and Nω-Nitro-L-arginine methyl ester (L-NAME) were purchased from Chemical Company (Sigma-Aldrich, St. Louis, MO, USA). Sodium pentobarbital (Nembutal) was purchased from Abbott (Minato-ku, Tokyo, Japan), and ethanol for pharmaceutical use was obtained locally. The reagent kits for the determination of creatinine and electrolytes were obtained from Human Diagnostics Worldwide Reagent (Magdeburgo, Sajonia-Anhalt, Germany).

Plant Collection and Identification

The fresh roots of *Solanum sisymbriifolium* Lam. were collected in Caacupé (Cordillera, Paraguay), and whole plant samples were processed and identified in the Department of Botany of the Facultad de Ciencias Químicas of the Universidad Nacional de Asunción (FCQ-UNA). One specimen of the sample was coded (Soria 5248) and deposited in the department's Herbarium. The crude ethanolic extract (EESs) and the saponins enriched fraction (SSs) were obtained from the dry powder of the root of *S. sisymbriifolium* Lam. and submitted to a subsequent phytochemical analysis through thin-layer chromatography (TLC) and ultra-performance liquid chromatography (HPLC), as previously described[11].

Animals

In the study were used male Wistar rats (12-week-old, 250-300 g) from the Animal Facility Prof. Dr. Yenny Montalbetti Moreno of the Department of Pharmacology of the Facultad de Ciencias Químicas of the Universidad Nacional de Asunción. The animals housed in the laboratory were kept in propylene boxes with a 12-hour cycle of light and darkness under temperature conditions of 24±2ºC, humidity 40-70%, and fed commercial feed (30 g/animal/day) and water *ad libitum*. All procedures were carried out in accordance with the ARRIVE and Animal Scientific Procedure UK (1986) guidelines[12,13]. The work protocol was evaluated and approved by the FCQ-UNA Research Ethics Committee, according to CEI 869/2022.

Diuretic Activity

The diuretic activity was determined according to the methodology described by Kau et al[14] (1984) with some modifications. The experimental animals were grouped into six groups of six animals each group. The rats were fasted overnight with free access to water prior to the administration of the different substances. The control group was administered 10 mL/100 g body weight of distilled water, while the reference group was administered 20 mg/kg of furosemide (positive diuretic control). The experimental groups were treated with doses of 50 and 100 mg/kg of the EESs, and doses of 1 and 10 mg/kg of the SSs, respectively. Immediately after dosing, the animals were sequentially introduced into the metabolic cages according to timing and treatment. After 5 and 24 hours of treatment, the urine was collected in graduated vials, and the volume was measured. The dose and dosing schema were selected based on previously published work[11], based on the acute oral toxicity test according to the OECD guidelines (Organization for Economic Co-operation and Development), where they determined that lethality occurs at doses greater than 1,000 mg/kg in mice orally.

Before treatment, all groups underwent a one-week adaptation period. During this period, the animals were individually placed in metabolic

cages for 24 hours, and their body weight and urine volume were recorded. This adaptation was necessary to reduce the stress to which the animals were subjected during the treatment period, and it was carried out until the volume of urine excreted remained constant.

The total urine volume of all rats was measured after 5 and 24 hours of treatment. Also, 24-hour urine was used for pH determination, and the urinary Na^+, K^+, and Cl^- concentrations were evaluated by Ion-Selective Electrode analysis (AVL 9180 Electrolyte analyzer, Roche, Germany). Electrolyte ratios Na^+/K^+ and $Cl^-/(Na^+ + K^+)$ were calculated to estimate natriuretic and carbonic anhydrase inhibitory activities (CAI), respectively[15]. The sum of Na^+ and Cl^- concentrations was performed to determine saluretic activity[16,17].

Effect of EESs and SSs from S. sisymbriifolium Lam. on Rat's Glomerular Filtration Rate (GFR)

Glomerular filtration rate was obtained using the creatinine clearance method described by Cortadellas et al[18] (2012), which consists of measuring the amount of creatinine eliminated from the circulation into the urine as it passes through the kidneys. Blood samples were obtained by puncture of the caudal vein from anesthetized rats with 50 mg/kg of sodium pentobarbital. The blood level of creatinine and the 24-hour urine of rats submitted to the metabolic cage was determined using a colorimetric method (BTS 350 semiautomatic analyzer, Biosystem, Barcelona, Spain) with HUMAN brand reagent kit and graded volume cylinder, respectively. GFR is calculated from the formula: GFR = (24-hour urine creatinine concentration x 24-hour urine volume/ plasma creatinine concentration x time x animal body weight) x 100[18].

