Diuretic Activity and Acute Oral Toxicity of *Caesalpinia Bahamensis* Lam. Extracts (Brasilete)

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ABSTRACT

Diuretics are drugs widely used in the therapy of several diseases. However, many synthetic diuretics are associated with adverse effects. *Caesalpinia bahamensis* Lam., known as "brasilete", is a medicinal plant utilized by the Cuban people to treat kidney diseases, but there is a lack of toxicological and pharmacological studies that support its traditional use. This research aimed to evaluate the diuretic activity and acute oral toxicity of the aqueous and hydroalcoholic extracts of the stems of *Caesalpinia bahamensis*. With regard to the diuretic activity, thirty Wistar rats were used and divided into five groups, receiving the following treatments by intragastric gavage: sodium chloride 0.9% (negative control), furosemide 20 mg/kg (positive control), hydrochlorothiazide 10 mg/kg (positive control), aqueous extract (10, 100, and 200 mg/kg) and hydroalcoholic extract (10, 100 and 200 mg/kg). The urine volume was measured every hour for four hours. The urinary flow was calculated and the urinary concentration of potassium and sodium was determined by flame photometry. The acute oral toxicity was evaluated by the class method and classified according to the Globally Harmonized System (GHS). The urinary flow and the natriuretic and kaliuretic activity were increased in the groups, which received the aqueous extract (200 mg/kg) and the hydroalcoholic extract (200 mg/kg), comparable to hydrochlorothiazide. The highest diuresis was observed at two hours after the administration. In addition, the extracts showed no sign of toxicity at a single dose of 2000 mg/kg. They were classified in category 4 according to GHS. The results obtained in this study support the traditional use of aqueous and hydroalcoholic extracts of *Caesalpinia bahamensis* and represent a contribution to the scientific knowledge about this plant species with regard to its potential future clinical use as a phytodiuretic drug.

Key Words: *Caesalpinia bahamensis; brasilete; toxicity; diuretic activity; aqueous extract; hydroalcoholic extract.*

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INTRODUCTION

The use of plants for medicinal purposes dates back to the very origins of human history when people had no other effective therapeutic resources to treat their diseases [1, 2]. This knowledge was transmitted through various monographs, pictographs, and legends until our days [3]. According to data from the World Health Organization (WHO), 80% of the population worldwide uses plants as a remedy to cure their diseases.[4, 5] On the other hand, it is known that around 20% -30% of the medicines available on the market are derived from natural products.[6] The traditional knowledge about medicinal plants is the first clinical evidence on the efficacy of herbal medicine; however, scientific studies are necessary to corroborate the ethnobotanical data. [7, 8]

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The Cuban population has a wide ethnobotanical knowledge about medicinal plants for the treatment of various diseases, and renal affections are among the most treated in ethnobotany. However, the phytotherapeutic potential of the island is still a virgin. For example, in ethnobotanical studies made in Cuba, 179 species have been used against diseases of the renal system, of which only 9% have been evaluated pharmacologically.[9]

Caesalpinia bahamensis Lam. is a medicinal plant used by the Cuban population to treat renal and hepatic diseases, diabetes, and peptic ulcers. [10] The cytotoxic [11] antioxidant, [12] diuretic, [13] and poor antimicrobial activities [14] have been reported previously. A total of 74 compounds have been identified in the non-polar fraction of a methanolic extract of the species, using gas chromatography-mass spectrometry (GC-MS). In this study, fatty acids, terpenoids, and phytosterols were reported as the major compounds of this fraction. [15] A comparative pharmacognostic study of the aqueous and hydroalcoholic extracts demonstrated the presence of flavonoids and phenolic compounds as the major metabolites. In addition, the phytochemical composition of both extracts was similar according to HPLC analysis. However, the total yield and quantity of the flavonoids were higher for the hydroalcoholic extract. [16] Apart from this, the scientific information about this species until now is very limited.

The aim of this study was to assess the diuretic effect and acute oral toxicity of the aqueous and hydroalcoholic extracts of the stems of *C. bahamensis* as a continuation of the studies to assess its safety and efficacy.

