Antihypertensive activity of *Hibiscus Sabdariffa* aqueous calyx extract in Albino rats

Abubakar M. G.\(^1\), Ukwuani A. N.\(^2\), and Mande U. U.\(^1\)

\(^1\)Department of Biochemistry, Faculty of Science, Usmanu Danfodiyo University, Sokoto.
\(^2\)Department of Biochemistry, Faculty of Science, Kebbi State University of Science and Technology, Aliero.

Accepted 3 September, 2015

The anti-hypertensive activity of aqueous calyx extract of *Hibiscus sabdariffa* on salt induce hypertensive albino rats was investigated for 28 days using the non invasive method. The extract and drug treated groups showed a significant \((P < 0.01)\) reduction in diastolic and systolic blood pressure when compared to the normotensive and hypertensive rats. There was no significant difference \((P > 0.05)\) between the drug treated group and the extract treated group during this treatment. Thus, this study further supports previous findings and the use of *H. sabdariffa* calyx extract in the treatment of hypertension.

**Key words:** Anti-hypertensive, *H. sabdariffa* calyx, diastolic, systolic, blood pressure.

INTRODUCTION

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (Ali et al., 1996). Diversity, flexibility, easy accessibility, broad acceptance in developing countries and increasing popularity in developed countries, relative low cost, relative low side effects and rising economic importance are some of the encouraging features of traditional medicines (Shahzad and Farnaz, 2013). Many African countries find the idea of research into traditional medicine enticing for it embodies with the hope that traditional medicines can supplement or even replace the orthodox form of health care which these countries are unable to provide adequately. In the recent times, there has been an increased awareness of the importance of traditional/alternative medicine in health care of human and animal population in developing countries (Adinya et al., 2012) and efforts are being made to integrate them with modern orthodox medicine.

Hypertension is the most common cardiovascular disease and major health problem in both developed and developing countries. It afflicts almost one billion people worldwide and is a leading cause for morbidity and mortality (Jawaid et al., 2011). In Nigeria, the true incidence of hypertension remains unknown but its prevalence amongst male and female is estimated to be 11.2% with age adjusted figure of 9.3%. This translates into approximately 13.4 million Nigerian hypertensive aged 15 years and above, using the projected National population census figure of 120 million (Akinkugbe, 1998). Despite of the large number of modern antihypertensive drugs, people largely depend on complementary and alternative medicine for treatment while some have the idea that combining it with conventional treatment works better.

*Hibiscus sabdariffa* is a medicinal herb belonging to the malvaceae family. The calyces of *H. sabdariffa* are prolific in many modern commercial blends of cold and hot drinks due to its pleasing taste, as well as having decorative, culinary and medicinal uses. Tender young leaves and stems - raw or cooked use in salads, as a pot-herb and as a seasoning in curries while fresh calyx (the outer whorl of the flower) is eaten raw in salads, is cooked and used for making soups, sauces, pickles, puddings and as flavoring agent (Arvind and Alka, 2011). In Nigeria, calyx infusion, called “zobo”, is used for treatment of hypertension. *H. sabdariffa* have been found to have antioxidant activity (Burton-Freeman., 2010), blood lipid lowering effect (Kuriyan et al., 2010), antimicrobial activity (Fullerton et al., 2011), anticancer activity (Lin et al., 2011) and hypotensive property.

*Corresponding author. E-mail: pinknnenna@yahoo.com.*
Table 1. Drug and *H. sabdariffa* calyx extract treatment table (7\textsuperscript{TH} to 10\textsuperscript{TH} week).

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal untreated</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>hypertensive untreated</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>hypertensive treated (nifedipine)</td>
<td>10mg/kg</td>
</tr>
<tr>
<td>IV</td>
<td>hypertensive treated (<em>H. sabdariffa</em>)</td>
<td>250mg/kg</td>
</tr>
<tr>
<td>V</td>
<td>hypertensive treated (<em>H. sabdariffa</em>)</td>
<td>500mg/kg</td>
</tr>
</tbody>
</table>

(Onyenekwe et al., 1999; Ajay et al., 2007; Mojiminiyi, 2012). This study uses non invasive method to evaluate the antihypertensive activity of aqueous calyx extract of *H. sabdariffa* in rats.

