Effects of Aqueous Seed Extract of *Hibiscus Sabdariffa Linn (Malvaceae)* on Isolated Perfused Rabbit Heart

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Abstract

The effect of aqueous seed extract of *Hibiscus sabdariffa l.* on heart rate and strength of contraction of an isolated perfused rabbit heart was investigated. The isolated heart were perfused through the aorta using the Langendorff's set-up. Heart rate and strength of contraction were determined using three concentrations of the extract (100µg/ml, 10mg/ml and 100mg/ml) after standardizing the tissue with three standard drugs (Adrenaline, Acetylcholine and Atropine). The extract decreases the strength of contraction significantly ($P<0.05$) in a dose dependent manner while the heart rate showed no level of significance. It was observed that the extract had a very strong negative inotropic effect on the isolated heart which may have blood pressure lowering effect with no chronotropic property. The interaction between the extract and adrenaline suggest that the extract may competitively inhibit the β-adrenergic receptors on the myocardium.

KEYWORDS: Acetylcholine, blood pressure, chronotropic, *Hibiscus sabdariffa*, inotropic.

Introduction.

Plants have been a good source of food for ages and they provide essential nutritional values, medicinal properties and notable physiological effect to life (Facciola, 1986; Chopra *et al.*, 1990). *Hibiscus sabdariffa linn* (Malvaceae) potential herbs that possess various medicinal properties used for different ailments (Dalziel, 1973). *Hibiscus sabdariffa l.* is grown in all parts of the world and it is taken as a beverage popularly known as zobo in Nigeria. *Hibiscus sabdariffa l.* has been reported to be antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, refrigerant, sedative, stomachic and tonic (Morton, 1987; Kunkel, 1984). The calyx is used in folk medicine for treatment of hypertension (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,1990; Olaleye, 2007), hypocholesterolemic (Chen *et al.*,2003; Bako *et al.*, 2013), anti-oxidative and hepatoprotective (Wang *et al.*, 2000) effects in animals. In recent times, focus on plant research

has increased all over the world and a large body of evidence has collected to show immense potentials of medicinal plants used in various traditional systems. Various medicinal plants have been studied using modern scientific approaches. The results from these plants have revealed the potentials of medicinal plants like *Hibiscus sabdariffa l.* However, there is dearth of literature supporting the effect of the seeds on cardiovascular system. In light of this, the study is designed to evaluate the anti-hypertensive effect of *Hibiscus sabdariffa l.* seed extract.

Materials.

*Chemicals and drugs***.**

All chemicals and drugs used were of analytical grade. Sodium Chloride, Sodium Bicarbonate, Calcium Chloride, Potassium chloride, D-glucose, Atropine, Acetylcholine, Adrenaline (Aldrich Chemical company, Gillingham England) were obtained from Department of pharmacology Ahmadu Bello University Zaria, Nigeria.

*Plant materials***.**

The samples of *Hibiscus sabdariffa l.* seed were collected in December 2010 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.* seed was done using Maceration method in Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria. The mixtures were then shaken for ten hours with mechanical shaker. The supernatant liquid (extract) was filtered through a plug of cotton or glass wool. The process was repeated for complete extraction. The extracts were then poured into evaporating dish to evaporate the solvent in the extract over the water bath at the temperature of 40ºC - 45 ºC.

Methodology.

*Animals used***.**

Three rabbits were used for the experiment. The first weighed 1,420g, the second weighed 1,396g while the third weighed 1408g. They were bought in Samaru Market, Zaria. They were kept in the animal house of the Department of Human Physiology for a week to acclimatize and were fed on carrots and spinach vegetables with tap water, *ad libitum.*

*Experimental design***.**

The isolated heart was setup based on the method of Langendorff (1895). The animals were sacrificed by cervical dislocation and the thoracic cavity was immediately opened and the heart together with pericardial sac was removed. The inflow cannula of the isolated heart setup was then inserted into the ascending aorta and tied with a thread which was secured against the side arm of the cannula to prevent the perfusion pressure from pushing it off. A hook was passed through the tip of the ventricles and attached to the transducer by a thread passed through a pulley. Another thread was attached to a stand to create tension in the suspended heart. The temperature of the perfusate (Ringer Locke solution) passing through the inner spiral bath to the heart was maintained at between 36°C-37°C. This was done by warming the outer bath to about 40°C. The perfusion pressure was maintained at 40mmHg. The

experimental setup was based on the constant pressure model of the Langendorff isolated heart setup.

Ringer Locke solution.

