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Effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* in patients with mild to moderate hypertension: a controlled and randomized clinical trial ☆

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Abstract

In order to compare the antihypertensive effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* with captopril, a controlled and randomized clinical trial was done. Patients from 30 to 80 years old with diagnosed hypertension and without antihypertensive treatment for at least 1 month before were included. The experimental procedure consisted of the administration of an infusion prepared with 10 g of dry calyx from *H. sabdariffa* on 0.5 l water (9.6 mg anthocyanins content), daily before breakfast, or captopril 25 mg twice a day, for 4 weeks. The outcome variables were tolerability, therapeutic effectiveness (diastolic reduction $\geqslant$10 mm Hg) and, in the experimental group, urinary electrolytes modification. Ninety subjects were included, 15 withdrew from the study due to non-medical reasons; so, the analysis included 39 and 36 patients from the experimental and control group, respectively. The results showed that *H. sabdariffa* was able to decrease the systolic blood pressure (BP) from 139.05 to 123.73 mm Hg (ANOVA $p<0.03$) and the diastolic BP from 90.81 to 79.52 mm Hg (ANOVA $p<0.06$). At the end of the study, there were no significant differences between the BP detected in both treatment groups (ANOVA $p>0.25$). The rates of therapeutic effectiveness were 0.7895 and 0.8438 with *H. sabdariffa* and captopril, respectively (X^2, $p>0.560$), whilst the tolerability was 100% for both treatments. A natriuretic effect was observed with the experimental treatment. The obtained data confirm that the *H. sabdariffa* extract, standardized on 9.6 mg of total anthocyanins, and captopril 50 mg/day, did not show significant differences relative to hypotensive effect, antihypertensive effectiveness, and tolerability.

Keywords: *Hibiscus sabdariffa*; Malvaceae; Captopril; Essential hypertension; Controlled and randomized clinical trial; Complementary and alternative medicine

Introduction

Hypertension is one of the most important health problems in developed countries. This is due to the numerous deaths secondary to cardiopathy, stroke, and renal failure, produced by vascular complications

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(Kannel, 2003; Du et al., 1997). Based on detected blood pressure (BP) rates, there are different classifications of hypertension; thus, the Mexican Official Norm (NOM 030-SSA2-1999) establishes three levels: mild or grade I (BP 140–159/90–99 mm Hg), moderate or grade II (160–179/100–109 mm Hg), and severe or grade III (BP > 180/110) (Secretaría de Salud, 2001). If the WHO-ISH threshold of 140/90 mm Hg is chosen, then approximately 20% of the adult population in most developed countries is hypertensive (Brown and Haydock, 2000). It has been calculated that, in Mexico, 26.6% of population between 20 and 69 years old is affected by arterial hypertension; in other words, in Mexico there are 13 millions persons affected by this illness, and 60% of them do not know it (Secretaría de Salud, 2001).

It has been widely demonstrated that the pharmacological control of hypertension decreases the morbidity–mortality produced by cardiovascular complications and prevents secondary brain hemorrhage and renal failure. Presently, antihypertensive therapeutic pharmacology includes different drugs, which, according to their action mechanisms, are grouped into six categories: diuretic, anti-adrenergic, vasodilator, calcium antagonist, angiotensine receptors antagonist, and angiotensine converting enzyme (ACE) inhibitor (Gerber and Nies, 1992). All of these drugs are able to reduce BP, but they produce undesirable side-effects; therefore, the treatment must be adapted to the particular conditions of each patient (Rivero-Serrano and Tanimoto-Weki, 1999). In different clinical trials, the ACE inhibitors have shown their antihypertensive activity. Particularly captopril, which is the prototype in this group of drugs, has demonstrated to be able to reduce the diastolic blood pressure (DBP) 8.7, 10.3, and until 14 mm Hg, with rates of therapeutic effectiveness (DBP < 90 mm Hg or DBP reduction > 10 mm Hg) between 54.7% and 70%, and 4% for side effects (Roca-Cusachs et al., 1997; Stimpel et al., 1996).