**MATERIALS AND METHODS**

**Plant material**

Dry calyx of *Hibiscus sabdariffa* were purchased from Sokoto state (Nigeria) old market in July, 2011 and taken to the botany unit, Department of Biological sciences, Usmanu Danfodiyo University, Sokoto, where it was identified and a specimen was deposited in the herbarium for future reference.

**Preparation of plant extract**

The dry calyx was crushed into coarse powder using pestle and mortar. 500 g of the powder was soaked in 2.5L of boiling water and allowed to stand for 30 min after which it was then filtered using muslin cloth. The filtrate was gradually evaporated at 45 °C in a drying cabinet. The dried extract was then weighed and stored in a clean dry container in a refrigerator for further use.

**Experimental animals**

Twenty five rats weighing between 200 – 300 g were randomly selected and used for this study. The animals were purchase from the Department of Pharmaceutical Sciences Animal House, Ahmadu Bello University, Zaria, Kaduna state, Nigeria. The animals were housed at Faculty of Veterinary Medicine Animal House, City Campus Complex of Usmanu Danfodiyo University, Sokoto and maintained on standard rodent pellet (rat chow) and water *ad libitum*. They were allowed to acclimatize for 14 days in the animal house prior to commencement of the experiment.

**Induction of hypertension**

All rats except the normotensive control were placed on a high-salt diet by adding 8% sodium chloride (NaCl) to their feed to accelerate the progression and severity of hypertension for 6 weeks. Weekly body weight, systolic and diastolic blood pressure was measured and recorded (Mojiminiyi et al., 2007; Niu et al., 2005).

**Experimental design**

The animals presenting high blood pressure measurement after six weeks of salt loading were randomly divided into groups of 5 rats each (Table 1). The calyx extract dose for this experiment was 0.2%, 5% and 10% of the already established LD\textsubscript{50} of *H. sabdariffa* (Onyenekwe et al., 1999). Nifedipine, the standard drug used in this study, is a known calcium channel block used to treat angina (heart pain), high blood pressure, and abnormal heart rhythms (Henry, 1980). Nifedipine and extract treatments commenced by the 7\textsuperscript{TH} week of salt loading. Systolic and diastolic blood pressure were also measured weekly and recorded.

**Measurement of blood pressure**

Systolic and diastolic blood pressures were measured by tail-cuff method using non-invasive Ugo Basile series 58500 blood pressure recorder. The rats were placed in the restrainers and kept in a scanner for 30 mins to warm the animals prior to obtaining pressure measurements. A cuff was placed on the base of the tail to occlude the blood flow. A transducer was placed close to the cuff which measures the pulse rate upon deflation. The non-invasive blood pressure sensor was utilized to monitor the blood pressure and an average of 6 readings was taken for each rat.

**Statistical analysis**

The data obtained was analyzed using one way analysis of variance (ANOVA) followed by Bonferoni Multiple Comparison Test using Instat® graph pad software (USA). The results were presented as Mean ± SEM and difference between means were considered significant at p < 0.05.
Table 2. Mean systolic blood pressure during induction.

