Hypotensive Effect of Ethanolic Seed Extract of *Hibiscus sabdariffa* Linn (*Malvaceae*) on Normotensive Cats

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**ABSTRACT**

The hypotensive effects of ethanolic seed extract of *Hibiscus sabdariffa* l. were evaluated in normotensive cats. The effects of the ethanolic extract were compared with normal basal rhythm and Acetylcholine. The standard drugs Ach and various doses of the extracts (1mg/ml, 5mg/ml and 10mg/ml) were injected through a cannula inserted in the femoral artery. The extract produced a significant (P<0.05) reduction in cat blood pressure. The potency of the extract seems to be high because it has activity at the minimum dose used. The 1mg/ml of the extract exhibited more effective response; however the standard drug Acetylcholine showed a greater potency than the extract. The seed extracts are characterized by low degree of toxicity with LD50 above 5000mg/kg in rats.

**Keywords:** Acetylcholine, blood pressure, *Hibiscus sabdariffa*, hypotensive.

**INTRODUCTION**

*Hibiscus sabdariffa* l. is taken as a common local drink popularly known as zobo in Nigeria. It is a herb belonging to the malvaceae family and cultivated for its leaf, fleshy calyx, seed or fibre (Dalziel, 1973). It is a medicinal herb, used in folk medicine in treatment of hypertension (Wang et al., 2000; Odigie et al., 2003). *Hibiscus anthocyanin*, a group of phenolic natural pigments present in the dried flower of *Hibiscus sabdariffa* and *Hibiscus rosasinensis* have been found to have cardioprotective (Jonadet et al., 1990), hypcholesterolemic (Chen et al., 2003), anti-oxidative and hepatoprotective (Wang et al.,2000; Amin and Hamza, 2005) effects in animals. Delphinidin 3-sambubioside, a *Hibiscus anthocyanin*, induces apoptosis in human leukemia cells through oxygen reactive species-mediated mitochondrial pathway.

Polysaccharides from *Hibiscus sabdariffa* flowers stimulate proliferation and differentiation of human keratinocytes (Brunold et al., 2004; Ali et al., 2005). *Hibiscus protocatechuic* acid has inhibitory and inductive effect on tumour promotion in mouse skin and in
human leukemia cells respectively. *Hibiscus sabdariffa* has been reported to be antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, refrigerant, sedative, stomachic and tonic (Morton, 1987; Olalere, 2007). In Nigeria, a decoction of the seeds is given to augment or induce lactation in cases of poor milk production, poor letdown and maternal mortality (Okasha et al., 2008). However, there is dearth of literature supporting hypotensive effect of the seeds. In light of this, the study is designed to evaluate the hypotensive effect of *Hibiscus sabdariffa* l. ethanolic seed extract.

**MATERIALS AND METHODS**

**Chemicals and drugs:** All chemicals and drugs used were of analytical grade. Heparin, sodium Thiopental, Atropine, Acetylcholine, Adrenaline (Aldrich Chemical Company, Gillingham England) was obtained from Department of pharmacology Ahmadu Bello University Zaria, Nigeria.

**Plant materials:** The samples of *Hibiscus sabdariffa* l. seeds were collected in December 2007 in Gaya Hong Local Government in Adamawa state of Nigeria. The plant was identified in the Department of Biological Sciences, Ahmadu Bello University, Zaria and authenticated voucher samples were deposited in the Herbarium section (code number 1056).

**Extract preparation:** The *Hibiscus sabdariffa* l. seeds were washed thoroughly, sun dried and ground into powder. The extraction of *Hibiscus sabdariffa* l. seeds was done using fifty grams (50g) of the powder seeds in soxhlet extractor with ethanol in Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria. The recycling of solvent (ethanol) was allowed to be repeated for complete extraction. The oil extracts were then poured into evaporating dish to evaporate the solvent in the extract over the water bath at the temperature of 40°C - 45 °C (Abdul, 1990) and a yield of 17ml of crude extract oil was obtained.

