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The Aqueous Calyx Extract of *Hibiscus sabdariffa* Lowers Blood Pressure and Heart Rate via Sympathetic Nervous System Dependent Mechanisms

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Summary: The antihypertensive effect of *Hibiscus sabdariffa* (HS) has been validated in animals and man. This study tested the hypothesis that its hypotensive effect may be sympathetically mediated. The cold pressor test (CPT) and handgrip exercise (HGE) were performed in 20 healthy subjects before and after the oral administration of 15mg/Kg HS. The blood pressure (BP) and heart rate (HR) responses were measured digitally. Mean arterial pressure (MAP; taken as representative BP) was calculated. Results are expressed as mean ±SEM. $P<0.05$ was considered significant. CPT without HS resulted in a significant rise in MAP and HR (111.1±2.1mmHg and 100.8±2.0/min) from the basal values (97.9±1.9mmHg and 87.8±2.1/min; $P<0.0001$ respectively). In the presence of HS, CPT-induced changes (ΔMAP=10.1±1.7mmHg; ΔHR= 8.4±1.0/min) were significantly reduced compared to its absence (ΔMAP= 13.2±1.2mmHg; ΔHR= 13.8±1.6/min; $P<0.0001$ respectively). The HGE done without HS also resulted in an increase in MAP and HR (116.3±2.1mmHg and 78.4±1.2/min) from the basal values (94.8±1.6mmHg and 76.1±1.0/min; $p<0.0001$ respectively). In the presence of HS the HGE-induced changes (ΔMAP= 11.5±1.0mmHg; ΔHR= 3.3±1.0/min) were significantly decreased compared to its absence (ΔMAP=21.4±1.2mmHg; ΔHR= 12.8±2.0/min; $P<0.0001$ respectively). The CPT and HGE -induced increases in BP and HR suggest Sympathetic nervous system activation. These increases were significantly dampened by HS suggesting, indirectly, that its hypotensive effect may be due to an attenuation of the discharge of the sympathetic nervous system.

Keywords*: Hibiscus sabdariffa*, Cold pesssor test, Hand grip exercise, Blood pressure, Heart rate

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INTRODUCTION

The aqueous calyx extract of *Hibiscus sabdariffa* (HS; Family: Malvaceae) is used in Nigerian folk medicine to treat hypertension (Oliver, 1960). Its folk reputation as an antihypertensive agent has been validated in hypertensive animals (Onyenekwe *et al.*, 1999; Odigie *et al.*, 2003; Mojiminiyi *et al.*, 2007; Mojiminiyi *et al.*, 2012) and man (Haji Faraji and Haji Tarkhani, 1999; Herrera-Arellano *et al.*, 2004; Herrera-Arellano *et al.*, 2007; Mckay *et al.*, 2009). The constituents of HS include protein, fat, carbohydrate and fibre (Da-Costa-Rocha et al., 2014). It is abundant in vitamin C, β-carotene, calcium and iron (Ismail et al., 2008). It also contains organic acids, anthocyanins, polysacharrides and flavonoids (Müller and Franz, 1990). Anthocyanins (e.g. cyanidin-3-sambubioside and delphindin-3-sambubioside) are believed to be the major bioactive compounds producing different antihypertensive and cardioprotective effects (Jonadet et al., 1990 and Meunier et al., 2009). Within 7 hours of post HS consumption cyanidin-3-sambubioside, delphindin-3-sambubioside and total anthocyanins appear in the urine (Frank *et al.*, 2005). HS calyces are considered to be relatively non-toxic. They have a low degree of toxicity with LD_{50} between 2,000 to 5,000mg/kg/day (Hopkins et al., 2013).