<table>
<thead>
<tr>
<th>Group</th>
<th>Base line</th>
<th>2nd week</th>
<th>4th week</th>
<th>5th week</th>
<th>6th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>122.0 ±0.63</td>
<td>123.0 ±1.05</td>
<td>118.4 ±2.09</td>
<td>122.2 ±0.80</td>
<td>121.6 ±1.21</td>
</tr>
<tr>
<td>II</td>
<td>120.6 ±2.42</td>
<td>130.0 ±1.58**</td>
<td>133.2 ±0.37**</td>
<td>134.0 ±0.55**</td>
<td>134.4 ±0.40*</td>
</tr>
<tr>
<td>III</td>
<td>122.0 ±0.84</td>
<td>130.4 ±1.57**</td>
<td>133.2 ±0.37**</td>
<td>133.8 ±0.37**</td>
<td>135.2 ±0.20**</td>
</tr>
<tr>
<td>IV</td>
<td>123.0 ±2.05</td>
<td>125.4 ±0.24</td>
<td>134.0 ±0.55**</td>
<td>133.4 ±0.40**</td>
<td>133.0 ±2.03**</td>
</tr>
<tr>
<td>V</td>
<td>124.2 ±0.37</td>
<td>124.8 ±0.37**</td>
<td>133.8 ±0.37**</td>
<td>134.2 ±0.58**</td>
<td>134.6 ±0.24**</td>
</tr>
</tbody>
</table>

Values are in Mean ± SEM. ** Significantly different (p<0.01) from group I (Normotensive).

Table 3. Mean diastolic blood pressure during induction.

<table>
<thead>
<tr>
<th>Group</th>
<th>Base line</th>
<th>2nd week</th>
<th>4th week</th>
<th>5th week</th>
<th>6th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>77.4 ±0.51</td>
<td>77.0 ±0.63</td>
<td>77.0 ±0.55</td>
<td>76.8 ±0.37</td>
<td>74.4 ±1.44</td>
</tr>
<tr>
<td>II</td>
<td>77.0 ±1.00</td>
<td>81.8 ±1.49</td>
<td>88.6 ±2.90*</td>
<td>90.6 ±1.33**</td>
<td>90.6 ±0.87**</td>
</tr>
<tr>
<td>III</td>
<td>74.8 ±1.74</td>
<td>78.8 ±1.24</td>
<td>78.4 ±0.48</td>
<td>85.4 ±3.03</td>
<td>85.8 ±2.85*</td>
</tr>
<tr>
<td>IV</td>
<td>77.0 ±2.87</td>
<td>80.2 ±1.80</td>
<td>82.0 ±1.92</td>
<td>84.8 ±2.08</td>
<td>85.4 ±1.72*</td>
</tr>
<tr>
<td>V</td>
<td>77.2 ±0.37</td>
<td>79.4 ±0.75</td>
<td>82.2 ±1.63</td>
<td>84.2 ±1.24</td>
<td>83.4 ±0.87**</td>
</tr>
</tbody>
</table>

Values are in Mean ± SEM. ** Significantly different (p<0.01) from group I (Normotensive). *Significantly different (p<0.05) from group I.

Table 4. Mean pulse rate during induction.

<table>
<thead>
<tr>
<th>Group</th>
<th>Base line</th>
<th>2nd week</th>
<th>4th week</th>
<th>5th week</th>
<th>6th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>169.5 ±2.62</td>
<td>195.0 ±3.02</td>
<td>232.5 ±5.46</td>
<td>195.2 ±25.05</td>
<td>240.8 ±51.37</td>
</tr>
<tr>
<td>II</td>
<td>278.0 ±50.81 *</td>
<td>292.4 ±30.01x</td>
<td>378.0 ±25.12**</td>
<td>196.8 ±6.32</td>
<td>209.4 ±10.75</td>
</tr>
<tr>
<td>III</td>
<td>173.2 ±19.03</td>
<td>287.0 ±24.82x</td>
<td>242.8 ±31.13</td>
<td>228.4 ±14.06</td>
<td>200.8 ±6.46</td>
</tr>
<tr>
<td>IV</td>
<td>173.0 ±4.58</td>
<td>296.0 ±32.66x</td>
<td>227.6 ±20.21</td>
<td>234.4 ±31.16</td>
<td>224.2 ±26.09</td>
</tr>
<tr>
<td>V</td>
<td>167.2 ±12.36</td>
<td>223.4 ±1.40</td>
<td>207.6 ±18.31</td>
<td>260.8 ±38.08</td>
<td>259.8 ±25.32</td>
</tr>
</tbody>
</table>

Values are in mean ± SEM. ** Significantly different (p<0.01) from group I (Normotensive). *Significantly different (p<0.05) from group I.