The physiological solution was prepared by dissolving the following constituents 45gNaCl, 5gD-glucose, 21ml of 10%KCl, 2.6g$NaHCO_3$, and 5.4ml$CaCl_2$ in 5 litres of distilled water. The solution was kept constantly aerated with pure oxygen throughout the duration of the experiment.

Principles of the Langendorff isolated perfused Heart apparatus.

The principle on which the Langendorff apparatus operates is maintenance of cardiac activity by perfusing the heart via the coronary arteries using an aortic cannula inserted into the ascending aorta. The perfusion solution may be blood or physiological solution (Ringer Locke solution) and it is constantly oxygenated. The perfusion solution is delivered to the heart in a retrograde manner from a pressurized reservoir via the cannula inserted into the ascending aorta. It closes the aortic valve and flows into the coronary arteries. After passing through the coronary circulation, the perfusate enters the right atrium via the coronary sinus and is driven out of the heart via the right ventricle and the pulmonary artery. The transducer was connected to the Physiograph which was run at a speed of 0.25cm/s. Administration of drugs was done through the inflow cannula just before its point of entry into the heart. The standard drugs were administered and interactions between them were tested before the various concentrations of the extract were administered. The extract was also interacted with the standard drugs adrenaline and atropine.

Statistical Analysis.

All data are expressed as Mean ± S.E.M. and were analyzed using the student's paired t-test. SPSS package version 20.0 and *post hoc* test for multiple comparisons. The ($P<0.05$) was accepted as significant (Betty and Jonathan, 2003).

Results.

The results obtained for both strength and rate of contraction are expressed in the following tables as mean ± SEM (standard error of mean). The strength of contraction in millivolts (mV) was obtained from the sensitivity of the machine while the rate of contraction in beats/min was calculated from the speed of the machine. In table 1, 100µg/ml of the extract showed a significant ($P<0.05$) increase in the rate of contraction. The scenario in both tables 2 and 3 where 10mg/ml and 100mg/ml of the extract did not significantly increase the rate of contraction. In figure 4, the standard drugs were seen to produce their expected effects, with adrenaline increasing the rate and acetylcholine decreasing it. Propanolol and atropine blocked adrenaline and acetylcholine respectively showing no significant increase in the rate of myocardial contraction. Table 5 shows interaction of the extract with standard drugs. It showed no increase in rate of contraction on interaction with any of the drugs. Table 6 shows the effect of 100µg/ml on the force of myocardial contractility. There is no significant change in the strength of contraction although 0.8ml and 1.0ml of 100µg/ml slightly decrease the force of contraction. In table 7, there is a progressive decrease in the force of myocardial contractility when progressively increasing volumes of 10mg/ml of the extract were administered. Figure 8 shows the same progressive decrease in volume with

administration of increasing volumes of 100mg/ml of the extract. Figure 9 shows the effect of the standard drugs. The standard drugs all showed the expected effect on the force of contraction of the heart. Finally, in table 10, extract interaction with standard drugs showed that all responses were reduced after administration.

Discussion.

The result of the present study showed that, aqueous seed extract of *Hibiscus sabdariffa l.* exhibited a negative inotropic effect on the perfused heart of the rabbit. On administration of 1µg/ml of adrenaline, there was a significant increase in both the rate and strength of contraction of the myocardium. This is due to stimulation of adrenergic receptors (β_1) present in the myocardium (Abu-sitta *et al*., 2000). 1µg/ml of acetylcholine produced a significant decrease in both rate and strength of contraction by stimulating Muscarinic (M_2) receptors present on the myocardium (Eisenberg, 1985; Katzung, 1998). The responses obtained for both adrenaline and acetylcholine shows that the heart responded to the known effects of the standard drugs. When an action potential passes over the cardiac muscle membrane, it spreads to the interior of the cardiac muscle fiber along the membranes of the transverse tubules. The tubule action potentials in turn act on the membranes of the longitudinal sarcoplasmic tubules to cause release of calcium ions into the muscle sarcoplasm from the sarcoplasmic reticulum (Bers, 2002; Ganong, 2010). These calcium ions now diffuse immediately into the myofibrils and catalyze the chemical reactions that promote sliding of the actin and myosin filaments along one another, which produces the muscle contraction. The aqueous seed extract of *Hibiscus sabdariffa l.* at 100µg/ml had significant effect on both the rate and strength of contraction of the myocardium. At both higher concentrations of 10mg/ml and 100mg/ml, the seed extract had no effect on the rate of contraction of the myocardium. In case of the strength of contraction of the myocardium, the extract significantly decreased the strength of contraction in a dose dependent manner. Free intracellular calcium comes from two sources, first is from outside the cell where opening of the voltage-sensitive calcium channels causes an immediate rise in free cytosolic calcium (Eisner and Smith, 1990). The second source is release of calcium from sarcoplasmic reticulum and mitochondria, which further increases the cytosolic level of calcium. The calcium rich seed extract of *Hibiscus sabdariffa l.* could enhance its free calcium through any of the two sources (Bako *et al.,* 2010). On administration of 10mg/ml of the extract with 100µg/ml of adrenaline, there was no significant increase in the heart rate and also in the strength of contraction. This implies that *Hibiscus sabdariffa l.* probably blocks the β-adrenergic action of adrenaline. On administration of the same 10mg/ml of *Hibiscus sabdariffa l.* with 20µg/ml of atropine, the same effect was observed as when 10mg/ml of *Hibiscus sabdariffa l.* was administered alone. From this, it could be said that atropine probably has no antagonistic effect on the receptors for *Hibiscus sabdariffa l.* Intravenous injection of aqueous extracts of *Hibiscus sabdariffa l.* to anaesthetized cats (Ali *et al.,* 1991; Bako *et al.,* 2009) and anaesthetized rats (Adegunloye *et al.,* 1996) lowered blood pressure in a dose-dependent manner. This effect was resistant to a number of

