Diuretic activity assessment of an aqueous extract of *Hibiscus sabdariffa* (Malvaceae) leaves on Wistar rats

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**INTRODUCTION**

The use of plants occupies an important place in the healthcare of many populations around the world.1 This is partly due to socio-cultural habits and partly to the wealth of organic compounds of therapeutic interest that plant species contain.2,3 As a result, 80% of the world’s population treat their health problems with pharmacopoeia plants.4 This practice was reinforced by the census of African pharmacopoeia plants, which intensified after independence.5 This survey showed that many traditional medicine plants are used to treat arterial hypertension (AH). Hypertension is a chronic non-communicable disease characterised by an increase in blood pressure (BP) above the normal range. This condition, which is common throughout the world, is the cause of numerous cardiovascular complications including strokes.6 According to7, the number of people suffering from hypertension worldwide could reach 1.56 billion by 2025. According to the Abidjan Heart Institute, Côte d’Ivoire recorded a prevalence rate of 38% in 2017. Apart from the modern treatment, people tend to use plants from the pharmacopoeia, particularly plants with diuretic properties.8 However, few scientific studies have been carried out on a large number of plant species used to treat hypertension. Among these plants, *Hibiscus sabdariffa* (Malvaceae) is a plant used in traditional medicine to treat a number of pathologies such as microbial infections and hypertension. This work was undertaken with a view to developing medicinal plants and establishing scientific basis for the use of *H. sabdariffa* (Malvaceae) as a diuretic in the treatment of hypertension.

**MATERIALS AND METHODS**

**Materials**

**Animal**

Wistar rats, *Rattus norvegicus* (Muridae), weighing between 150 and 250 g were used. These animals are from the Animal Physiology Laboratory house at a temperature between 28±3°C with a 12-hour light/dark cycle. They were fed ad libitum with standard pellets produced and sold by IVOGRAIN® in Abidjan, Côte d’Ivoire, and had free access to water. The experimental protocols were followed in accordance with the protocol for the protection of experimental animals of the European Council legislation 2012/707 (EU, 2012).9

**Plant**

These are *Hibiscus sabdariffa* (Malvaceae) leaves, locally known as “dah”. The leaves were collected from a field in Agboville, capital of the Agney-Brassa Region (Côte d’Ivoire). The plant has been identified and registered at the National
The urine of the rats was collected; the quantity was measured every two hours and accumulated for twenty-four hours in each group. The diuretic activity was calculated as follows:

\[ UE (\frac{ml}{kg}) = \frac{UV (ml)}{P (kg)} \]

With, \( UE \) = urine excretion, \( UV \) = urine volume and \( P \) = weight of the animal.

\[ UVE (\%) = \frac{VE (ml)}{VA (ml)} \times 100 \]

With, \( UVE \) = urinary volumetric excretion, \( VE \) = volume excreted and \( VA \) = volume of the test substance administered.

\[ DI=Treated/VEControl \]
\[ SI=UECTreated/UEControl \]
\[ NI=UCNa+/UCK+ \]

Statistical analysis

The statistical analysis of the results and the graphical representation of the data were carried out using Graph Pad Prism 7 software (San Diego, California, USA). Statistical differences between the results were determined using analysis of variance (ANOVA) followed by the Turkey-Kramer multiple comparison test, with a significance level of \( p<0.05 \). All values are presented as mean ± SEM (Standard Error on the Mean).

RESULTS AND DISCUSSION

Results

Phytochemical study of the aqueous extract of Hibiscus sabdariffa leaves

Table 1 shows the results of the phytochemical screening of the aqueous extract of Hibiscus sabdariffa leaves (EAHS). It revealed the presence of several chemical compounds including sterols, polyterpenes, polyphenols, flavonoids, saponosides, alkaloids, gallic tannins and alkaloids. On the other hand, the absence of quinonic compounds and catechic tannins in EAHS was noted.
Table I: Phytochemical screening of the aqueous extract of *Hibiscus sabdariffa* leaves.

<table>
<thead>
<tr>
<th>Compounds researched</th>
<th>Test or reagents</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterols and polyterpenes</td>
<td>Liebermann</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>Ferric chloride</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Cyanidine</td>
<td>+</td>
</tr>
<tr>
<td>Saponosides</td>
<td>Foam test</td>
<td>+</td>
</tr>
<tr>
<td>Quinonic compounds</td>
<td>Borntraäger</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Dragendorff</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Bouchardat</td>
<td>+</td>
</tr>
<tr>
<td>Catechic tannins</td>
<td>Stiasny</td>
<td>-</td>
</tr>
<tr>
<td>Gallic tannins</td>
<td>Hydrochloric acid</td>
<td>+</td>
</tr>
</tbody>
</table>

(+): Presence of the compound; (-): Compound not present

Diuretic activity of the aqueous extract of *Hibiscus sabdariffa* leaves in rats

Effect of the aqueous extract of *Hibiscus sabdariffa* leaves on the rats' urine excretion