**Experimental design:** The experiment was an in vivo and two male cats weighing 1.72kg and 1.95kg were used respectively. The cats were anesthetized by injecting thiopental sodium via intraperitoneal route. The animal lost consciousness after 20 minutes of administration, after which it was properly secured to the dissecting table by using twine to tie its limbs, so as to prevent unwanted movement of the animal during the experiment. The left femoral vein was exposed and tied with peripheral ligature. Also the right carotid artery was exposed and cannulated for blood pressure measurement. The blood pressure was recorded on a filter paper of the micro-dynamometer after the administration of the heparinized normal saline to prevent blood clotting. The standard drugs and extract were administered through the cannula inserted through the left femoral vein. The speed and sensitivity of the machine were 95mm/minute and 1mV respectively. The first set of drugs recorded were the standard drugs (Ach and Adrenaline), followed by graded dose response for each extract, and the dose which caused maximum effect was chosen as experimental dose. Flushing was properly done after every administration of extract and drugs till it was brought back to normal.

**Phytochemical Analysis:** The ethanolic seed extract of *Hibiscus sabdariffa* l. were subjected to preliminary phytochemical screening to identify the chemical constituents. The methods of analysis employed were those described by Brain and Turner (1975).

**Acute toxicity study:** The lethal dose (LD50) of the plant extract was determined by the method of Lorde (1983) using 13 rats. In the first phase rats were divided into 3 groups of 3 rats each and were treated with the ethanolic extract of the seed at doses of 10, 100 and 1000 mg/kg body weight intraperitoneal. They were observed for 24 h for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat each and were also treated with the aqueous extract at doses of 1000, 1600, 2900 and 5000 mg/kg bodyweight (i. p). The median lethal dose (LD50) was calculated using the second phase.

**Statistical Analysis.** All data are expressed as Mean ± S.E.M. The data obtained were analyzed using one way analysis of variance (ANOVA) and Turkey-Kramer post hoc test for multiple comparisons. The (P<0.05) will be accepted as significant (Betty and Jonathan, 2003).

**RESULTS**

**Acute toxicity study (LD50):** The seed extracts are characterized by a very low degree of toxicity. The acute toxicity LD50 of *Hibiscus sabdariffa* l. ethanolic seed extract in albino rats...
was found to be above 5000 mg kg\(^{-1}\) according to the method of Lorke (1983).

**Phytochemical Analysis:** The preliminary phytochemical screening of the ethanolic seed extract of *Hibiscus sabdariffa l.* found the presence of alkaloids, saponins, Cardenolides, Deoxy sugar, tannins, steroidal rings, cardiac glycosides, flavonoids and anthraquinones.

**Blood pressure values:** The blood pressure values of the cats for normal saline, the injected standard drugs and ethanolic extract of *Hibiscus sabdariffa l.* as obtained from the micro-dynamometer are shown in the tables and figures below. One centimeter (1cm) of the standard ruler used in measuring the systolic and diastolic blood pressure change corresponds to 10mmHg pressure change in glass sphygmomanometer. On the tracing the values from the baseline to the lowest border of the tracing represent the diastolic pressure while from the baseline to upper border represent the systolic pressure. The blood pressure (systolic and diastolic), pulse pressure and mean arterial pressure in all the three doses (1mg/ml, 5mg/ml and 10mg/ml) of seed extract decreased significantly (P<0.05) when compare to normal basal rhythm, except in 5mg/ml of the pulse pressure there was insignificant decrease (P>0.05) when compare to normal basal rhythm. The standard drug Acetylcholine also when compared to the normal basal rhythm the blood pressure (systolic and diastolic), pulse pressure and mean arterial pressure in all the doses of Acetylcholine decreased significantly (P<0.05) as shown in table 1. The tracings of 1mg/ml in fig.2, 5mg/ml in fig.3 and 10mg/ml in fig.4 showed hypotensive effect *Hibiscus sabdariffa l.* when compared to normal basal rhythm in fig. 1.

**Table 1.** Comparison between Normal Basal Rhythm, Acetylcholine and ethanolic extract of *Hibiscus sabdariffa l.*

<table>
<thead>
<tr>
<th>Blood pressure [mm Hg]</th>
<th>Basal Rhythm</th>
<th>Acetylcholine</th>
<th>Ethanol extract of <em>Hibiscus sabdariffa l.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 mg/ml</td>
<td>5 mg/ml</td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>48.0±1.4</td>
<td>39.0±3.8(^{a})</td>
<td>29.8±3.2(^{b})</td>
</tr>
<tr>
<td>Diastolic pressure</td>
<td>32.5±1.6</td>
<td>16.3±3.4(^{a})</td>
<td>29.8±0.2(^{a})</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>15.5±2.5</td>
<td>19.5±0.9(^{a})</td>
<td>5.3±1.5(^{a})</td>
</tr>
<tr>
<td>MAB pressure</td>
<td>36.5±2.4</td>
<td>35.3±1.1(^{a})</td>
<td>33.0±1.0(^{a})</td>
</tr>
</tbody>
</table>

NS= Not significant, S= Significant.