Diuretic Activity and Evaluation of The Mechanism of Action

The possible mechanisms of action of the extracts were evaluated by determining the diuretic activity of the extracts with the co-administration of substances inhibiting cyclooxygenase and nitric oxide synthase[19]. Briefly, three different groups of male rats were pretreated orally with saline, 100 mg/kg of EESs, and 10 mg/kg of SSs, respectively. After 30 minutes, each group received sequentially saline, N(ω)-nitro-L-arginine methyl ester (60 mg/kg, L-NAME, p.o.), as nitric oxide synthesis inhibitor[20], or indomethacin (5 mg/kg, i.p.), as prostaglandin synthesis inhibitor[21], respectively by oral route. The doses of the extracts that presented the greatest diuretic activity in the trials cited above were chosen for pretreatment. After 24 h, urine was collected in a graduated cylinder, and the excreted volume was measured.

Statistical Analysis

The results were expressed as mean ± standard deviation (SD); One-way ANOVA followed by Dunnett's multiple comparisons test was performed using GraphPad Prism version 9.0 for Windows (GraphPad Software, La Jolla, CA, USA). The level of probability (*p*) lower than 0.05 was considered statistically significant.

Results

Acute Oral Administration of EESs and SSs in Rats Presented a Pronounced Diuretic Effect After 24 Hours

The results of the effect of the administration of both extracts of *S. sisymbriifolium* Lam. (EES and SSs) on diuresis in rats are shown in Table I. Rats treated with the standard diuretic drug (furosemide, 20 mg/kg) presented a statistically significant increase in urinary volume at 5 and 24 hours after treatment compared to the control group. The oral administration of both doses of the EESs provoked a significant increase in the volume of urine excreted within 24 hours after treatment. However, at 5 hours of treatment, there were no significant changes in excreted urine with both doses compared to the control group. Additionally, a significant increase in urinary volume at 5 and 24 hours after treatment was denoted with 10 mg/kg of SSs. However, a statistically significant increase in urinary volume was observed with 1 mg/kg of SSs only at 24 hours compared to the control group.

Concerning the diuretic action, the dose of 10 mg/kg of the SSs, when compared with the dose of 100 mg/kg of EESs, showed higher values in diuretic potency. The higher dose of SSs (10 mg/kg) produced a diuretic action of 3.01, while the higher dose of EEss (100 mg/kg) produced a diuretic action of 2.82 at 24 hours of treatment. Additionally, the diuretic activity of all doses of EESs and SSs denoted a comparable diuretic activity to 20 mg/kg of furosemide (reference diuretic drug) at 24 hours of treatment. The higher dose of EESs (100 mg/kg) produced a diuretic action of 0.79, while the higher dose of SSs (10 mg/kg) produced a diuretic action of 0.84.

Table I. Effect of EESs and SSs of *S. sisymbriifolium* Lam. on urine volume in rats restrained individually in metabolic cages and measured at 5 and 24 h after treatment.

Groups	5 h			24 h		
	Volume of urine (mL)	Diuretic action[a]	Diuretic activity[b]	Volume of urine (mL)	Diuretic action[a]	Diuretic activity[b]
Control	1.23±0.75	1.00	-	6.05±1.30	1.00	-
Furosemide (20 mg/kg)	10.82±3.34****	8.80	1.00	21.59±5.61****	3.57	1.00
EESs (50 mg/kg)	1.66±1.58	1.35	0.15	13.73±4.06***	2.27	0.64
EESs (100 mg/kg)	1.79±0.45	1.46	0.16	17.05±4.31****	2.82	0.79
SSs (1 mg/kg)	1.85±0.99	1.50	0.17	13.58±4.45***	2.24	0.63
SSs (10 mg/kg)	3.62±1.03*	2.94	0.33	18.20±5.57****	3.01	0.84

Values are expressed as mean ± SD. [a]Diuretic action = urine volume of test group/urine volume of control group. [b]Diuretic activity = urine volume of test group/urine volume of furosemide group. Significant change at *p<0.05, ***p<0.001 and ****p<0.0001 with respect to control rats. EESs: crude ethanolic extract of *S. sisymbriifolium* Lam.; SSs: saponins enriched fraction from *S. sisymbriifolium* Lam.