The results showed that, after two hours (2h), the urine volumes collected in the rats batches treated with the extract different doses were not significantly different (p>0.05) to the control (5.63±3.1 ml/kg). However, the urine excreted volume obtained with furosemide (46.94±0.47 ml/kg) was much higher (p<0.0001) than those obtained in the control batch. After six (6), eight (8) or 12 hours of experimentation, the dose of 500 and 1000 mg/kg bw doses of EAHS induced significant (p<0.05) and very significant (p<0.001) increases in urine excretion compared with the control respectively. For 500 mg/kg bw of EAHS, this increase was 259.33% ; 306.67% and 157.07% for urine excretions of 34.46±0.74 ; 39±0.76 ; 43.06±1.47 ml/kg at 6, 8, 12 h respectively. As for 1000 mg/kg bw, the extract increased by 227.33 ± 2.03 and 237 ± 2.08 mmol/L respectively for the extract doses of 500 and 1500 mg/kg bw. However, in rats treated with 1000 mg/kg bw of EAHS, the natriuresis determined were comparable to those of the control batch. The urine Na⁺ excretion concentrations were 220±3.61 mmol/L, 207.67±1.33 and 213.33±1.2 respectively. In addition, EAHS induced non-significant (p>0.05) increases in urinary potassium and chlorine excretion at all extract doses compared to the control group. The 1000 mg/kg bw dose of the extract produced the highest diuretic activity. In fact, the diuretic index obtained was 2.9 at this dose extract, that was statistically similar to that obtained with furosemide (3.9) (Table II).

Effect of EAhs and furosemide on urine electrolyte excretion

In order to monitor urinary electrolyte (Na⁺, K⁺ and Cl⁻) leakage, three electrolytes were measured 24 hours after the administration of the various substances (Table III). EAHS, at doses of 500 and 1500 mg/kg bw, produced a significant increase (p<0.001) in urine Na⁺ excretion after 24 hours compared with the control (213.33±1.2 mmol/L). The Na⁺ elimination was 227.33 ± 2.03 and 237 ± 2.08 mmol/L respectively for the extract doses of 500 and 1500 mg/kg bw. However, in rats treated with 1000 mg/kg bw of EAHS, the natriuresis determined were comparable to those of the control batch. Urine Na⁺ excretion concentrations were 220±3.61 mmol/L, 207.67±1.33 and 213.33±1.2 respectively. In addition, EAHS induced non-significant (p>0.05) increases in urinary potassium and chlorine excretion at all extract doses compared to the control group. The 1000 mg/kg bw dose of the extract produced the highest diuretic activity. In fact, the diuretic index obtained was statistically similar to that of furosemide.
Graph 1: Urine excretion induced by different doses of EAHS and furosemide.
Values are represented as $M\pm SEM$; $n = 4$; *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$

Table II: Urinary excretion (UE) and urinary volumetric excretion (UVE).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NaCl</th>
<th>Furo</th>
<th>EAHS 500</th>
<th>EAHS 1000</th>
<th>EAHS 1500</th>
</tr>
</thead>
<tbody>
<tr>
<td>UE (ml/kg)</td>
<td>16.3±1.1</td>
<td>73.38±11.8***</td>
<td>44.5±3.45**</td>
<td>47.41±3.33**</td>
<td>24.17±1.8*</td>
</tr>
<tr>
<td>UVE (%)</td>
<td>34.99±8.46</td>
<td>123.06±11.07***</td>
<td>66.11±6.19**</td>
<td>112.22±4.36***</td>
<td>61.39±10.28**</td>
</tr>
<tr>
<td>DI</td>
<td>-</td>
<td>3.9</td>
<td>1.89</td>
<td>2.99</td>
<td>1.75</td>
</tr>
</tbody>
</table>

DI: Diuretic index; EAHS: Aqueous extract of *Hibiscus sabdariffa* leaves; UE: Urine excretion; UVE: Urinary volumetric excretion
Table III: Urine electrolyte concentrations and urinary indices.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NaCl</th>
<th>Furo</th>
<th>EAHS 500</th>
<th>EAHS 1000</th>
<th>EAHS 1500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺ (mmol/L)</td>
<td>213.33± 1.2</td>
<td>207.67 ± 1.3</td>
<td>227.33 ± 2**</td>
<td>220 ± 3.61</td>
<td>237 ± 2.08**</td>
</tr>
<tr>
<td>K⁺ (mmol/L)</td>
<td>28.56 ± 4.01</td>
<td>13.17 ± 1.11**</td>
<td>30.53 ± 9.87</td>
<td>25.30 ± 0.92</td>
<td>40.40 ± 4.9</td>
</tr>
<tr>
<td>Cl⁻ (mmol/L)</td>
<td>201.68 ± 1</td>
<td>167±1**</td>
<td>219.33±1.8</td>
<td>205± 1.1</td>
<td>220.68 ± 2</td>
</tr>
<tr>
<td>SI</td>
<td>-</td>
<td>0.97</td>
<td>1.06</td>
<td>1.03</td>
<td>1.11</td>
</tr>
<tr>
<td>NI</td>
<td>7.47</td>
<td>15.77**</td>
<td>7.45</td>
<td>8.69</td>
<td>5.87</td>
</tr>
</tbody>
</table>

