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Pharmacological characterization of the diuretic effect of *Hibiscus sabdariffa* Linn (Malvaceae) extract

Javier Alarcón-Alonso[a,b], Alejandro Zamilpa[a], Francisco Alarcón Aguilar[c], Maribel Herrera-Ruiz[a], Jaime Tortoriello[a], Enrique Jimenez-Ferrer[a,*]

[a] Centro de Investigación Biomédica del Sur, Instituto Mexicano del Seguro Social (IMSS), Argentina No. 1 Xochitepec, Morelos CP 62790, Mexico
[b] Doctorado en Ciencias Biológicas y de la Salud, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Iztapalapa, Mexico
[c] División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Iztapalapa, Mexico

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ABSTRACT

Ethnopharmacological relevance: Hibiscus sabdariffa L. (Malvaceae) popularly known in Mexico as "Jamaica", "flor de Jamaica", has widely used in Mexican Traditional Medicine as antihypertensive and diuretic, although the latter activity has been reported the present work show evidence about the diuretic, natriuretic and potassium-sparing effects.
Aim of the study: To evaluate the diuretic activity of *Hibiscus sabdariffa* aqueous extract on *in vivo* and *in situ* models.
Materials and methods: The *Hibiscus sabdariffa* aqueous extract was administrated in increasing doses and evaluated the diuresis produced and disposal of electrolytes. Moreover, in isolated kidney was determined the renal filtration rate with plant extract, furosemide and amiloride.
Results: The yield of *Hibiscus sabdariffa* aqueous extraction was 28.3% and the chemical standardization from 1 g of extract was: 56.5 mg delphinidin-3-O-sambubioside, 20.8 mg/g cyanidin-3-O-sambubioside, 3.2 mg/g quercetin, 2.1 mg/g rutin and 2.7 mg/g chlorogenic acid. The diuretic and natriuretic effect of *Hibiscus sabdariffa* aqueous extract showed a dose-dependent behavior. The pharmacological constants of natriuretic effect was ED50 = 86 mg/kg and Emax = 0.9 mEq/100 g/5 h. In the model of kidney *in situ* was observed that renal filtration increased 48% with the aqueous extract of *Hibiscus sabdariffa* and an additive effect when was perfuse with furosemide.
Conclusion: The compound presents in *Hibiscus sabdariffa* as quercetin had effect on the vascular endothelium causing oxide nitric release, increasing renal vasorelaxation by increasing kidney filtration. Therefore, the diuretic effect of *Hibiscus sabdariffa* may be mediated by nitric oxide release.

1. Introduction

Hibiscus sabdariffa L. (Malvaceae), popularly known in Mexico as "Jamaica" or "flor de Jamaica", has been widely used in Mexican traditional medicine as an antihypertensive, diaphoretic, diuretic and a colagogue (Argueta, 1994). Pharmacological studies have demonstrated the antihypertensive effect produced by *Hibiscus sabdariffa* extracts (Onyenekwe et al., 1999; Odigie et al., 2003; McKay et al., 2010). Plant extracts have also been found capable of relaxing vascular smooth muscle by calcium antagonism effects (Ali et al., 1991; Adegunloye et al., 1996; Onyenekwe et al., 1999; Ajay et al., 2007), and activation of endothelial path of the nitric oxide/cGMP (Ajay et al., 2007). In addition, the extract from this plant has been found to act as an inhibitor of angiotensin-converting enzyme (ACE) (Jonadet et al., 1990; Ojeda et al., 2010). The diuretic effect of *Hibiscus sabdariffa* has been pharmacologically characterized by several research groups, both in clinical trials (Kirdpon et al., 1994; Mojiminiyi et al., 2000; Prasongwatana et al., 2008) and in preclinical experiments in rats (Aguwa et al., 2004). Diuretic activity has also been reported as a beneficial side-effect in clinical trials evaluating the antihypertensive effect (Herrera-Arellano et al., 2004). Similarly, the antihypertensive effect of *Hibiscus sabdariffa* was found together with diuretic activity in spontaneous hypertensive rats (Onyenekwe et al., 1999).

The diuretic effects of *Hibiscus sabdariffa* remain controversial, despite many attempts, to characterize them. Some authors reported an increase in diuresis (Cáceres et al., 1987; Mojiminiyi et al., 2000; Aguwa et al., 2004), while other research groups found no increase in urinary volume (Odigie et al., 2003; Prasongwatana et al., 2008). The controversy extends to urinary sodium excretion, as some researchers found an increase in this parameter (Cáceres et al., 1987; Herrera-Arellano et al., 2004), while other investigators

* Corresponding author. Tel.: +52 777 361 2155; fax: +52 777 361 2155.
E-mail address: enriqueferrer_mx@yahoo.com.mx (E. Jimenez-Ferrer).

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observed no change (Prasongwatana et al., 2008) still other working groups reported a decrease in sodium elimination (Aguwa et al., 2004; Kirdpon et al., 1994), and Mojiminiyi et al. (2000) described hyponatremia. The elimination of potassium is another such controversial item: some authors found a slight increase (Aguwa et al., 2004), others observed no changes in urinary potassium excretion (Prasongwatana et al., 2008) and still others showed a decrease in this parameter (Kirdpon et al., 1994). In short, the antihypertensive activity of *Hibiscus sabdariffa* has been amply demonstrated; however, the diuretic effect has not been defined beyond controversy. This paper contributes to the attempt of defining the characteristics of the diuretic effect of *Hibiscus sabdariffa*. In this work used two experimental models, such as rat diuresis and renal filtration rate in kidney "*in situ*". In these tests, were able to observe the diuretic, natriuretic and potassium-sparing effects.