Effect of Acute Oral Administration of EESs and SSs on Urinary Excretion of Electrolytes and pH

Urine samples from rats individually restrained in metabolic cages and collected after 24 h were used for the urinary electrolyte analysis. The main electrolyte profiles are described in Table II.

As expected, the diuretic control group produced a significant increase in the urinary excretion of Na^+ and Cl^- at 24 hours, compared to the control group, without affecting the elimination of K^+. In addition, a significant increase in Na^+ and Cl^- excretion was induced by all doses of EESs and SSs when compared with the control (saline) group, without affecting K^+ clearance. Besides, higher elimination indexes of the different ions were observed in comparison with those of the control group.

Moreover, Table III shows the results for natriuretic, saluretic activity, and carbonic anhydrase inhibition. The sum of the urinary excretion values of Na^+ and Cl^- was used to indicate saluretic activity, while the Na^+/K^+ ratio was calculated as a parameter of natriuretic activity[15]. The furosemide (20 mg/kg), EESs (50 and 100 mg/kg), and SSs (1 and 10 mg/kg) showed potent saluretic and natriuretic activity compared to the saline control group. Additionally, the $Cl^-/(Na^+ + K^+)$ ratio was calculated as an index of carbonic anhydrase inhibition[15]. All doses of the extracts used presented a higher index of CAI compared to the control group.

The urine pH of rats submitted, respectively, to EESs and the SSs treatments was not modified compared to the saline group. In contrast, a statistically significant decrease in urine pH was induced in animals treated with a control diuretic drug compared to the saline-treated group (Figure 1A).

Acute Oral Administration of EESs and SSs Improved GRF

As depicted in Figure 1B, groups treated with the positive diuretic drug (20 mg/kg furosemide),

Table II. Effect of EESs and SSs obtained from *S. sisymbriifolium* Lam., on urinary electrolyte excretion level of rats restrained individually in metabolic cages and measured in sample collected at 24 h.

Groups	Urinary Na^+ (mEq/24 h)[a]	Urinary K^+ (mEq/24 h)[a]	Urinary Cl^- (mEq/24 h)[a]	Na^+ index[b]	K^+ index[b]	Cl^- index[b]
Control	0.41±0.10	1.02±0.36	1.23±0.07	1.00	1.00	1.00
Furosemide (20 mg/kg)	1.84±0.51***	1.21±0.52	4.41±1.06****	4.49	1.19	3.58
EESs (50 mg/kg)	1.04±0.05**	1.56±0.65	2.93±0.60***	2.54	1.53	2.38
EESs (100 mg/kg)	1.23±0.24**	1.46±0.08	2.60±0.26***	3.00	1.43	2.11
SSs (1 mg/kg)	0.94±0.24*	1.35±0.86	2.31±0.44**	2.29	1.32	1.87
SSs (10 mg/kg)	1.02±0.38*	0.77±0.21	2.11±0.71*	2.49	0.75	1.71

[a]Values are expressed as mean ± SD. [b]Index = excretion in test group/excretion in control group. Significant change at *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 with respect to control rats. EESs: crude ethanolic extract of *S. sisymbriifolium* Lam.; SSs: saponins enriched fraction from *S. sisymbriifolium* Lam.

