Assessment of the effect of aqueous extract of calyx of *Hibiscus sabdariffa* on some biochemical indices of renal function in rats

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ABSTRACT

The calyx of *Hibiscus sabdariffa* is used to prepare a beverage popularly known as ‘zobo’ in Nigeria. In spite of its acclaimed medicinal and nutritional values, studies on its potential toxicity on vital organs such as the kidney, liver and heart are few. In this study, the effect of aqueous extract of calyx of *Hibiscus sabdariffa* on some biochemical indices of renal function in rats was investigated. Rats were grouped and treated with graded doses of the extract for 28 days. Thereafter, blood samples were taken for biochemical assays. There was no significant difference in the levels of total protein, urea, creatinine, creatinine clearance, serum amylase, and kidney thiobarbituric acid -reactive substances (TBARS) of the treated rats when compared with the control. The study showed that the extract was not nephrotoxic at the doses administered.

Keywords: Kidney, *Hibiscus sabdariffa*, toxicity, bioassay, beverage.

INTRODUCTION

*Hibiscus sabdariffa* Linn is a shrub that belongs to the family Mallowaceae. It is widely grown in the tropics for its nutritional and medicinal values. Its common names include Roselle, Sorrel, Jamaica sorrel, India sorrel, Jelly okra and Florida cranberry [1]. In Nigeria, the aqueous extract of the calyx is prepared and taken as beverage after adding spices and flavoring agents. This beverage popularly known as ‘zobo’ has received widespread acceptance and has replaced canned and bottled carbonated drinks in many communities in Nigeria. The aqueous extract of the calyx has been reported to lower blood pressure [2]. It has also been shown to reduce lipid peroxidation in carbon tetrachloride-induced liver damage in experimental animals [3]. Zobo is consumed by all sorts of people including male and female, young and old, and even pregnant women. It is generally assumed that zobo is safe for consumption. However, in spite of its acclaimed nutritional and medicinal properties, safety is not guaranteed. The plant needs to be subjected to more toxicological studies to establish its toxicity profile. After such studies, some medicinal plants that were once considered safe for consumption have been found to cause damage to vital organs of the body [4]. This underscores the need to screen food and drinks for toxic effects before they are approved for consumption. It is especially important to appreciate the fact that all chemicals, including those naturally found in foods, become toxic at some doses. For instance, consumption of glucose, salt and even nutrients like vitamin A are hazardous at intakes only a few times greater than normal human requirements. In addition, there are many examples of potentially dangerous toxins in natural food products. Cyanide is found in plants such as cassava, sorghum and almond kernel while alkaloids are found in some herbal teas [5]. Puffer fish which contains a potentially fatal neurotoxin is considered a delicacy in Japan. It was recently reported that about 100 people died in Kenya after consuming a poisonous locally brewed beer [6]. Toxicity testing of food and drinks reveals what the likely adverse effects are and at what level of consumption they may occur. This will go a long way in preventing toxicity tragedies among the consumers. In view of the widespread acceptance of aqueous extract of *Hibiscus sabdariffa* (zobo) as a refreshing beverage...
without considering its potential toxic effects, more toxicological studies of the plant are needed. In the present study, we investigated the effects of zobo drink on some biochemical indices of renal function.

**MATERIALS AND METHODS**

**Preparation of zobo drink**

Dried samples of *Hibiscus sabdariffa* calyx were obtained from a popular market in Osogbo, Nigeria. The plant was identified and authenticated in the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria where voucher specimen was deposited. Aqueous extract of the plant was prepared according to the method of Iyare and Adegoke (2008). Two hundred grams (200 g) of dried *Hibiscus sabdariffa* calyx was boiled in 1000 mL of distilled water for 15 min. The boiled sample was allowed to cool and then filtered. The filtrate was evaporated to dryness in an oven at 40 °C to produce a dark red residue (yield = 6.8%).

**Experimental animals**

Healthy male Wistar rats (180 - 200 g) used in the study were obtained from the Animal House of the College of Health Sciences, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. They were kept in rat cages and maintained under standard laboratory conditions. The animals were acclimatized for one week, fed on rat pellets (Livestock Feeds PLC, Ibadan, Oyo State, Nigeria) and allowed free access to clean water *ad libitum*. They were fasted overnight before the experiment was carried out. The study was approved by the Animal Welfare and Ethics Committee of LAUTECH, Ogbomoso, Nigeria. All conditions of animal use were also as approved by United States National Institute of Health (NIH) guide for Care and Use of Laboratory Animals and in accordance with the recommendation of IASP [7].

**Experimental protocol**

Twenty rats were randomly divided into four groups of 5 rats each. Group I which served as the control received distilled water (5mL/kg). Groups II, III and IV were given 100, 200 and 400 mg/kg body weight of the extract respectively. The animals were acclimatized once a day for 28 days. After the 28-day treatment, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg body weight). Before the rats were sacrificed, blood was collected from the abdominal aorta for serum assay. Blood samples were centrifuged at 1,800 rpm for 10 min at 4 °C. Serum obtained was immediately stored at -70 °C until it was assayed. The kidney of each rat was excised, homogenized and then centrifuged and the supernatant was used for biochemical assays.