The plant species *Hibiscus sabdariffa* has been, for many years, used in different countries around the world as a culinary and therapeutic resource. It has been utilized for preparing candies, jelly, and hot and cold beverages. According to different ethnobotanical studies, some traditional medicines use the aqueous extract of this plant as diuretic, for treating gastrointestinal disorders, and hypercholesterolemia, and as diaphoretic, and antihypertensive. In Mexico, this plant is known as "flor de jamaica" or simply "jamaica". Actually, the dry calyx from this plant possesses great commercial value because its use as a plant colorant, but principally due to its use as beverage and, recently, for its antihypertensive properties (Rovesti, 1936; Bhaaskara and Seshadri, 1945; Sharaf, 1962; Haji-Faraji and Haji-Tarkhani, 1999).

Chemical studies have shown the presence, in dry calyx of this plant, of aluminum, chromium, copper, iron (Wrobel et al., 2000), hibiscus acid and its 6-methyl ester (Hansawasdi et al., 2000), protocatechuic acid, a polyphenolic compound (Lin et al., 2003; Liu et al., 2002; Tseng et al., 2000), different anthocyanidins as delphinidin and cyaniding and their glycoside forms (Kahkonen and Heinonen, 2003; Lazze et al., 2003), sabdaretin and hibiscretin, in which the common aglicone is the hibiscetin (Sankara and Nair, 1972); heterogeneous acid polysaccharides and phenolic compounds including gossypetine-3-glycoside; flavanoids, water-soluble polysaccharides (Muller and Franz, 1992); flavones and anthocyanins (Jonadet et al., 1990). The characteristic red color elicited by the decoction of this plant is produced by the anthocyanins (Guo, 1986; Wang et al., 2000), hibiscin (Gowali, 1982), and, to a lesser degree, by the delphinidin-3-glucoside and cyanidine-3-glucoside. The presence of β-carotene has also been reported, as well as riboflavin, thiamine, niacin, and the ascorbic, malic and hibiscic acids (Salama, 1979; El-Merzabani et al., 1979). The pharmacological properties of these compounds have not been completely explored. Nevertheless, the medicinal effects that could be produced by this plant have been attributed to the flavonoids. Jonadet et al. (1990) published the evaluation of a hydroalcoholic extract from *H. sabdariffa* on an in vitro model, which showed that the flavonoids possess an inhibitory effect on the ACE. There are other reports about the activity of different extracts from this plant on the isolated smooth muscle, where it showed spasmolytic and vasodilator effects, but without establishing the type of extract or their chemical compounds (Salah et al., 2002). Two clinical studies show that a decoction of the dry calyx from this plant possesses hypotensive, diuretic, and laxative properties, but they did not show the preparation form and dosage of the utilized product (Leclerc, 1938; Perry, 1980). Recently, another clinical study utilized the administration of a tea preparation from *H. sabdariffa* for 12 days in hypertensive patients; the systolic blood pressure (SBP) and DBP decreased from 158–140 and 101–90 mm Hg, respectively, without showing side effects. This study did not include the quantification of any compound present in the experimental product (Haji-Faraji and Haji-Tarkhani, 1999). In another clinical trial, a decoction of the calyx from this plant was administered to hypercholesterolemic patients for 1 year; there were no side-effects reported, which confirms the safety of this plant species, even when used over a long period of time (Aquino et al., 1998).

The aim of the present study was to compare the antihypertensive effectiveness and tolerability of a decoction prepared with the dry calyx from *H. sabdariffa* (standardized on 9.6 mg of anthocyanins/dose) with captopril, 25 mg twice/day for 4 weeks, as well as to determine the modifications on the urinary electrolytes induced by the experimental treatment.

Materials and methods

Subjects

The study was carried out (in the second semester of 2001) with primary care outpatients of the "Hospital General Regional No. 1" of the Mexican Institute of Social Security (IMSS) in Cuernavaca, Morelos. Subjects of both sexes between 30 and 80 years of age were included based on the following criteria: (a) they must be diagnosed with mild to moderate hypertension, (b) have not received antihypertensive treatment at least for the last month prior to the study, and (c) must have granted consent for participation in this clinical investigation. Patients with diabetes mellitus, nephropathy, cardiopathy, hepatic disease, cancer, pregnant women, as well as patients with evidence of secondary hypertension or those with known intolerance to captopril or to the *H. sabdariffa* extracts were not included. All patients who did not conclude the study or who withdrew from the investigation due to non-medical reasons were excluded.