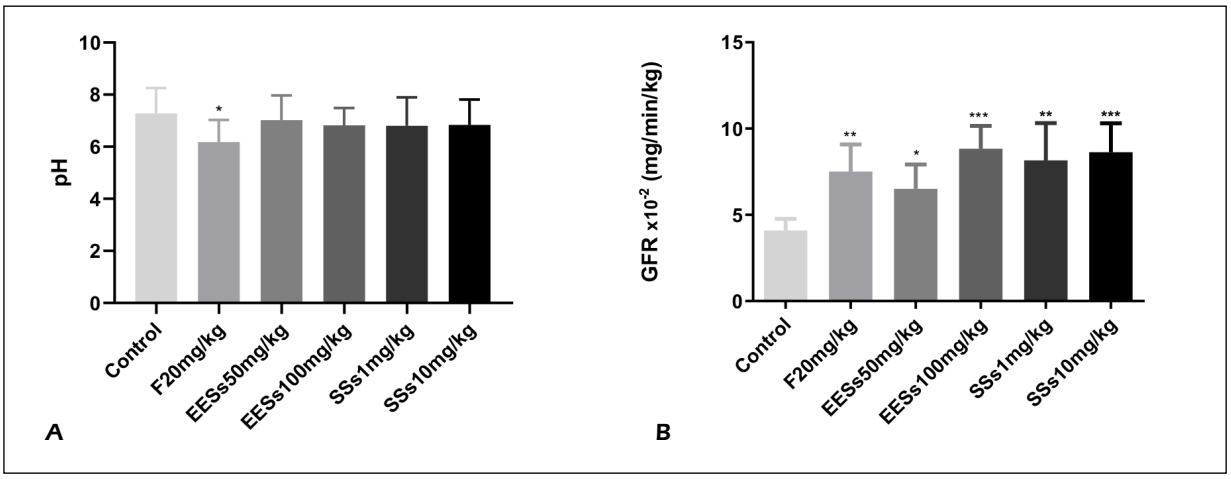

Figure 1. Effect of EESs and SSs obtained from *S. sisymbriifolium* Lam. on urinary pH (**A**) and glomerular filtration rate (GFR) (mg/min/kg) (**B**) of rats, restrained in metabolic cages. The urine was collected at 24 h of treatment (n=8). Values are expressed as mean ± SD. **p*<0.05, ***p*<0.01 and ****p*<0.001, significant against the control group. F20 mg/kg: furosemide; EESs: crude ethanolic extract of *S. sisymbriifolium* Lam.; SSs: Saponins enriched fraction from *S. sisymbriifolium* Lam.

doses of EESs, and SSs showed a diuretic effect accompanied by a significant increase in the glomerular filtration rate (GFR) when compared to the control group (saline). Also, the GFR potency of EESs and SSs is very similar to those induced by furosemide.

Evaluation of Potential Mechanism of Diuretic Property of EESs and SSs

A significant reduction of the diuretic effect of EESs and SSs when co-administered with indomethacin (5 mg/kg) was observed. However, the co-administration with L-NAME (60 mg/kg) did not modify the diuretic effect when compared with the control group (Figure 2).

Discussion

Diuretics may be helpful for treating hypertension and fluid retention due to renal, hepatic, or cardiac causes or in cases of hydroelectrolytic imbalances[2]. Thus, it is important to demonstrate the efficacy of extracts from *S. sisymbriifolium* Lam. as a potential diuretic agent.

The diuretic activity of the ethanolic extracts and fraction enriched in saponins of the root of *S. sisymbriifolium* Lam. was evaluated in rats using furosemide as a reference standard[14]. The implemented methodology was successfully validated since the reference standard drug used demonstrated the characteristic biological effect. Oral

Table III. Effect of EESs and SSs obtained from *S. sisymbriifolium* Lam. on natriuretic effect, saluretic effect and carbonic anhydrase inhibition of rats restrained individually in metabolic cages and measured in sample collected at 24 h.

Groups	Saluretic effect (Na^+ Cl^-)[a]	Natriuretic effect (Na/K)[a]	CAI (Cl^-/[Na^+ + K^+])[a]	Saluretic index[b]	Natriuretic index[b]	CAI index[c]
Control	1.61±0.04	0.39±0.12	0.83±0.17	1.00	1.00	1.00
Furosemide (20 mg/kg)	6.25±1.33****	2.07±0.58****	1.49±0.51	3.88	5.31	1.80
EESs (50 mg/kg)	3.79±0.82**	0.80±0.25*	1.32±0.30	2.35	2.05	1.59
EESs (100 mg/kg)	3.75±0.50**	0.95±0.31*	1.05±0.02	2.33	2.44	1.26
SSs (1 mg/kg)	3.30±0.64*	1.29±0.25**	1.25±0.40	2.05	3.31	1.51
SSs (10 mg/kg)	3.13±1.14*	1.43±0.36**	1.16±0.38	1.94	3.67	1.40

[a]Values are expressed as mean ± SD. [b]Index = excretion in test group/excretion in control group. [c]CAI: carbonic anhydrase inhibition. Significant change at **p*<0.05, ***p*<0.01, and *****p*<0.0001 with respect to control rats. EESs: crude ethanolic extract of *S. sisymbriifolium* Lam.; SSs: saponins enriched fraction from *S. sisymbriifolium* Lam.