**Table 2.** Comparison between Acetylcholine and ethanolic extract of *Hibiscus sabdariffa l.*

<table>
<thead>
<tr>
<th>Blood pressure [mm Hg]</th>
<th>Acetylcholine</th>
<th>Ethanol extract of <em>Hibiscus sabdariffa l.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>39.0±3.8</td>
<td>29.8±3.2(^{a})</td>
</tr>
<tr>
<td>Diastolic pressure</td>
<td>16.3±3.4</td>
<td>29.8±0.2(^{a})</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>19.5±0.9</td>
<td>5.3±1.5(^{a})</td>
</tr>
<tr>
<td>MAB pressure</td>
<td>35.3±1.1</td>
<td>33.0±1.0(^{a})</td>
</tr>
</tbody>
</table>

NS= Not significant, S= Significant.

The standard drug Acetylcholine also when compared to the various doses of the extract decreased blood pressure (systolic and diastolic), pulse pressure and mean arterial pressure in all the doses of Acetylcholine significantly (P<0.05) as shown in table 2.
### Table 3. Drug Antagonistic Studies.

<table>
<thead>
<tr>
<th>Drug + Aqueous Extract</th>
<th>0.2 ml Atropine [100µg/ml] + 0.4ml Eth. extract [1mg/ml]</th>
<th>0.1ml Ach [10µg/ml] + 0.4ml Eth. Extract [1mg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure parameters [mm Hg]</td>
<td>Atropine</td>
<td>Atropine + eth. ext</td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>Diastolic pressure</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>MAB pressure</td>
<td>33</td>
<td>33</td>
</tr>
</tbody>
</table>

In the drug antagonist studies in table 3, both Atropine and Acetylcholine blocked the effect of the ethanolic seed extract. Although the percentage change of Atropine was less than that of Acetylcholine, this indicates that Atropine has a stronger blocking effect.

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**Fig. 1.** Tracing of normal saline.

**Fig. 2.** Tracing of 1mg/ml of ethanolic seed extract of *Hibiscus sabdariffa l.* with volume of 1.6mls
Fig. 3. Tracing of 5mg/ml of ethanolic seed extract of *Hibiscus sabdariffa* l. with volumes of 0.2 and 0.4mls.

Fig. 4. Tracing of 10mg/ml of ethanolic seed extract of *Hibiscus sabdariffa* l. with volumes of 0.4 and 0.8mls.

Fig. 5. Tracing of drug antagonist studies of 0.4ml of 5mg/ml ethanolic seed extract of *Hibiscus sabdariffa* l. blocked by 0.1ml of 100µg/ml of Atropine.
DISCUSSION

The results of the present study reported that, the ethanolic seed extract *Hibiscus sabdariffa* l. showed blood pressure lowering effect in normotensive cat with significant statistical difference (P<0.05). The blood pressure (systolic and diastolic), pulse pressure and mean arterial pressure in all the three doses (1mg/ml, 5mg/ml and 10mg/ml) of seed extract decreased significantly (P<0.05) when compared to normal basal rhythm, except in 5mg/ml of the pulse pressure there was insignificant decrease (P>0.05) when compared to normal basal rhythm. Intravenous injection of extracts of *Hibiscus sabdariffa* calyx to anaesthetized cats (Ali et al., 1991) and anaesthetized rats (Adegunloye et al., 1996) lowered blood pressure in a dose-dependent manner. Aqueous seed extract of *Hibiscus sabdariffa* l. decreases blood pressure (Bako et al., 2009). The blood pressure (systolic and diastolic), pulse pressure and mean arterial pressure in all the doses of standard drug Acetylcholine decreased significantly (P<0.05) when compared to the normal basal rhythm. These effects was completely blocked by Atropine and Acetylcholine in seed extract while the hypotensive effect was resistant to a number of standard receptor blocking agents, but the hypotensive effect was partially blocked by atropine (Ali et al., 1991; Olatunji et al., 2005). Therefore, the hypotensive action of *Hibiscus sabdariffa* l. may be mediated through cholinooceptors as cholinergic antagonist, because Atropine blocked the effect of seed extract. From this study it can be inferred that, *Hibiscus sabdariffa* l. seed has an appreciable blood pressure lowering effect with mechanism of action through cholinooceptors as cholinergic antagonist.

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REFERENCES