Preparation of treatments

For the experimental treatment, dry calyx from *H. sabdariffa* L. (Malvaceae) was used. Plant material was authenticated by M.C. Abigail Aguilar, Director of the IMSSM Herbarium, where the voucher specimens were stored for future reference (IMSSM-14290). *H. sabdariffa* calyx was collected from a controlled crop in the town of Xochitepec, in the state of Morelos (Mexico). The collected material was dried under dark conditions at room temperature and then ground in an electric mill to obtain particles <2 mm. This material was packed in paper envelopes (10 g each), which were given to the patients, as well as oral and written instructions about the preparation of an infusion with the envelope's contents (add the contents to 0.5 l of boiling water and let stand for 10 min), and the administration procedure (to drink daily before breakfast for 4 weeks). A member of the family (previously instructed), in a shadow study analogy, supervised the preparation and administration of this treatment. For the control treatment, tablets with 25 mg of captopril (Capotena®, Bristol-Meyers Squibb) were used and administered every 12 h for 4 weeks (Stimpel et al., 1996; Roca-Cusachs et al., 1997). Since the control treatment (Captopril) was not soluble in water, it was not possible to blind the treatments; nevertheless, all BP measurements were done by a designated physician, who did not know the treatment group assigned.

Extract standardization

The standardization of the experimental treatment was carried out through a colorimetric method. It was based on the anthocyanin ability to produce a color at pH 1.0 that disappears at pH 4.5. This characteristic is produced by a pH dependent structural transformation of the chromophore. The colored oxonium ion predominates at pH 1.0, while the non-color hemiketal is present at pH 4.5. This method allows the accurate and fast determination of total anthocyanins, still with the presence of polymeric pigments and other compounds. This procedure was done with 1 ml of the treatment solution (10 g of dried *H. sabdariffa* calyx extracted with 500 ml of water). Two samples were gauged to 5 ml solution at pH 1.0 and 4.5, respectively. These solutions were filtered through a 0.45 μm membrane (Gelman acrodisk LCPVDF), and analyzed with a spectrophotometer at 510 and 700 nm, respectively. The total anthocyanins concentration is obtained by using the next formula: concentration (mg/ml) $=(A \times \mathrm{MW} \times FD \times 1000)/(\varepsilon \times 1)$. With this method, it was observed that every envelope of the experimental treatment supplied 9.62 mg of total anthocyanins (Fuleki and Francis, 1968).

Measurements

Therapeutic tolerability was measured through the presence and intensity of side effects produced by the administration of the treatments. The hypotensive effect was calculated as the difference from the basal BP and that obtained at the end of the study. The therapeutic effectiveness was achieved when, at the end of the study, the DBP diminution was $\geqslant 10$ mm Hg (Roca-Cusachs et al., 1997). Finally, those cases where there was therapeutic effectiveness and tolerability were considered as being a "therapeutic success". At the beginning and end of the experimental treatment, a general urine test and urine electrolytes determination were done at 24 h. These tests were done in a certified external laboratory, with a standardized and automatic system (Easy Lyte Plus. Medica Corp., MA).

Statistical analysis

The obtained results were analyzed with the STATA statistical software (Hamilton, 1993). Data were classified by group and week of treatment. In order to determine the median differences, the ANOVA test was used, and the X^2 test was used for differences between proportions ratios. Values of $p<0.05$ were considered for rejecting the nullity hypothesis.