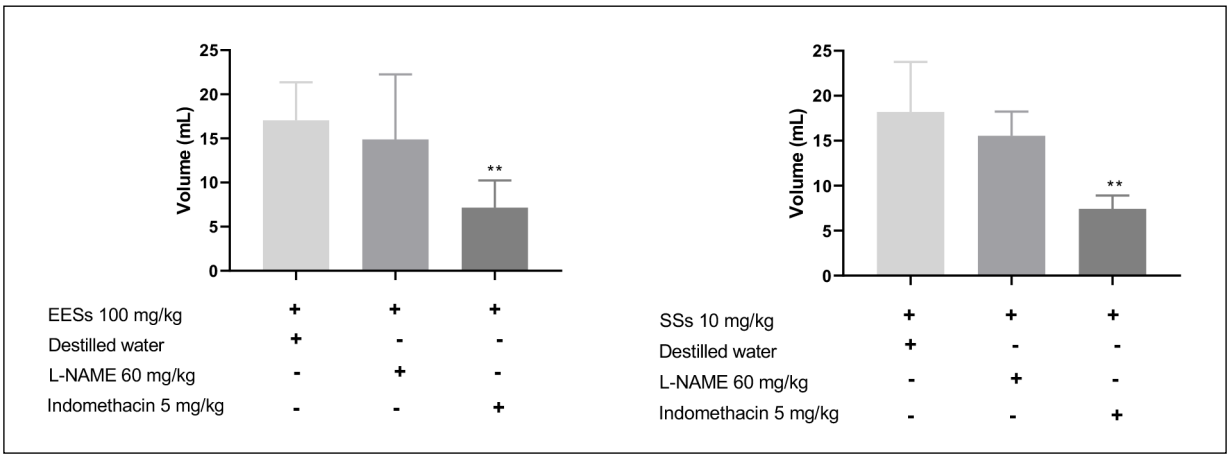

Figure 2. Diuretic activity of EESs and SSs. The diuretic activity of EESs and SSs was evaluated with the co-administration of L-NAME (60 mg/kg) and indomethacin (5 mg/kg). Data are representative of two independent experiments (n=8). Values are expressed as mean ± SD. ** $p<0.01$, significant against the control group (groups co-administered with the extracts and distilled water). EESs: crude ethanolic extract of *S. sisymbriifolium* Lam.; SSs: Saponins enriched fraction from *S. sisymbriifolium* Lam.

administration of 20 mg/kg of furosemide caused an increase in urinary volume at 5 and 24 hours, a decrease in urinary pH (increased H^+ excretion), and an increase in GFR and renal excretion of electrolytes (Na^+, Cl^-). Furosemide is a known loop diuretic, which blocks the $Na^+/K^+/2Cl^-$ symporter, a cotransporter system in the thick ascending limb of the loop of Henle, thereby increasing water elimination[22,23].

The fraction enriched in saponins with the highest dose (10 mg/kg) demonstrated a greater diuretic effect compared to the other doses of the extracts (Table I). Therefore, it is possible to suggest that the greater diuretic effect of SSs 10 mg/kg is attributed to its higher concentration of active components of saponins. These results support our previous study where a superior diuretic effect of saponins extracts of *S. sisymbriifolium* was demonstrated compared to the ethanolic extract in hypertensive rats in a chronic treatment protocol[11].

The diuretic action value evaluates the diuretic potentials of any substance. Diuretic action and activity are considered good if greater than 1.50, moderate if between 1.00-1.50, low or little if between 0.72-1.00, and null if less than 0.72[24,25]. In this way, the fraction enriched in saponins at the highest dose (10 mg/kg) presented a good diuretic action at 5 and 24 hours, which was similar to the reference drug; while the other doses of both EESs and SSs presented a good diuretic action only at 24 hours, and a moderate diuretic action at 5 hours (Table I). According to these results, both EESs and SSs have demonstrated good diuretic potential with all doses used after 24 hours of treatment. Additionally, the diuretic activities of the different experimental groups were calculated to compare the diuretic effect with the standard drug (Table I). The dose of 100 mg/kg of EESs and 10 mg/kg of SSs presented a diuretic activity comparable to the reference drug after 24 hours of treatment. This demonstrates that the active ingredients of *S. sisymbriifolium* could induce increased urine formation comparable to the effects of synthetic diuretics, such as furosemide.

