Effects of aqueous extract of *Hibiscus sabdariffa* on renal Na\(^+\)-K\(^+\)-ATPase and Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase activities in Wistar rats

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**OBJECTIVE:** To investigate the effects of oral administration of aqueous extract of *Hibiscus sabdariffa* on renal Na\(^+\)-K\(^+\)-ATPase and Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase activities in rats.

**METHODS:** The 25 and 50 mg/(kg • d) of aqueous extracts of *H. sabdariffa* were respectively given to rats in the experimental groups for 28 d, and rats in the control group received an appropriate volume of distilled water as vehicle. Na\(^+\)-K\(^+\)-ATPase and Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase activities in the kidney were assayed by spectrophotometric method.

**RESULTS:** Administrations of 25 and 50 mg/(kg • d) of aqueous extract of *H. sabdariffa* significantly decreased the Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase activity in the kidney of rats \((P < 0.05)\). However, the renal Na\(^+\)-K\(^+\)-ATPase activity of the experimental rats was not affected by either dose of the extract. And the plasma Na\(^+\), K\(^+\) and Ca\(^{2+}\) levels of the experimental rats had no significant changes. Administration of either dose of the extract did not result in any significant changes in body and kidney weights, the concentrations of plasma albumin and total protein, and alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activities. However, concentrations of creatinine and urea were significantly reduced by 50 mg/kg of the extract \((P < 0.05)\).

**CONCLUSION:** The present study indicates that oral administration of aqueous extract of *H. sabdariffa* may preserve the renal function despite a decreased renal Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase activity.

**KEYWORD:** *Hibiscus*; Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase; sodium-potassium-exchanging ATPase; kidney function tests; rats
Studies showed that a high-plant-food diet is associated with a lower risk of chronic diseases, such as cardiovascular diseases, metabolic disorders and some forms of cancer\(^{[1,5]}\). Plant foods contain many microconstituents other than vitamins and minerals that are known to be biologically active. The health benefits of plant foods may not simply attribute to the micronutrient content alone but also to the biologically active phytochemicals\(^{[31]}\). However, further investigation is necessary to evaluate the roles of plant foods, particularly those that are widely used for health promotion and disease prevention.

*Hibiscus sabdariffa* L. (Malvaceae) has been employed as a herbal medicinal agent in many countries. The aqueous extract of *H. sabdariffa* is now used as a local drink material in many developing countries, including Nigeria, where it is commonly called “ZOBO”. Interestingly, *H. sabdariffa* is one of the most common ingredients found in commercial herbal tea blends sold in the United States. Beverages made by *H. sabdariffa* calyces are called hibiscus tea, bissap, roselle, red sorrel, agua de Jamaica, Lo-Shen, Sudan tea, sour tea, or karkade. The beneficial effects of the aqueous extract of *H. sabdariffa* petals are blood pressure-lowering\(^{[6,13]}\), antidiabetes\(^{[14,15]}\) and antioxidant properties\(^{[8,16-19]}\) and hypocholesterolemic\(^{[20-23]}\) in both rats and humans.

Experimental studies have demonstrated that aqueous extract of *H. sabdariffa* could enhance the cardiac and vascular Na\(^+\)-K\(^+\)-ATPase and Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase activities\(^{[24,25]}\) which can possibly explain its cardio-protective effect.

An energy-dependent integral membrane enzyme, Na\(^+\)-K\(^+\)-ATPase that is in all mammalian cells, including renal epithelial cells\(^{[26]}\), is responsible for the maintenance of intracellular Na\(^+\) and K\(^+\) balances. It is widely considered to be an essential driving force for active transport of various ions and water across electrolyte-transporting epithelial tissues\(^{[27]}\). Regulation of tubular Na\(^+\) reabsorption is a major determinant of total body electrolyte homeostasis and blood pressure control\(^{[28]}\). The basolateral arrangement is associated with apical-to-basal transport of Na\(^+\), Na\(^+\)-coupled electrolytes such as Ca\(^{2+}\) transport, osmotic fluid reabsorption and maintenance of transmembrane potential or stabilization of membrane\(^{[29]}\). However, abnormalities in activities of Na\(^+\)-K\(^+\)-ATPase and Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase have been implicated in several chronic clinical conditions such as hypertension, hyperglycemia, kidney disease\(^{[29-31]}\) and renal dysfunction\(^{[32,33]}\). A reduction in Na\(^+\)-K\(^+\)-ATPase activity has been shown to enhance the myocardial contractility due to increased intracellular Ca\(^{2+}\) concentration although a cellular load of Ca\(^{2+}\) may be associated with cardiac cell damage and death after ischemia\(^{[34]}\).