Attempts have been made to delineate its mode of action. It has been reported to have cardioprotective (Jonadet, 1990), hypocholesterolemic (Chen *et al*, 2008), antioxidant (Wang *et al*, 2000, Amin and Hamza, 2005) and diuretic effects (Mojiminiyi *et al.*, 2000; Ajay *et al.*, 2007 and Alarcon-Alonso *et al.*, 2011). In addition it has vasorelaxant (Obiefuna *et al.*, 1993; Adegunloye *et al.*, 1996) and angiotensin-converting enzyme inhibitory effects (Ojeda *et al.*, 2010). Three recent excellent reviews (Hopkins *et al.*, 2013; Da-costa-Rocha *et al.*, 2014; Guardiola and Mach, 2014) have appeared in the literature suggesting a gratifyingly heightened interest in HS.

However, the role of the sympathetic nervous system, if any, has not been widely investigated. This is in spite of its primacy in both the control of normal blood pressure (Guyenet, 2006) or its dysfunction in hypertension (Guyenet, 2006) and other cardiometabolic diseases such as diabetes, syndrome

(metabolic syndrome) X e.t.c. Several classes of cardiovascular sympathetic efferents: thermosensitive, glucosensitive and barosensitive regulate arterial blood pressure (Dempsy *et al.*, 2002; Janig, 2003; Vallbo *et al.*, 2004; Guyenet, 2006). The barosensitive sympathetic efferents control the heart and kidneys and are responsible for short-term blood pressure fluctuations (Blessing, 1997; Janig, 2003). These efferents are also likely to be the key determinants of the long-term neural control of blood pressure. This is because renin secretion, kidney tubular reabsorption and renal blood flow are all controlled by barosentitive sympathetic efferents (DiBona and Kopp, 1997)

Consequently, the present study tested the hypothesis that the hypotensive effect of HS may occur through the inhibition of the sympathetic nervous system. This was achieved by using two maneuvers, cold pressor test (CPT) and hand grip exercise (HGE) that are known to activate the sympathetic nervous system (Victor *et al.*, 1987: Delaney *et al.*, 2010 respectively).

MATERIALS AND METHODS

Plant materials

The dried red calyces of HS were purchased in Talata Mafara central market, Zamfara state, Nigeria. They were identified and a voucher specimen (voucher number PCG/UDUS/MLV 001) was deposited in the herbarium of the Department of Pharmacognosy & Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

Extraction procedure

The dried red calyces of HS were pounded into fine coarse powder. The powder (500g) was dissolved in 2.7 litres of hot water (50 ^{0}C) in a conical flask and mixed thoroughly by means of a magnetic stirrer. It was left overnight and later filtered using a filter paper. It was then decanted and evaporated to dryness in a water bath at 60 ^{0}C leaving a powdery extract.

Tableting of powdery extract of HS

The powdery extract was then prepared into tablets containing 500mg of extract per tablet using the wet granulation method. Table 1 gives the batch manufacturing formular. The granules were tableted using single pouch machine type (ART 400 Eureka, Gmbh, Germany)

Ethical Clearance

Before the commencement of the study, approval was obtained from the Ethical Committee of Specialist Hospital Sokoto, Nigeria. Thereafter 20 apparently healthy human (male) volunteers were randomly selected following informed consent. They were 29.9±1.6 years old and weighed 67.3±2.7 kg respectively. They were not on any medication that affects BP and HR, nor were they consuming alcohol or caffeine-containing beverages. Also, they were not involved in strenuous exercise 24 hours before the test nor did they suffer from any cardiovascular, renal or endocrine diseases.

Cold pressor test

Hine's protocol for cold pressor test as described by Wood *et al.* (1984) was used. The nature of the test was explained to the volunteers. They were weighed using the Camry Mechanical Personal Scale (Model: BR 9012, China**)**. The subjects rested for at least 30 minutes to acclimatize before recordings were made. They were then asked to lie in the supine position in a quiet room. The blood pressure and heart rate were measured using the HuBDIC EchoMax plus BP-400 digital sphygmomanometer (HuBDIC Co. Ltd., Gyeonggi-do, Korea). These were taken as the casual BP and HR. Serial blood pressure and heart rate measurements were taken at 10-minute intervals until three almost similar readings were obtained. The last of these measurements were taken as the basal blood pressure and heart rate. The subject was then asked to immerse one hand into iced water (4^0-5^0 C) just above the wrist for 1-2 minutes (Wood *et al.*, 1984). During the period of immersion, blood pressure and heart rate readings were measured in the other arm at one minute interval. The highest of these recordings was designated as peak or ceiling blood pressure and heart rate. The subjects were allowed to rest for one hour. At the end of the rest, they were given tablets of HS orally at a dose of 15mg/kg and sat down for an hour. Thereafter the procedure was repeated.