RESULTS

Effect of *H. sabdariffa* on physical activity and body weight

All of the rats treated with the drug and extract showed normal general behavior. The extract treated groups were less interested in food but consumes larger amount of water when compared to the normotensive rats. The body weights of treated groups before, during and after treatment in this study showed no significantly different (p > 0.05) from those of the control.

Effect of *H. sabdariffa* on blood pressures

In the first six (6) weeks of high salt diet there was a progressive increase (p<0.01) in mean arterial pressure (MAP), systolic blood pressure (SBP) and Diastolic blood pressure (DBP) when compared to the normotensive group (Tables 2 - 4). Upon commencement of treatment (7th to 10th week), MAP, SBP and DBP decreased in the extract treated groups while nifedipine treated group also showed a decrease in blood pressure but not significantly (p > 0.05) greater than the extract treated group (Table 5). In the *H. sabdariffa* treated groups, the mean systolic blood pressure decreased from 134.0 to 129.0 mmHg and 134.8 to 129 mmHg for 250 and 500 mg/kg of aqueous Calyx extract of *H. sabdariffa* respectively. The mean reduction in systolic and diastolic blood pressure in the drug treated group were slightly, but not significantly (p > 0.05) greater than the reductions in the extract treated groups. At the end of treatment (11th to 12th week), there was no significant (p > 0.05) increase or decrease in MAP, SBP and DBP in all the groups (Table 6).

Discussion

Salt induced hypertension was used to assess the antihypertensive effects of the aqueous calyx extract of *H. sabdariffa*. Results obtained showed that chronic consumption of salt increased blood pressure. These findings are in agreement with previous studies demonstrating that salt can cause hypertension (Theophile, 2005; Nui et al., 2005; Ogaihara et al., 2002).
Results show that the aqueous calyx extract is efficient as an antihypertensive agent by significantly preventing the increase of blood pressure and heart rate in salt-induced hypertensive rats.

Hypertension has been identified as one of the risk factors for the emergence of cardiovascular diseases and is associated with substantial morbidity and mortality, estimated to account for 35% of myocardial infarction and stroke, 49% of heart failure, and 24% of premature mortality (Padwal et al., 2001). Past hypertension intervention trials have revealed that the risk treatment of hypertension could reduce the risks of stroke by 42% and a 14% reduction in coronary heart disease (Hobbs, 2004). In order to treat hypertension, synthetic drugs have been developed by the pharmaceutical industry. However, such drugs have been reported to produce side effects including insomnia, angioedema, cough, and fetal abnormalities (Brown and Vaughan, 1998).

Nifedipine, the standard drug used in this study, is known to decrease arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through calcium channels. Inhibition of the initial influx of calcium inhibits the contractile processes of the smooth muscle cells, causing dilation of the coronary and systemic arteries, increased oxygen delivery to the myocardial tissue, decreased total peripheral resistance, decreased afterload. The vasodilatory effect of nifedipine results in an overall decrease in blood pressure (Drug bank, 2013). The findings of this study show the effect of nifedipine to be comparable to the calyx extract treated groups suggesting the extract could be acting via the same mechanism as nifedipine. The potential mechanisms of action for the blood pressure lowering effect of *H. sabdariffa* were not determined in our study but have been explored by others. In vitro and animal studies show that *H. sabdariffa* is a vaso-relaxant (Ali et al., 1991), perhaps via action on calcium channels (Owolabi et al., 1995), an angiotensin converting enzyme (ACE) inhibitor (Jonadet et al., 1990), and a diuretic (Caceres et al., 1987; Mojiminiyi et al., 2000).

### Conclusion

In conclusion, the present study reveals that aqueous calyx extract of *H. sabdariffa* possessed antihypertensive activity against salt-induced hypertension in rats. These results further support previous in vivo findings and the traditional use of *H. sabdariffa* as an antihypertensive agent. Further research is required to isolate and characterize the active constituent of this plant responsible for its antihypertensive effects.

### REFERENCES