standard receptor blocking agents, but the hypotensive effect was partially blocked by atropine (Ali *et al.*, 1991; Sharma, 1992). The effectiveness of an aqueous extract of *Hibiscus sabdariffa l.* on mild to moderate hypertension was confirmed in a clinical trial (Herrera-Arellano *et al.*, 2004). These results were not significantly different from those obtained by captopril treatment. No adverse effects were found with either treatment, confirming the effectiveness and safety of the extract. It was observed that the extract had a very strong negative inotropic effect on the isolated heart which may have blood pressure lowering effect. The interactions between the extract and adrenaline suggest that the extract may competitively inhibit the β-adrenergic receptors on the myocardium.

Acknowledgement.

The authors are grateful to the following; Muhammad Nura, Bala Muhammad and John Move of the Department of Human physiology Ahmadu Bello University, Zaria for the role he played in the laboratory during this research. We also acknowledge Alhaji Adamu Muhammad of the Department of Pharmacognosy and Drug Development Ahmadu Bello University, Zaria for the extraction of *Hibiscus sabdariffa l.* seed. We also acknowledge mcArthur foundation Ahmadu Bello University, Zaria and University Board of Research of Ahmadu Bello University, Zaria for supporting this research work.

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Table 1: Rate of contraction of 100µg/ml seed extract of *Hibiscus sabdariffa l.*

***Hibiscus sabdariffa* seed extract**	**MEAN ± SEM – Before**	**MEAN ± SEM –After**
100µg/ml (0.1ml)	87.5 ± 2.5	92.5 ± 2.5[b]
100µg/ml (0.2ml)	90.0 ± 2.0	93.0 ± 3.0[b]
100µg/ml (0.4ml)	91.0 ±1.0	107.0 ±1.0[a]
100µg/ml (0.8ml)	91.0 ±1.0	106.0 ±1.0[a]
100µg/ml (1.0ml)	92.0 ±1.0	106.5 ±1.0[a]

Significant ($P<0.05$)[a]; Not Significant ($P>0.05$)[b]

Table 2: Rate of contraction of 10mg/ml seed extract of *Hibiscus sabdariffa l.*

***Hibiscus sabdariffa* seed extract**	**MEAN ± SEM – Before**	**MEAN ± SEM –After**
10mg/ml (0.1ml)	117.5 ±2.5	115 ±5.0[b]
10mg/ml (0.2ml)	107.5 ±2.5	90.0 ±20[a]
10mg/ml (0.4ml)	105.0 ±1.0	105 ± 1.0[b]
10mg/ml (0.8ml)	109.0 ±10	105 ± 5.0[b]

Significant ($P<0.05$)[a]: Not Significant ($P>0.05$)[b]

Table 3: Rate of contraction of 100mg/ml seed extract of *Hibiscus sabdariffa l.*

***Hibiscus sabdariffa* seed extract**	**MEAN ± SEM - Before**	**MEAN ± SEM –After**
100mg/ml (0.1ml)	89 ±1.0	90 ±5.0[b]
100mg/ml (0.2ml)	75 ±5.0	77 ±5.0[b]
100mg/ml (0.4ml)	77±2.5	79±4.0[b]
100mg/ml (0.8ml)	85±2.4	78±3.0[b]

Significant (P<0.05)[a]; Not Significant (P>0.05)[b]

Table 4: Rate of contraction of standard drugs.