SI: salidiuretic index; NI: natriuretic index; EAHS: aqueous extract of Hibiscus sabdariffa leaves

Assessment of renal function

24 hours after the oral administration of the substances to the rats, the results of the levels of creatinine and urea excreted by urine route were measured and presented in Graph 2. In animals administered with furosemide (40 mg/kg bw), 500, 1000 and 1500 mg/kg bw of EAHS, creatinine values were 2.34±1.17, 4.10±0.41, 4.65±0.98 and 6.54±0.48 mmol/L respectively. Urea levels were 139.70±70.48, 255.50±16.01, 234.41±40.99 and 382.03±32.14 mmol/L respectively. Compared to the control, whose creatinine level was 11.27±0.49 11 mmol/L and urea 590.63±53.11 mmol/L, these values in treated animals showed high significant variations (p<0.001). These values decreased by 79.24%; 63.62%; 58.74%; 41.97% for creatininuria and by 70.63%; 56.74%; 60.31%; 35.32% for urine urea content with furosemide, EAHS 500, 1000 and 1500 mg/kg bw respectively.

Graph 2: Urine concentration of urea and creatinine after administration of different doses of EAHS and furosemide over 24 hours.

**p<0.01; ***p<0.001; ****p<0.0001

DISCUSSION

Phytochemical screening revealed the presence of polysterols, polyprenenes, polyphenols, flavonoids, saponosides, alkaloids and gall tannins. These results are comparable to those obtained from calyces.14 These authors showed the presence of anthocyanins, polysters and polyprenenes. A study of the diuretic activity of aqueous extract of hibiscus sabdariffa leaves in rats revealed an increase in urinary volumetric excretion (UVE) comparable to that of furosemide. Diuretics that mimic the effect of furosemide act on the Na⁺/K⁺/Cl⁻ pump at the loop of Henle, and inhibit sodium and water reabsorption at the loop of Henle, as does furosemide.15 They influence the urine dilution-concentration mechanism to encourage high diuresis. These diuretics mayantagonise the action of antidiuretic hormone (ADH). ADH is responsible for regulating the reabsorption of water from the filtrate in the renal collecting tubules in order to maintain the body's osmolarity by inhibiting urinary excretion. EAHS also causes urinary leakage of sodium and potassium. These urinary electrolyte excretions were greater than those caused by furosemide. The effects of EAHS are similar to those induced by the aqueous extract Ficus exasperata (Moraceae), Rosmarinus officinalis (Lamiaceae) and Centaurium erythraea (Gentianaceae).16,17 The natriuresis and chloruresis induced by EAHS are greater than those induced by furosemide. This would explain the relatively high salidiuretic and natriuretic properties of the studied extract. The urine potassium
concentration induced by EAHS was low, as was that induced by furosemide. This low potassium concentration suggests that potassium excretion is spared. This "potassium-sparing" property has also been reported in the diuretic effect of furosemide. EAHS is thought to contain a large amount of potassium, giving it the advantage of apotassium-sparing diuretic effect.18 EAHS induces normal urine excretions of creatinine and urea similar to those of the control and relatively lower than those induced by furosemide. These different eliminations of urea and creatinine are thought to result from an increase in their glomerular filtration rate. The effects of EAHS are similar to those induced by the aqueous extract of Spergularia purpurea leaves (Caryophyllaceae).19 These authors reported that this extract resulted in an increase in glomerular filtration rate, associated with an increase in creatinine clearance. The leaves of the H. sabdariffa plant have a diuretic effect. Not only do they promote better excretion of water and electrolytes (Na+, Cl-, K+), but above all they increase glomerular filtration rate due to their effects on the renal purification function, as do Ficus exasperata (Moraceae) and Bridelia ferruginea (Euphorbiaceae). These diuretic plants are used as first-line treatments to moderate hypertension.20,21

CONCLUSION

The studies of the pharmacological effects of aqueous extract of Hibiscus sabdariffa leaves on diuresis have shown that it has a diuretic and natriuretic activity comparable to that of furosemide, the reference diuretic. This activity could be attributed to the presence of alkaloids, steroids and flavonoids, compounds known for their diuretic effects. These results support the use of this plant as a diuretic in the treatment of high blood pressure.

Author’s contribution:

All authors have contributed equally to the work

Conflicts of Interest:

The authors declare no conflicts of interest involved in this study

REFERENCES