Study description

After approval was obtained from the institutional committee, patients were referred by the institutional physicians to our research group, or identified through

clinical screening. Hypertension diagnosis was confirmed by a designated physician in the research group, based on the mean of two pressure measurements obtained in resting conditions with a 5 min difference (Secretaría de Salud, 2001). The treatment group assignation was done through a sequential list prepared based on a randomized numbers table. In the initial interview, the basal pressure measurements were done, and a week's worth of medication was handed out, along with oral and written instructions. For all of the patients, the clinical evaluation, the outcome variables, and the treatment adherence (counting the number of empty envelopes) were evaluated weekly. Patients who, at any point of the study, showed BP increments together with vascular-spasmodic symptoms were submitted to the emergency service for specialized attention, and were considered as therapeutic failures.

Results

At the beginning of the study, the experimental (*H. sabdariffa*) and control (captopril) group contained 53 and 37 patients, respectively. Table 1 shows the distribution of the basal clinical characteristics in both treatment groups. In the basal record, only the continuous variables—body mass index (BMI), SBP, and differential BP or pulse pressure (PP)—showed significant differences (ANOVA, $p < 0.03$); the BMI was higher in the experimental group, while the SBP and PP were higher in the control. Regarding categorical variables, the familiar antecedent of hypertension, smoking (tobacoism) and the hypertension level, showed significant differences (X^2, $p < 0.03$); these antecedents were more frequent in the control group, as were the number of cases of moderate hypertension.

Table 1. Basal clinical characteristics of the population under study

Parameters	*H. sabdariffa* $n = 53$ mean ± SD	Captopril $n = 37$ mean ± SD	ANOVA p
Age (years)	51.13 ± 10.77	55.27 ± 13.22	> 0.10
Evolution (months)	69.20 ± 78.05	91.54 ± 84.15	> 0.19
Weight (kg)	73.44 ± 13.10	69.11 ± 12.54	> 0.12
BMI (kg/m^2)	29.46 ± 4.25	27.53 ± 4.33	< **0.03**
SBP (mm Hg)	139.05 ± 7.23	143.51 ± 8.88	< **0.01**
DBP (mm Hg)	90.81 ± 2.19	91.62 ± 4.26	> 0.24
PP (mm Hg)	48.24 ± 7.45	51.89 ± 8.36	< **0.03**
	Freq. (%)	Freq. (%)	X^2p
Sex			> 0.14
Masculine	10 (18.8)	12 (32.4)	
Feminine	43 (81.1)	25 (67.5)	
Relatives on HBP			< **0.01**
Positive	12 (22.6)	19 (51.3)	
Negative	41 (77.3)	18 (48.6)	
Alcoholism			> 0.71
Positive	2 (3.7)	2 (5.4)	
Negative	51 (96.2)	35 (94.5)	
Tobacoism			< **0.03**
Positive	1 (1.8)	5 (13.5)	
Negative	52 (98.1)	32 (86.4)	
Exercise			> 0.71
> 1 h/week	28 (52.8)	21 (56.7)	
Negative	25 (47.1)	16 (43.2)	
Hypertension level			< **0.02**
Grade I	50 (94.3)	29 (78.3)	
Grade II	3 (5.6)	8 (21.6)	

Note: Values are means ± SD and frequencies (%).
SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, differential pressure or pulse pressure; HBP, high blood pressure.

At the end of 4 weeks of treatment, 70 patients concluded the study: 38 of the experimental group and 32 of the control group. Fourteen subjects withdrew from the experimental group for non-medical reasons (it was due to the bitter flavor of the extract), and one other subject was excluded due to rising BP. In the control group, four patients were excluded due to BP elevation periods and one other withdrew from the study for non-medical reasons.

Table 2 illustrates different urinary parameters evaluated in the experimental group. Urinary chlorine and potassium, as well as the urinary pH, showed no significant diminution after the treatment with *H. sabdariffa*, while a significant (ANOVA $p<0.001$) increase of the urinary sodium excretion was observed (19.3 mEq/l in 24 h). Although density showed statistically significant differences, they are not significant from a clinical point of view.