2. Material and methods

2.1. Plant material

Calyces of *Hibiscus sabdariffa* L. were obtained from a controlled crop established in Xochitepec, Morelos, Mexico. A voucher specimen was prepared for reference, and deposited in the IMSSM herbarium with registration number IMSSM-14290 and identified. The dried and ground plant material was extracted in water at 55 °C for 2 h. The extract (HsAq) was then concentrated in a rotary evaporator, and finally dried by lyophilization.

2.2. Animals

Male Albino Sprague–Dawley Rats (250–280 g) (Harlan, México City) were housed and maintained under laboratory conditions at 25 °C, normal 12 h:12 h light/dark schedule with lights on at 07:00 a.m., and free access to water and food (pellets from Harlan rodent lab diet). The animals were allowed at least three weeks to adapt to the laboratory environment before experiments. All studies were carried out in accordance with the official Mexican regulations NOM-062-ZOO-1999 (technical specifications for production, care and use of laboratory animals). The protocol was submitted and accepted by the scientific and institutional Ethics Committee and registered with number: R-2006-1701-5.

2.3. Extract standardization by HPLC technique

The aqueous extract from *Hibiscus sabdariffa* was standardized based on the concentration of delphinidin-3-sambubioside, cyanidin-3-sambubioside, quercetin, rutin, and chlorogenic acid by HPLC method.

Standard curves were built using commercial compounds from Sigma–Aldrich, Mexico. Anthocyanins were isolated and purified following a previously reported procedure (Herrera-Arellano et al., 2007; Ojeda et al., 2010).

2.4. Diuresis in rats

Rats were divided into seven groups of equal number ($n = 6$), and kept in water withdrawal conditions for 18 h with free access to food. Following this period, an isotonic saline solution (ISS) was administered at 7.5 ml/100 g with gastroesophageal probe. After 45 min of administration of ISS, all treatments were administered orally: the negative control group received 1.5 ml of distilled water/200 g; the positive control group was administered the diuretic drug furosemide (13 mg/kg); the experimental groups received the extract (HsAq) dissolved in distilled water at doses of 500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2000 mg/kg and, 2500 mg/kg. After this, the animals were individually placed in metabolic cages and kept under observation for 5 h. All the urine produced in the 5 h period was collected. For analysis the total volume was divided obtain the urine volume excreted per hour (ml/h).

Sodium, potassium and chloride content in the excreted urine were determined spectrophotometrically, following the manufacturer's instructions (Spinreact S.A., Spain).

2.5. Diuresis in the in situ kidney model

Rats were anesthetized by administration of urethane 1.5 g/kg (i.p.). Animals were placed in ventral position on a surgery platform and the kidney was exposed by a left lateral surgical incision. The renal artery was tied at the level of the aortic bifurcation; the renal artery and vein, as well as the ureter were channeled. Urine samples were collected with the catheter. Through the channeled renal artery kidney was perfused with Ringer–Krebs buffer (KRB) (123.3 mM NaCl; 6.17 mM KCl; 3.29 mM $CaCl_2{\cdot}7H_2O$; 0.78 mM $MgSO_4{\cdot}7H_2O$; 32.14 mM $NaHCO_3$; 1.54 mM KH_2PO_4; 6.4 mM sodium piruvate; 6.4 mM sodium glutamate; 7 mM sodium fumarate and 11.1 mM glucose) at a rate of 5 ml/min for 10 min. Thereafter, the rate was decreased to 0.7 ml/min for 30 min, corresponding to the period of the test. During this period the kidney was perfused and the samples collected. Afterwards, the kidney was removed, weighed and homogenized to determine protein content by the Bradford method (Sigma–Aldrich). The glomerular filtration rate (FR) of each kidney was calculated using the following formula:

$$\mathrm{FR} = \frac{\text{urine volume excreted in 30 min}}{\text{protein (mg)}}$$

The kidneys were perfused ($n = 6$), with the following liquid perfusion: (a) basal condition or control group: Krebs Ringer buffer (KRB), (b) sodium-free KRB (NaCl replaced by choline chloride, and $NaHCO_3$ replaced by potassium carbonate), (c) potassium-free KRB (choline chloride instead of KCl and KH_2PO_4 replaced by NaH_2PO_4), (d) KRB with 1 mM amiloride, (e) KRB with 0.6 mM furosemide, (f) KRB with 10 mg/ml of HsAq, (g) sodium-free KRB and 10 mg/ml of HsAq, (h) potassium-free KRB with 10 mg/ml of HsAq, (i) KRB with 1 mM amiloride and 10 mg/ml of HsAq, and (j) KRB with 0.6 mM furosemide plus 10 mg/ml of HsAq.

2.6. Statistical analysis

Results were analyzed with analysis of variance (ANOVA). Statistically significant differences were identified with Tukey's test. Both tests were applied to all groups. Statistical significance was considered with $p < 0.05$. The SPSS 11.0 program was used for this statistical analysis.

3. Results

3.1. Plant extract

The yield obtained for *Hibiscus sabdariffa* aqueous extract was 28.3%. The chemical standardization analysis showed the presence and concentration of the following compounds: delphinidin-3-O-sambubioside 56.5 mg/g, cyanidin-3-O-sambubioside 20.8 mg/g, quercetin 3.2 mg/g, rutin 2.1 mg/g, chlorogenic acid 2.7 mg/g.

3.2. Diuresis in rat

The diuretic drug furosemide administered at 13 mg/kg, caused urine excretion of 4.8 ml/h (data not shown). On the other