Diuresis consists of two components: the first is an increase in urine volume (water excretion), and the second is a net loss of solutes (electrolytes)[2]. These processes result from the suppression of renal tubular reabsorption of water and electrolytes into the bloodstream[2]. Therefore, urinary electrolytes were also measured to evaluate the concentrations of the ions excreted by the experimental groups (Table II). The results demonstrated that both doses of EESs and SSs increased the urinary elimination of Na^+ and Cl^-, without affecting the elimination of K^+. Once again, these results are in accordance with our recent studies where a significant increase in the urinary excretion of Na^+ and Cl^- was observed in hypertensive rats treated with the ethanolic extract and saponin extract of *S. sisymbriifolium*, without affecting the K^+ levels[11]. Similar studies reported the increased urinary excretion of Na^+, K^+ and Cl^- with the administration of saponin extracts from *Herniaria glabra* L., a plant with diuretic and antihypertensive properties[17].

The value of sodium and chloride ion excretion was determined as an indicator for saluretic activity[15]. All the doses of the EESs and SSs increased significantly Na^+ and Cl^- excretion compared to the control (Table II). According to the results, it is reasonable to suggest that the diuretic effect of *S. sisymbriifolium* extracts is saluretic type in contrast to aquaretic type which is typical of most phytodiuretic agent[26].

The ratio Na^+/K^+ was calculated as a parameter for natriuretic activity[15]. Values greater than 2.00 indicate a favorable natriuretic effect, while proportions greater than 10.00 indicate a potassium-sparing effect[17]. Our results showed that both doses of EESs and SSs presented a ratio greater than 2.00 and less than 10.00, which translates into a favorable natriuretic effect of the extracts. These results indicate (Table III) that EESs and SSs extracts exhibit a good diuretic effect without too much urinary K^+ loss (Na^+/K^+ ratio greater than 1.00). In other words, the extracts increase the excretion of Na^+ more than that of K^+, which is considered a very good safety profile for diuretic agents because it avoids one of the possible adverse effects of synthetic diuretics, such as hypokalemia[3,23].

The higher CAI index of the groups treated with EESs, and SSs compared to the control group suggests that one of the possible mechanisms of action of the active ingredients of the extracts used could be the inhibition of carbonic anhydrase. It should be noted that, under physiological conditions, carbonic anhydrase plays an important role in the proximal reabsorption of HCO_3^-, Na^+, and Cl^- at the level of the proximal convoluted tubule of the renal glomerulus[23]. Therefore, this anhydrase inhibitory effect asserts that there is an increase in urinary volume with increased excretion of these ions[27], which agrees with our results since it is observed that the renal excretion rate of Na^+ and Cl^- is much higher compared to the control group (Table III).

Neither dose of both extracts affected urinary pH (Figure 1A). On the other hand, the administration of the different doses of EESs and SSs presented a diuretic effect accompanied by a significant increase in the glomerular filtration rate compared to the control group (Figure 1B). Similar results were obtained when using the ethanolic extract enriched in saponins of *Herniaria glabra* L. in rats, observing a significant increase in GFR after 24 hours of administration[17]. These results suggest several pathways of physiological action, such as a direct effect on blood pressure[7-11] that could affect GFR or glomerular blood flow.