After comparing the BP values within treatment groups, significant reductions were detected in both groups. The hypotensive effect was slightly better in the control group (Table 3). When comparisons of antihypertensive effects were made between groups, no significant differences in any of the three evaluated parameters were observed (Table 4). Regarding the outcome variables, no differences were found in therapeutic tolerability, as both treatments were equally well tolerated by all of the patients. When the effectiveness of the treatments was evaluated, it was found that 30 patients (78.95%) from the experimental group, and 27 (84.38%) from the control, showed therapeutic effectiveness (X^2, $p>0.56$). Thus, the percentages of therapeutic success (tolerability plus therapeutic effectiveness) are the same as that found for therapeutic effectiveness. Eight patients from the experimental group and five from the control group were considered as therapeutic failures, because they did not reach therapeutic success, while one patient from the experimental group and four from the control were considered therapeutic failures because they showed elevations of BP before finishing the study. In summary, nine (23.08%) patients from the experimental group and nine (25%) from the control group were considered therapeutic failures, without significant differences between groups (X^2, $p>0.84$).

Discussion

Different phytochemical studies have allowed the identification of different primary groups of compounds in extracts prepared from *H. sabdariffa*. It is known that the anthocyanins (abundant flavonoids of red color) are one of the major groups of compounds present in the aqueous extract of this plant, and they could be the bioactive compounds producing different antihypertensive action mechanisms, such as the inhibition of the angiotensine I and angiotensine II converting enzyme, as well as the angio- and cardio-protector effect (Meunier et al., 1987; Jonadet et al., 1990). This group of compounds has also been isolated from other plant species in which different antihypertensive activities have been described. This is the case of the dose-dependent hypotensive effect of procyanidine-B2, present in the leaves of *Melastoma candidum* (Cheng et al., 1993), and the vasodilator effect of the polymeric proanthocyanidins identified in *Pistacia lentiscus* (Sanz et al., 1992). Moreover, another antihypertensive action mechanism of the proanthocyanidins was recently identified which inhibited the binding of angiotensine II to the AT_1 receptor. This study included the screening of different plants from Panama (Caballero-George et al., 2001), and particularly an extract obtained from the bark of *Guazuma ulmifolia* (Caballero-George et al., 2002). Other pharmacological activities have been detected in the anthocyanins group, in which antioxidant (Bahorun et al., 1994) and hypo-cholesterolemic (Lee et al., 2002) effects are prominent. Within this group of anthocyanins, it has been possible to identify numerous compounds, the most important of which are: hibiscin, delphinidin-3-glucoside, cyanidin-3-sambubioside, sabdaretin, and hibiscretin. In spite of this, none of the pharmacological studies—in which the antihypertensive activity of this plant has been demonstrated—have identified the responsible compounds. Our study allowed the quantification of the total anthocyanin content in the utilized *H. sabdariffa* extract (9.62 mg, total anthocyanins/dose); this point is interesting because in future studies it will be possible to measure the dosage of the anthocyanins present in the extracts or phytopharmaceuticals prepared from this plant species. Nevertheless, pharmacological and phytochemical

Table 2. Urinary parameters detected in patients before and after treating with the *H. sabdariffa* extract

Parameter	Initial	Final	ANOVA p
Chlorine (mEq/l/24 h)	136.90 ± 55.48	132.86 ± 57.13	> 0.39
Sodium (mEq/l/24 h)	106.11 ± 45.04	125.42 ± 53.43	< **0.001**
Potassium (mEq/l/24 h)	52.16 ± 22.74	47.74 ± 21.46	> 0.33
pH	5.68 ± 0.73	5.66 ± 0.67	> 0.11
Density	1017.84 ± 4.87	1016.77 ± 5.25	< **0.03**

Note: Values are means ± SD.

Table 3. Blood pressure rates (mm Hg) detected on patients, before and after treating with the *H. sabdariffa* extract and captopril

Parameter	Basal BP	Final BP	Reduction	ANOVA *p*
H. sabdariffa				
SBP	139.05 ± 7.23	123.73 ± 12.10	14.15 ± 11.76	< **0.03**
DBP	90.81 ± 2.19	79.52 ± 7.25	11.18 ± 6.91	< **0.06**
PP	48.24 ± 7.45	44.21 ± 10.89	2.97 ± 10.23	< **0.008**
Captopril				
SBP	143.51 ± 8.88	127.0 ± 11.32	16.43 ± 9.56	< **0.001**
DBP	91.62 ± 4.26	78.43 ± 7.88	13.12 ± 7.23	< **0.01**
PP	51.89 ± 8.36	48.56 ± 11.17	3.31 ± 9.47	< **0.0006**

Note: Values are means ± SD.
SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, differential pressure or pulse pressure.