MATERIALS AND METHODS

Plant material and preparation of the extracts

Stems of *Caesalpinia bahamensis* Lam. (Leguminosae) were collected in March 2017 from Cañada Arroyón, Artemisa, Cuba (22°46'45.7"N 83°04'18.6"W). The materials were identified in the National Botanical Garden of Cuba, where a voucher specimen (No. 85369) was deposited. The material was dried at 40°C in an oven (AI-SET-DNE 600, Shanghai, China) for 7 days and milled (Manesti, Italy) until the particles' size was below 2mm. Aqueous and hydroalcoholic extracts were obtained by maceration at room temperature in the dark, during 24 h. Five milliliters of the solvent was used for each gram of dry plant material. After that, the extracts were dried in a rotary evaporator (IKA) under reduced pressure. The plant material and the extracts were previously characterized. [15]

Animals

Wistar rats (200 – 250 g body weight) were obtained from the animalarium of CENPALAB (Havana, Cuba) and kept in collective cages at 22 °C under a 12-h light/dark cycle (lights on at 07:00) with ad libitum access to laboratory food and tap water. This investigation followed Cuban laws for this type of preclinical studies in agreement wth the international regulations for animal care and was approved by the Ethical Committee of the Institute of Basic and Preclinical Sciences "Victoria de Girón", Medical University of Havana, Cuba (Agreement 28-2019; December 2019).

Diuretic activity

The diuretic activity was evaluated in Wistar rats using metabolic cages. Fifty-four male Wistar rats were divided into 5 groups of equal size (n=6) as follow:

Group 1: Physiological solution of sodium chloride 0.9% (negative control)
Group 2: Furosemide in CMC (20 mg/kg) (positive control)
Group 3: Hydrochlorothiazide in CMC (10 mg/kg) (positive control)
Group 4: Dried aqueous extract of *C. bahamensis* in CMC (10 mg/kg)
Group 5: Dried aqueous extract of *C. bahamensis* in CMC (100 mg/kg)
Group 6: Dried aqueous extract of *C. bahamensis* in CMC (200 mg/kg)
Group 7: Dried hydroalcoholic extract of *C. bahamensis* in CMC (10 mg/kg)
Group 8: Dried hydroalcoholic extract of *C. bahamensis* in CMC (100 mg/kg)
Group 9: Dried hydroalcoholic extract of *C. bahamensis* in CMC (200 mg/kg)

All treatments were suspensions prepared with carboxymethyl cellulose (CMC) and administered by intragastric gavage using a volume of 3 mL per rat. After that, the rats were placed in metabolic cages and the excreted urine volume was measured every hour for four hours. The urinary flow was calculated and the urinary concentration of potassium and sodium was determined by flame photometry (Corning 400, USA).

Acute oral toxicity

The *in vivo* toxicological properties of the aqueous and hydroalcoholic extracts for the stems of *C. bahamensis* were evaluated according to OECD Guideline 423, which consists of a 14-day single-dose acute oral toxicity investigation. The GHS was used for the classification. [16] Six male and six female Wistar rats were randomly assigned into four independent blocks of experiments (3 rats of each sex per block; n = 3). According to the OECD Guideline, three animals of each sex were treated with the extracts at a dose of 2000mg/kg body weight as a starting dose to study acute toxicity. After treatment, the toxicity symptoms, including changes in behavioral pattern, respiration, eyes, fur and skin, and/or death in all rats were periodically observed during the first 24h (0.5, 1, 6, and 24 h), and then once daily for 14days. After the treatment period, the rats

were weighed once a week. At day 15, all animals were anesthetized by petroleum ether inhalation, sacrificed by cervical dislocation, and their internal organs were observed. [17]

Statistical analysis

All statistical comparisons between the groups were made by means of One-way Analysis of Variance (ANOVA) with the post hoc Student-Newman-Keuls test. A p-value <0.05 was regarded as significant.

RESULTS

Diuretic activity

The diuretic effect of the aqueous and hydroalcoholic extracts was evaluated and compared with furosemide and hydrochlorothiazide, well-established diuretic drugs. The rats receiving the extracts exhibited a significant increase ($p < 0.05$) in the urinary flow and the levels of Na^+ and K^+ compared to the negative control group; similar values were obtained as for the hydrochlorothiazide group. Furosemide showed the highest values for the mentioned parameters [Table 1]. The groups of animals that received 100 mg/kg of the studied extracts also showed statistically significant results, which demonstrated diuretic pharmacological efficacy at this dose. The 10 mg/kg dose did not produce statistically significant results for both extracts, demonstrating a dose-dependent effect.