Cyclooxygenase 1 (COX-1) is found in the glomerulus, medullary, and cortical region of the collecting ducts and in the afferent and efferent arterioles[28,29], ensuring the maintenance of the kidney's physiological functions, such as hemodynamic regulation and glomerular filtration rate (GFR)[30,31]. This isoform produces prostaglandin E2 (PGE_2) and prostaglandin D2 (PGD_2), which antagonize the vasoconstrictive action of angiotensin II (AngII), and inhibit the release of norepinephrine, respectively. In this way, these prostanoids promote vasodilation, increasing the perfusion of the organ and causing redistribution in the blood flow from the renal cortex to the nephrons in the intramedullary region[32]. Additionally, the PGE_2, alongside the $PGF_2\alpha$, possesses diuretic and natriuretic effects, and the PGE_2, like the PGI_2, antagonizes the action of vasopressin[33,34]. Although COX-2 has been initially considered pathological, it is also found constitutively in the kidneys. Like in COX-1, prostaglandins (PGs) also derive from COX-2, being important physiological modulators of vascular tone and hydric balance in the kidneys[35]. The prominent PGs in renal tissue are PGE_2 and PGI_2, where PGE_2 is synthesized by the tubular epithelium and interstitial cells, expressed in the renal tubules regulating the transport of chloride and sodium in the Henle loop, besides helping water transport and blood flow. PGI_2 is located in the renal cortex, controlling the glomerular filtration rate and renin secretion[36]. For this reason, it was decided to evaluate the diuretic effect of the extracts in the presence of a cyclooxygenase inhibitor, such as indomethacin. The results demonstrated that the diuretic activity of both EESs and SSs was totally inhibited with the co-administration of indomethacin (Figure 2). This suggests that prostaglandins participate in the diuretic effect of *S. sisymbriifolium*. These findings, in addition to supporting the possible diuretic mechanism of the extracts, also support their antihypertensive effect. By involving the COX/PG pathway, it also opens a range for the study of the anti-inflammatory activities of the extract. Currently, the role that inflammation plays in the development of kidney and cardiovascular diseases is known[37-39], so the extracts used have the potential for the search for new therapeutic alternatives.

On the other hand, the participation of nitric oxide, a powerful physiological vasodilator, was estimated with a nitric oxide synthetase inhibitor (L-NAME), with no alteration observed with oral co-administration (Figure 2).

The extracts used show a strong diuretic effect comparable to the standard drug used, and the natriuretic and saluretic effects observed with the different doses used lead us to propose the inhibition of Na^+-Cl^- symport and the carbonic anhydrase enzyme of the renal tubules as one of the possible mechanisms of action. The cyclooxygen pathway is possibly involved in the diuretic effect of the extracts since diuresis was prevented with the co-administration of a COX inhibitor. However, these statements are subject to more detailed pharmacotoxicological and molecular studies.

The diuretic activity of *S. sisymbriifolium* extracts may be due to the presence of active saponins molecules since the best diuretic effect was observed in extracts containing a higher concentration of saponins. There are several studies that have reported the diuretic activity of saponins, among them are triterpene saponins isolated from the root of *Ampelozizyphus amazonicus*[40] and saponins isolated from the leaves of *Herniaria glabra*[17]. Hence, further studies are necessary to confirm the mechanisms of action and the active substances responsible for the diuretic activity of the *Solanum sisymbriifolium* Lam.

Conclusions

The study examines the diuretic properties of *Solanum sisymbriifolium* Lam., which bridges the gap between traditional phytotherapy and modern pharmacology. We confirmed the ethnopharmacological use of *S. sisymbriifolium* Lam. as a diuretic agent. Both extracts presented natriuretic and saluretic effects with a possible mechanism of action mediated, at least partially, by the inhibition of carbonic anhydrase. Furthermore, the possible participation of prostaglandins synthesized from COX expressed constitutively at the kidney level is observed in the diuretic effect. Additionally, the increase in the glomerular filtration rate supports this biological effect. The diuretic activity of *S. sisymbriifolium* extracts may be due to the presence of active saponin molecules. Finally, the diuretic effect supports the potential antihypertensive effect of this plant species.

Conflict of Interest
The authors declare that they have no conflict of interest.

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Ethics Approval
The working protocol was evaluated and approved by the Facultad de Ciencias Químicas of the Universidad Nacional de Asunción Research Ethics Committee with No. CEI 869/2022 on June 13, 2022.

Informed Consent
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Authors' Contribution
W.J. Arrúa was involved in the design of the study, the experimental implementation, and data collection. J.G. Duarte was involved in experimental implementation, data collection, analysis, and interpretation of data. M.C. Hellión-Ibarrola and D.A. Ibarrola made the critical revision related to the relevant intellectual content of the manuscript. W.J. Arrúa was involved in coordinating the study, supervising the work, and involved in writing the final form of the manuscript. All authors read and approved the final manuscript.

Availability of Data and Materials
The data underlying this article will be shared at a reasonable request by the corresponding author.

AI Disclosure
The authors declare that they have not used any artificial intelligence tools to write the manuscript or perform any other procedure.

ORCID ID
W.J. Arrúa: 0000-0003-4057-5186
J.G. Duarte: 0009-0006-8691-8198
M.C. Hellión-Ibarrola: 0000-0001-5970-8300
D.A. Ibarrola: 0000-0002-3427-9877

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