Table 4. Blood pressure reduction obtained with the aqueous extract from *H. sabdariffa* compared with captopril in patients with mild to moderate hypertension

Parameters	*H. sabdariffa*	Captopril	ANOVA *p*
SBP	14.15 ± 11.76	16.43 ± 9.56	> 0.38
DBP	11.18 ± 6.91	13.12 ± 7.23	> 0.25
PP	2.97 ± 10.23	3.31 ± 9.47	> 0.88

Note: Both treatments were administered orally, daily for 4 weeks. Values are mm Hg (means ± SD).
SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, differential pressure or pulse pressure.

parallel studies (bioguided studies) are necessary in order to identify and elucidate the responsible compounds of the different antihypertensive biological activities.

Another study has already evaluated the effect of *H. sabdariffa* on hypertensive patients, in which a tea prepared from dry calyx was used for 12 days. Nevertheless, in this work there was no quantification of any group of compounds as a marker of the utilized extract. This study showed a decrease of 11.2% and 10.7% of the SBP and DBP, respectively (Haji-Faraji and Haji-Tarkhani, 1999). The present study confirms the antihypertensive capability of the aqueous extract from *H. sabdariffa*; nevertheless, when comparing our results with that of the aforementioned study, the reduction of diastolic pressure rates was better in our study, reaching a 12.31% reduction. Moreover, the present clinical trial allows us to establish comparisons between the *H. sabdariffa* extract with the prototype drug of the ACE inhibitors, captopril, which is the first election drug for the treatment of patients suffering mild to moderate hypertension. Relative to captopril, the obtained results are of the same magnitude as that from the international literature (Stimpel et al., 1996; Roca-Cusachs et al., 1997). The antihypertensive effect observed in both treatment groups (demonstrated by a decrease of DBP, SBP, and PP, as well as the rates of therapeutic effectiveness, measured by the diminution of at least 10 mm Hg) was of the same rank. This fact allows us to say that regarding antihypertensive capability, at doses utilized, there are no significant differences between the aqueous extract from *H. sabdariffa* (standardized on 9.62 mg of total anthocyanins/dose/day) and captopril (25 mg every 12 h.).

There are reports that allow us to suppose that the aqueous extract from this plant exerts its antihypertensive activity by at least three specific action mechanisms: diuretic (Onyenekwe et al., 1999), vasodilator (Adegunloye et al., 1996), and ACE inhibitor (Meunier et al., 1987; Jonadet et al., 1990). It is also possible that this effect was due to the blockage of the AT_1 receptor binding to angiotensine II, like the anthocyanins have done in other plant species (Caballero-George et al., 2002). Another possible antihypertensive mechanism could be the Ca^{2+} channel modulation, exerted by quercetin and eugenol (Salah et al., 2002). It is possible that oligomeric procyanidines, which are known for their ACE-inhibiting effect (Lacaille-Dubois et al., 2001) and, in addition potassium acetate (contained in the water extract), which has a moderate diuretic effect, could contribute to the antihypertensive effect.

On the other hand, at the end of the present study, patients treated with the *H. sabdariffa* extract showed an increment of the urinary excretion of sodium, without substantially modifying other urinary electrolytes, including potassium. A similar behavior is found with the administration of diuretics of the spironolactone type or aldosterone antagonists, which are potassium savers

(Weiner, 1992). Therefore, the diuretic activity of *H. sabdariffa* is probably analogues to that of this group of drugs, but this hypothesis must be probed with more basic pharmacological experiments.

Our study contributes further evidence of the innocuousness of the short- and long-term administration of aqueous extract from *H. sabdariffa*, because no side-effects or intolerability was detected. Based on the obtained results, it is necessary to develop assays in order to identify the bioactive compounds responsible for the different action mechanisms, as well as clinical trials with standardized phytopharmaceuticals of *H. sabdariffa*.

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