Table 1: Effect of *C. bahamensis* extracts on the urinary flow and urinary concentration of sodium and potassium in Wistar rats (n=6)

Group	Urinary flow (ml/min)	C(Na) (mEq)	C(K) (mEq)
G1. Sodium chloride 0.9%	0.011 ± 0.005[a]	47.67 ± 4.50[a]	26.67 ± 3.04[a]
G2. Furosemide	0.043 ± 0.007[b]	85.67 ± 2.53[b]	40.67 ± 2.65[b]
G3. Hydrochlorothiazide	0.032 ± 0.004[c]	76.33 ± 9.18[c]	33.33 ± 2.16[c]
G4. Aqueous extract 10 mg/kg	0.013 ± 0.008[a]	45.23 ± 3.17[a]	23.85 ± 2.83[a]
G5. Aqueous extract 100 mg/kg	0.021 ± 0.007[d]	59.00 ± 4.13[d]	28.57 ± 1.18[d]
G6. Aqueous extract 200 mg/kg	0.028 ± 0.008[c]	73.00 ± 2.24[c]	35.28 ± 3.07[c]
G7. Hydroalcoholic extract 10 mg/kg	0.015 ± 0.004[a]	44.72 ± 5.18[a]	22.49 ± 2.56[a]
G8. Hydroalcoholic extract 100 mg/kg	0.019 ± 0.003[d]	61.06 ± 4.19[d]	27.28 ± 1.03[d]
G9. Hydroalcoholic extract 200 mg/kg	0.030 ± 0.005[c]	77.15 ± 3.23[c]	34.15 ± 1.28[c]

Values are expressed as mean ± SD. Different superscripts indicate significant differences (p <0.05) between groups.

The highest diuresis was observed 2h after the administration of the extracts (200 mg/kg), similar to hydrochlorothiazide. In contrast, furosemide showed the highest diuresis after one hour [Figure 1].

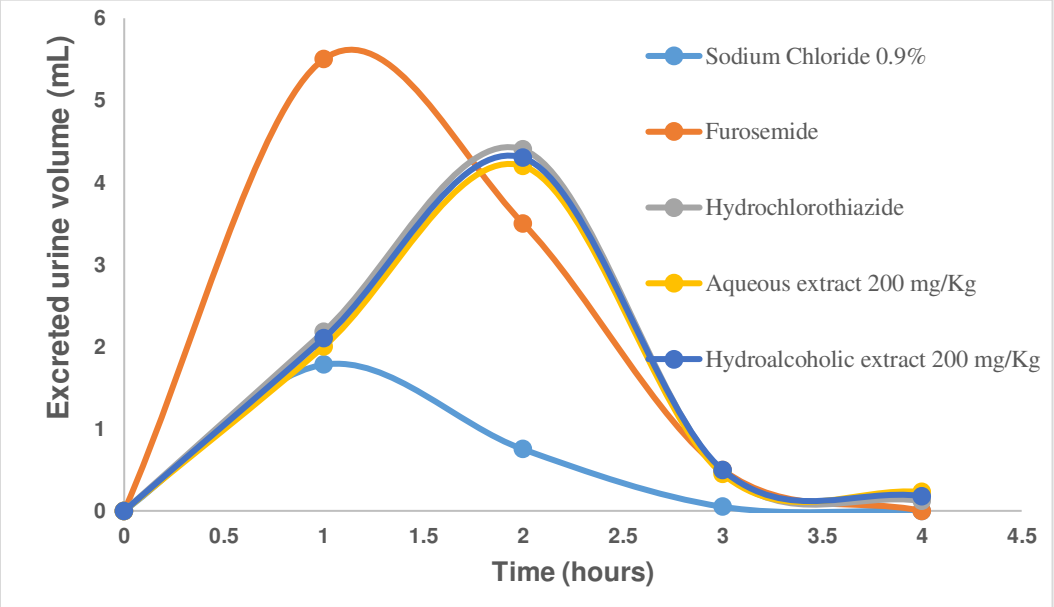

Fig. 1. Kinetics of the elimination of urine for each group

In summary, the extracts of the stems of *C. bahamensis* showed a diuretic activity similar to hydrochlorothiazide in Wistar rats at a dosage of 200 mg/kg.