Hence, any agent that can stimulate the Na\(^+\)-K\(^+\)-ATPase activity may be useful in the prevention of initiation or progression of cardiorenal dysfunction. The present study is therefore aimed at evaluating the effects of oral administration of aqueous extract of *H. sabdariffa* on renal Na\(^+\)-K\(^+\)-ATPase and Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase activities in rats, and to examine the effects of the extract on renal and liver functions.

### 1 Materials and methods

#### 1.1 Animals
A total of 18 male Wistar rats weighing 120 to 150 g were obtained from the Animal Breeding Unit of the Department of Biochemistry, University of Ilorin, Nigeria. The rats were housed in a well-ventilated room under a 12 to 12 h light-dark cycle; food (Bendel Feeds and Flourmills Ltd. Benin city, Nigeria) and water were available ad libitum. Rats were randomly divided into three groups (\(n = 6\)) of equal mean body weight.

#### 1.2 Preparation of extract of *H. sabdariffa* petals
Fresh samples of the petals of *H. sabdariffa* were obtained from Maiduguri in Borno State, Nigeria. The plant materials were botanically authenticated by

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Prof. F. A. Oladele of the Department of Botany, University of Ilorin, Nigeria. The preparation of the plant material was as previously reported\cite{4,25,24}. The extract was then filtered through Whatman No. 1 filter paper and the residue was discarded. The resultant filtrate was then evaporated to dryness and stored in capped bottles at 4 °C until use\cite{25,26}. The desired amount of the extract was dissolved in distilled water to make 25 and 50 mg/mL of stock solutions for lower and higher doses, respectively.

1.3 Administration of extract of *H. sabdariffa* Rats in the control group received an appropriate volume of distilled water per day as vehicle by gavage for 28 d. and the experimental rats respectively received 25 and 50 mg/kg of the plant extract by gavage. The vehicle of *H. sabdariffa* administration was stopped 24 h before the animals were killed in order to evaluate the chronic effects of the extract rather than the effects of acute administration.

1.4 Tissue preparation At the end of the experimental period, the animals were killed by cervical dislocation. The blood was collected from jugular vein into heparinized sample bottle and the plasma was extracted after centrifuged at 3,000 × g for 10 min, stored at 4 °C and used within 12 h. The kidneys were quickly excised, decapsulated and transferred into ice-cold 0.25 mol/L of sucrose solution. The kidneys and livers were later blotted and weighed. The kidney weight indexes were shown as the ratio of kidney weight to body weight. The kidney was sectioned and the homogenate was prepared in ice-cold 0.25 mol/L of sucrose solution (the percentage concentration by weight is 1:5) as in the previous study\cite{25}.

1.5 Biochemical assays Na⁺-K⁺-ATPase and Ca⁺⁺-Mg⁺⁺-ATPase activities were assayed spectrophotometrically by measuring the amount of inorganic phosphate release, following incubation of the kidney homogenate with diosidase adenosine triphosphate (Sigma Chemical Co. Ltd., UK) as reported in the previous study\cite{26}. Alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in the kidney were determined by standard methods using assay kits (Quinchia Clinica Aplicada, Spain). Plasma total protein concentration was estimated by Biuret’s method\cite{27} while albumin was measured by bromocresol green dye-binding method\cite{28}. Plasma urea and creatinine concentrations were determined by standard spectrophotometric method. Plasma total protein, albumin, urea and creatinine concentrations were estimated by standard assay kits (Quinchia Clinica Aplicada, Spain).

1.6 Statistical analysis Data were presented as means±standard error of mean. Significance was determined by analysis of variance followed by Duncan’s multiple range post-hoc test, using SPSS program (SPSS, Chicago, USA). When P<0.05, it was taken as significant.