Standard drugs	**MEAN ± SEM - Before**	**MEAN ± SEM –After**
1µg/ml Adrenaline (0.1ml)	90 ± 5.0	150 ±10[a]
1µg/ml Ach (0.1ml)	105 ±5.0	60 ± 5.0[a]
100µg/ml Adrenaline(0.1ml) +100µg/ml Propanolol (0.2ml)	105 ±5.0	120 ±5.0[b]
10µg/ml Ach(0.1ml) + 10µg/ml Atropine (0.2ml)	104.5 ±2.5	106 ±1.0[b]

Significant (P<0.05)[a]; Not Significant (P>0.05)[b]

Table 5: Rate of contraction of *Hibiscus sabdariffa l.* interaction with standard drugs.

Standard drugs + *H. sabdariffa*	**MEAN ± SEM - Before**	**MEAN ± SEM –After**
10mg/ml *H. sabdariffa*(0.8ml) + 100µg/ml Adrenaline(0.2ml)	75 ±1.0	60 ±5.0[b]
10mg/ml *H. sabdariffa*(0.8ml) + 100µg/ml Adrenaline(0.4ml)	78 ±4.0	77.5 ±7.5[b]
10mg/ml *H. sabdariffa*(0.8ml) + 20µg/ml Atropine(0.1ml)	90 ±5.0	75.5 ±0.5[b]

Not Significant (P>0.05)[b]

Table 6: Strength of contraction of 100µg/ml seed extract of *Hibiscus sabdariffa l.*

***Hibiscus sabdariffa* seed extract**	**MEAN ± SEM - Before**	**MEAN ± SEM –After**
100µg/ml (0.1ml)	250 ±10	255 ±5.0[b]
100µg/ml (0.2ml)	250 ±5.0	255 ± 5.0[b]
100µg/ml (0.4ml)	252.5 ± 2.5	257.5 ± 2.5[b]
100µg/ml (0.8ml)	247.5 ± 2.5	205 ± 5.0[a]
100µg/ml (1.0ml)	300 ±5.0	215 ± 5.0[a]

Significant (P<0.05)[a]; Not Significant (P>0.05)[b]

Table 7: Strength of contraction of 10mg/ml seed extract of *Hibiscus sabdariffa l.*

***Hibiscus sabdariffa* seed extract**	**MEAN ± SEM - Before**	**MEAN ± SEM –After**
10mg/ml (0.1ml)	250 ±10	250 ±5.0[b]
10mg/ml (0.2ml)	250 ±32	97.5 ±28[a]
10mg/ml (0.4ml)	250 ±1.0	210 ±1.0[a]
10mg/ml (0.8ml)	100 ±2.5	25 ± 1.0[a]

Significant (P<0.05)[a] ; Not Significant (P>0.05)[b]

Table 8: Strength of contraction of 100mg/ml seed extract of *Hibiscus sabdariffa l.*

***Hibiscus sabdariffa* seed extract**	**MEAN ± SEM - Before**	**MEAN ± SEM –After**
100mg/ml (0.1ml)	150 ±5.0	50 ±1.0[a]
100mg/ml (0.2ml)	150 ±10	25 ±5.0[a]
100mg/ml (0.4ml)	120±2.5	35±4.0[a]
100mg/ml (0.8ml)	130±3.5	55±6.0[a]

Significant (P<0.05)[a]

Table 9: Strength of contraction of standard drugs.

Standard drugs	**MEAN ± SEM - Before**	**MEAN ± SEM –After**
1µg/ml Adrenaline (0.1ml)	400 ±5.0	800 ±10[a]
1µg/ml Ach (0.1ml)	400 ±5.0	350 ±10[a]
100µg/ml Adrenaline(0.1ml) +100µg/ml Propanolol (0.2ml)	450 ±50	300 ±50[a]
10µg/ml Ach(0.1ml) + 10µg/ml Atropine (0.2ml)	300 ±10	300 ±10[b]

Significant (P<0.05)[a]

Table 10: Strength of contraction of *Hibiscus sabdariffa l.* with standard drugs.

Standard drugs + *H. sabdariffa*	**MEAN ± SEM - Before**	**MEAN ± SEM –After**
10mg/ml *H. sabdariffa*(0.8ml) + 100µg/ml Adrenaline(0.2ml)	400±20	300 ±10[a]
10mg/ml *H. sabdariffa*(0.8ml) + 100µg/ml Adrenaline(0.4ml)	500±10	400±5.0[a]
10mg/ml *H. sabdariffa*(0.8ml) + 20µg/ml Atropine(0.1ml)	450±5.0	150±10[a]

Significant (P<0.05)[a]