Acute oral toxicity

The results of the study showed that 14 days after the administration of a high dose of the aqueous and hydroalcoholic extracts, no signs of toxicity and mortality were observed. Body weight increased in the time, a parameter indicative of the absence of or low toxicity [data not shown]. Since vital organs, including spleen, lungs, liver, heart, and kidneys are functionally crucial and toxic substances often impair them, gross examination of these organs was conducted to detect potential symptoms of organ-targeted toxicity. No lesion was found upon microscopic examination of the internal organs of any animals treated with the extracts. According to the Globally Harmonized System, the extracts of *C. bahamensis* were classified as drugs of category 4. These results suggest that extracts of *C. bahamensis* are not acutely toxic after oral administration.

DISCUSSION

Diuretics are defined as any substance that increases urine flow and thereby water excretion. The majority acts by decreasing NaCl reabsorption at different sites in the nephron, thereby increasing urinary Na, and thus, water loss. [18] They are classified as loop diuretics, distal convoluted tubule diuretics or thiazides, potassium-sparing diuretics, carbonic anhydrase inhibitors, and osmotic diuretics. This classification is based on the mechanism by which they inhibit transport and their predominant action site along the nephron. [19] They are among the most commonly used drugs for disease conditions such as congestive heart failure, nephrotic syndrome, cirrhosis, renal failure, hypertension, and pregnancy toxemia. However, many chemical diuretic medicines have adverse effects on the quality of life including fatigue, gout, ototoxicity, hyperglycemia, and impotence. [20] So, it is essential to search for alternative treatments with less toxicity.

In this research, the diuretic effect of the aqueous and hydroalcoholic extracts of *C. bahamensis* was evaluated and compared with furosemide and hydrochlorothiazide. They are well-established drugs and their mechanism of action can be explained according to the differences in the urinary flow, time of action, and ion concentrations in urine.[21] For example, furosemide has the highest excretion of water and sodium in the urine but a short duration of action; for this reason, it is used to treat edemas and acute diseases. In contrast, hydrochlorothiazide excretes less water and sodium with respect to furosemide, but it has a long duration of action and it is used mainly for chronic diseases.[22]

The rats that received the extracts of *C. bahamensis* showed similar values of urinary flow and concentration of sodium and potassium as the rats of the hydrochlorothiazide group. This suggests that *C. bahamensis* acts with a similar mechanism as hydrochlorothiazide at the distal convoluted tubule, where about 5-10% of filtered NaCl is reabsorbed. However, other studies are necessary to corroborate this hypothesis.

In a previous study, the diuretic activity of the aqueous extract of the stems of *C. bahamensis* was evaluated. [11] In general, aqueous extracts are used in traditional medicine as tea or decoction, but these formulations are not stable for a long time and the dosage is not exact. Therefore the use of hydroalcoholic extracts is more common in the pharmaceutical industry for the development of herbal medicines. For this reason, it was necessary to evaluate also the diuretic effect of the hydroalcoholic extract of the stems of *C. bahamensis*. Also, the hydroalcoholic extract was richer in flavonoids than the aqueous extract. [16] The presence of flavonoids can be associated with the diuretic activity of the drug, because flavonoids enhance the prostacyclin synthase activity, leading to the release of renal prostaglandin that has been concerned in diuresis. [23]

In addition, the acute oral toxicity of the extracts was evaluated in order to start safety studies of this herbal preparation. In this sense, neither of the two extracts showed acute toxicity in the experimental conditions. Therefore, *C. bahamensis* can be a safe candidate drug to be used as a diuretic; however, it is necessary to evaluate the effects after chronic administration taking into account the pharmacological similarity with hydrochlorothiazide.

CONCLUSION

The hydroalcoholic and aqueous extracts of the stems of *Caesalpinia bahamensis* showed a diuretic activity similar to hydrochlorothiazide and they did not show acute oral toxicity. These studies contribute to endorsing the popular use of this plant species for diuretic purposes and are part of the set of studies that may contribute to its future development as a herbal formulation in the treatment of kidney diseases.

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Conflicts of interest

There are no conflicts of interest.

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