2 Results

2.1 Effects of aqueous extract of *H. sabdariffa* on body and kidney weights and ALP, AST and ALT activities Administrations of *H. sabdariffa* extract at both doses did not produce any significant effects on the body and kidney weights, and ALP, AST and ALT activities (P > 0.05). See Tables 1 and 2.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effects of aqueous extract of <em>Hibiscus sabdariffa</em> on body and kidney weights of rats (Mean±standard error of mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>n</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
</tr>
<tr>
<td>Extract of <em>Hibiscus sabdariffa</em> (25 mg/kg)</td>
<td>6</td>
</tr>
<tr>
<td>Extract of <em>Hibiscus sabdariffa</em> (50 mg/kg)</td>
<td>6</td>
</tr>
</tbody>
</table>

2.2 Effects of aqueous extract of *H. sabdariffa* on plasma Na⁺, K⁺ and Ca⁺⁺ concentrations and renal Na⁺-K⁺-ATPase and Ca⁺⁺-Mg⁺⁺-ATPase activities Administrations of 25 and 50 mg/kg of aqueous extract of *H. sabdariffa* significantly decreased the Ca⁺⁺-Mg⁺⁺-ATPase activity (P<0.05, Figure 1). There was no significant change in Na⁺-K⁺-ATPase activity or plasma Na⁺, K⁺ and Ca⁺⁺ concentrations (Figure 2 and Table 3). The decrease in Ca⁺⁺-Mg⁺⁺-ATPase activity induced by 50 mg/kg of *H. sabdariffa* extract was not significantly greater than that induced by 25 mg/kg of *H. sabdariffa* extract (P>0.05, Figure 1).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effects of aqueous extract of <em>Hibiscus sabdariffa</em> on plasma ALP, AST and ALT activities in rats (Mean±standard error of mean, IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>n</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
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<tr>
<td>Extract of <em>Hibiscus sabdariffa</em> (25 mg/kg)</td>
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</tr>
<tr>
<td>Extract of <em>Hibiscus sabdariffa</em> (50 mg/kg)</td>
<td>6</td>
</tr>
</tbody>
</table>

ALP: alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

2.3 Effects of aqueous extract of *H. sabdariffa* on plasma concentrations of total protein, albumin, urea and creatinine The effects of administration of aqueous extract of *H. sabdariffa* petals on plasma total protein, albumin, urea and creatinine concentrations are summarized in Table 4. Plasma urea and creatinine concentrations were signifi-
significantly reduced in rats treated with 50 mg/kg of *H. sabdariffa* extract, when compared with the control rats (*P* < 0.05). However, administration of aqueous extract of *H. sabdariffa* petals did not produce any significant effect on the plasma total protein and albumin concentrations (*P* > 0.05).

![Figure 1: Effects of aqueous extract of *Hibiscus sabdariffa* on Ca²⁺-Mg²⁺-ATPase activity in rat kidney](image)

Data are presented as mean ± standard error of mean. *n* = 6; *P* < 0.05, vs control group.

![Figure 2: Effects of aqueous extract of *Hibiscus sabdariffa* on Na⁺-K⁺-ATPase activity in rat kidney](image)

Data are presented as mean ± standard error of mean, *n* = 6.

### Table 3: Effects of aqueous extract of *Hibiscus sabdariffa* on plasma sodium, potassium and calcium levels in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Sodium (mmol/L)</th>
<th>Potassium (mmol/L)</th>
<th>Calcium (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>142.2±1.2</td>
<td>5.0±0.1</td>
<td>2.4±0.1</td>
</tr>
<tr>
<td>Extract of <em>Hibiscus</em></td>
<td>140.0±1.1</td>
<td>5.3±0.2</td>
<td>2.1±0.2</td>
</tr>
<tr>
<td><em>H. sabdariffa</em> (25 mg/kg)</td>
<td>143.4±1.1</td>
<td>5.2±0.2</td>
<td>2.2±0.1</td>
</tr>
<tr>
<td>Extract of <em>Hibiscus</em></td>
<td>140.0±1.1</td>
<td>5.3±0.2</td>
<td>2.1±0.2</td>
</tr>
<tr>
<td><em>H. sabdariffa</em> (50 mg/kg)</td>
<td>143.4±1.1</td>
<td>5.2±0.2</td>
<td>2.2±0.1</td>
</tr>
</tbody>
</table>

### Table 4: Effects of aqueous extract of *Hibiscus sabdariffa* on plasma total protein, albumin, creatinine and urea concentrations in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Plasma total protein (g/L)</th>
<th>Albumin (g/L)</th>
<th>Creatinine (g/L)</th>
<th>Urea (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>6.48±0.06</td>
<td>3.65±0.07</td>
<td>6.05±0.20</td>
<td>0.47±0.01</td>
</tr>
<tr>
<td>Extract of <em>Hibiscus</em></td>
<td>6</td>
<td>6.78±0.11</td>
<td>3.83±0.12</td>
<td>5.95±0.38</td>
<td>0.46±0.03</td>
</tr>
<tr>
<td><em>H. sabdariffa</em> (50 mg/kg)</td>
<td>6</td>
<td>6.62±0.06</td>
<td>3.64±0.06</td>
<td>4.89±0.12</td>
<td>0.37±0.03</td>
</tr>
</tbody>
</table>

* *P* < 0.05, vs control group.