**Biochemical assays**

For the estimation of kidney thiobarbituric acid-reactive substances (TBARS), the method of Gutteridge and Quinlan [8] was used. Briefly, 150 µL of the tissue supernatant was diluted to 500 µL with deionized water. Then 1.34% thiobarbituric acid (250 µL) was added. This was followed by the addition of 250 µL of trichloroacetic acid. The mixture was thoroughly shaken and incubated for 30 min in a boiling water bath. The mixture was allowed to cool to room temperature after which the absorbance was read at 532 nm. In order to estimate creatinine clearance, rats were individually kept in metabolic cages for 24 hours before they were sacrificed. During this time, they were allowed free access to clean water and standard rat pellets. Urine volume in 24 hours was calculated for individual rat. Different biochemical parameters viz. serum and urine creatinine, blood urea, total protein and activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using an autoanalyzer (Hitachi Model 905, Tokyo, Japan). Serum amylase level was measured by an enzymatic method, using the Abbott spectrum analyzer (Abbott, Chicago, IL, USA). Creatinine clearance was calculated using the formula:

\[
\text{Creatinine clearance} = \frac{\text{urine creatinine} \times \text{urine volume per day} \times \text{body weight}}{\text{serum creatinine} \times \text{body weight}} \times 100
\]

**Statistical analysis**

All values were expressed as mean ± SEM. Differences between groups were evaluated by one-way ANOVA followed by Tukey multiple comparison tests. Results were considered significant at *P* < 0.05

**RESULTS AND DISCUSSION**

The kidneys play a very important role in the regulation of electrolytes and intracellular fluid volume as well as the excretion of metabolic waste from the body. As such, overall body homeostasis is dependent on the functional integrity of the kidneys (Kamal, 2010). Any substance that is toxic to the kidney would adversely affect the total body metabolism. It is therefore important to establish the safety of food, drink and drugs before they are ingested. In this study, the effect of aqueous extract of *Hibiscus sabdariffa* on the biochemical markers of kidney function was investigated.

Total protein is a measure of all plasma proteins in the blood. The level of total protein may be affected by alteration in hepatic synthesis, protein distribution, dehydration or overhydration, and protein breakdown or excretion. An increase in total protein is usually the result of tissue damage [10]. In this study, all doses of the extract administered did not cause any significant change in the level of total protein. This suggests that the extract did not cause significant tissue damage in the rats. It is also an indication that excretion of protein via the kidney was not altered. Urea is a waste product of protein metabolism. It is formed in the liver and carried by the blood to the kidneys for excretion. Because urea is cleared from the blood by the kidneys, it can be used as a test of renal function [11]. In the present study, blood urea was slightly increased in the treated animals but this was not significant (*P* > 0.05) when compared with control. This suggests that renal function was not compromised following the administration of the extract. Serum creatinine is

http://ijps.alzeonpublishers.net/content/2014/3/ijps587-590.pdf
another parameter for determining kidney function. Creatinine is a protein produced by muscle and released into the blood stream. Creatinine level in the serum is proportional to the rate at which it is excreted and is therefore a measure of kidney function [12].

**Table 1. Effects of extract of Hibiscus sabdariffa on biochemical parameters in rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>100</th>
<th>200</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (mg/dL)</td>
<td>8.53 ± 1.64</td>
<td>8.62 ± 1.55</td>
<td>8.70 ± 1.19</td>
<td>8.55 ± 1.83</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.82 ± 0.12</td>
<td>0.76 ± 0.17</td>
<td>0.69 ± 0.21</td>
<td>0.66 ± 0.24</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>28.43 ± 4.31</td>
<td>28.85 ± 3.53</td>
<td>29.72 ± 4.20</td>
<td>31.22 ± 3.34</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>126.53 ± 5.72</td>
<td>127.23 ± 6.60</td>
<td>127.84 ± 5.06</td>
<td>127.51 ± 6.22</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>72.66 ± 4.61</td>
<td>69.72 ± 5.34</td>
<td>72.81 ± 4.77</td>
<td>70.78 ± 5.62</td>
</tr>
<tr>
<td>TBARS (nmol/mg protein)</td>
<td>0.82 ± 0.27</td>
<td>0.60 ± 0.14</td>
<td>0.64 ± 0.33</td>
<td>0.62 ± 0.16</td>
</tr>
<tr>
<td>Serum amylase (x 103 U/L)</td>
<td>1.62 ± 0.51</td>
<td>1.54 ± 0.32</td>
<td>1.68 ± 0.74</td>
<td>1.65 ± 0.48</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM (n = 5)

In rats treated with the extract of Hibiscus sabdariffa, there was a slight reduction in the level of serum creatinine which was not significant (P > 0.05) compared with control. This again indicates that the extract did not affect kidney function adversely at the doses administered. Enzymes are important for many biochemical reactions in the living cell. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are good examples of these cytosolic enzymes. Although they are more concentrated in some organs like liver and heart, they are widely distributed in other parts of the body. Damaged tissues usually spill these enzymes into the plasma. Elevation of the enzymes in parts of the body. Damaged tissues usually spill these enzymes into the plasma. Elevation of the enzymes in the blood is therefore an indication of tissue damage and altered membrane permeability [13]. The extract did not cause any significant change in the activities of AST and ALT. This also suggests that the functions of vital organs like liver, heart and kidney are not impaired. In terms of metabolic risk factors, only renal dysfunction contributes to increase in serum levels of amylase because circulating amylase is mainly excreted by the kidney [14]. *Hibiscus sabdariffa* did not cause any significant increase or reduction in the level of serum amylase, suggesting that it is not toxic to the kidney (Table 1). Oxidative stress, accompanied by an increase in production of TBARS, may contribute to hypertrophy of nephron and impaired kidney function via increased production of angiotensin II [15]. In this study, the level of TBARS in the kidney of the treated rats was slightly reduced but the reduction was not significant (P > 0.05) when compared to the control. Creatinine clearance in the treated rats was slightly increased but not significantly different from that of the control (Figure 1). All these indicate that the functional integrity of the kidney is maintained in rats treated with extract of *Hibiscus sabdariffa*.

**CONCLUSION**

In conclusion, the results of this study suggest that aqueous extract of *Hibiscus sabdariffa* does not impair kidney function in rats. Clinical study is necessary to confirm its low toxicity in human.

**REFERENCES**