### 3 Discussion

The main findings of the present study demonstrate that oral administration of aqueous extract of *H. sabdariffa* petals decreased the Ca²⁺-Mg²⁺-ATPase activity but preserved the Na⁺-K⁺-ATPase activity in kidney of rats. The study also indicates that administration of the extract resulted in insignificant changes in plasma Na⁺, K⁺, Ca²⁺, total protein and albumin concentrations but led to significant decreases in plasma urea and creatinine concentrations. The effects of aqueous extract of *H. sabdariffa* petals on renal function have not received serious attention, despite kidney being an important organ responsible for the excretion of foreign substances including the extract of the plant materials.

It has been observed that impaired activities of Na⁺-K⁺-ATPase and Ca²⁺-Mg²⁺-ATPase are associated with abnormal renal function[32,33]. On the other hand, enhanced activities of these enzymes have been reported to impact on cardiovascular and renal functions positively[28,31]. The decreased Ca²⁺-Mg²⁺-ATPase activity may imply the abnormal renal calcium handling during the use of *H. sabdariffa*. Nonetheless, the speculation is not supported by the unchanged plasma calcium level observed in these animals. Furthermore, the fact that renal Na⁺-K⁺-ATPase activity was preserved further ruled out the possibility that the observed impaired Ca²⁺-Mg²⁺-ATPase activity would lead to abnormal calcium renal handling since Na⁺-K⁺-ATPase is also involved in renal calcium handling. It was also observed that the extract did not have a significant effect on biochemical “markers” for cell lysis or death such as ALP, AST and ALT activities, as well as kidney and body weights, indicating that the extract may not have any deleterious effect on body or organs. The observation that aqueous extract of *H. sabdariffa* at 50 mg/kg caused a significant reduction in serum urea and creatinine concentrations further corroborates the view that aqueous extract of *H. sabdariffa* preserves renal function. This was similar to what was observed by Kirdpon *et al*[39], although their findings were in humans receiving a higher dose of 24 g/d of *H. sabdariffa*.

In conclusion, this study demonstrates that oral administration of aqueous extract of *H. sabdariffa* petals is associated with decreased renal Ca²⁺-Mg²⁺-ATPase activity and unaltered renal calcium handling. And it also supports that aqueous extract of *H. sabdariffa* petals enhances renal function due to its reduction of serum urea and creatinine.
concentrations.

4 Acknowledgements
The authors are grateful to Prof. G.A. Olutunji, Chemistry Department, University of Ilorin, Nigeria for assistance in the extraction process.

5 Competing interests
The authors declare that they have no competing interests.

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玫瑰茄提取物对 Wistar 大鼠肾 Na⁺-K⁺-ATP 酶及 Ca²⁺-Mg²⁺-ATP 酶活性的影响

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目的：研究口服玫瑰茄水提取物对大鼠肾 Na⁺-K⁺-ATP 酶和 Ca²⁺-Mg²⁺-ATP 酶活性的影响。
方法：连续 28 d 分别给予实验大鼠口服 25 和 50 mg/kg 的玫瑰茄水提取物，同时给予对照组大鼠灌胃适当剂量的蒸馏水。用光谱测定法分析大鼠肾脏中 Na⁺-K⁺-ATP 酶和 Ca²⁺-Mg²⁺-ATP 酶的活性。
结果：口服 25 和 50 mg/kg 的玫瑰茄提取物后，实验组大鼠肾 Ca²⁺-Mg²⁺-ATP 酶活性显著降低（P<0.05），然而肾 Na⁺-K⁺-ATP 酶的活性未受到任何影响。实验组大鼠体质量、肾脏质量，血浆白蛋白和总蛋白浓度，碱性磷酸酶、天冬氨酸氨基转移酶、丙氨酸氨基转移酶活性以及血浆钠、钾、钙离子的浓度与对照组大鼠相比无明显变化；但大鼠的肌酐以及尿素水平在服用 50 mg/kg 的玫瑰茄提取物后明显降低（P<0.05）。
结论：尽管口服玫瑰茄提取物会引起肾 Ca²⁺-Mg²⁺-ATP 酶活性的减弱，但同时可以起到保护肾脏功能的作用。
关键词：木槿属；Ca²⁺-Mg²⁺-ATP 酶；钠钾交换 ATP 酶；肾功能试验；大鼠