Hand grip exercise

The BP pressure and heart rate were measured at rest in the supine position. Static exercise was performed by means of hand grip which was done by asking the subject to hold a pair of pliers (Gripp plier, India) forcefully in a sustained manner for a period of 1-2 minutes, or until fatigue was felt, and the parameters were measured again. A pair of pliers was used in this study because a handgrip dynamometer was not available. The subjects were allowed to rest for one hour. HS tablets were then given orally at a dose of 15mg/kg and the procedure repeated after an hour.

RESULTS

The Blood pressure and heart rate of the subjects before and during the cold pressor test (without HS) are presented in table 2. The peak systolic (SBP), diastolic (DBP), mean arterial blood pressure (MABP) and Heart rate (HR) obtained during the cold pressor test were significantly ($P<0.0001$) higher than the basal values.

Table 1: Showing the Batch manufacturing formular for *HS* tablets

Materials	Qtty/tablet	Qtty/300 tablets
Extract (water HS)	500mg	150g
Lactose	160mg	48g
Starch	40mg	12g
Starch (mucilage)	82.4mg	24.72g
Talc	16mg	4.0g
Magnesium stearate	1.6mg	0.48g
Total	800mg	240g

Table 2: The Blood pressure and Heart rate of apparently healthy subjects before (basal) and during the Cold pressor test (peak) without HS. SBP= Systolic Blood Pressure, DBP= Diastolic Blood Pressure, MAP= Mean Arterial Pressure, HR= Heart Rate

Parameter	Basal (N= 20)	HGE; PEAK (n=20)
SBP (mmHg)	124.9±2.2	146.8±2.7*
DBP (mmHg)	79.8±1.5	101.6±1.8*
MAP (mmHg)	94.8±1.6	116.3±2.1 *
HR (beats/min	76.1±1.0	87.4±1.2*

*= P<0.0001 vs Basal values

Table 3: The Blood pressure and Heart rate of apparently healthy subjects before (basal) and the during the Cold pressor test (Peak) in the presence of 15mg/kg HS

Parameter	CPT (n=20)	CPT + HS (n = 20)
SBP (mmHg)	147.1±2.4	137.1±1.7*
DBP (mmHg)	93.3±2.1	84.4±1.8*
MAP (mmHg)	111.1±2.2	92.4±2.1*
HR (beats/min	100.8±2.0	101.5±1.7*

*= P<0.0001 vs Basal

Table 4: The Blood pressure and Heart rate of apparently healthy subjects before (basal) and during the Static hand grip exercise (peak) without HS

Parameter	HGE (n=20)	HGE +HS (n = 20)
SBP (mmHg)	146.8±2.7	139.1±2.0*
DBP (mmHg)	101.6±1.8	87.6±2.1*
MAP (mmHg)	116.3±2.1	104.8±1.9*
HR (beats/min	78.6±2.1	87.9±1.4*

*= p<0.0001 vs Basal

Table 5: The Blood pressure and Heart rate of apparently healthy subjects before (basal) and during Static hand grip exercise (peak) in the presence of 15mg/kg *HS*

Parameter	Basal (N= 20)	CPT; PEAK (n=20)
SBP (mmHg)	131.8±2.2	147.1±2.4*
DBP (mmHg)	81.0±2.1	93.3±2.1*
MAP (mmHg)	97.9±2.0	111.1±2.2*
HR (beats/min	87.8±2.1	100.8±2.0*

*= p<0.0001 vs Basal

Also the peak values of these parameters obtained during the cold pressor test in the presence of HS were significantly (P<0.0001) higher than the basal values (Table 3). Figure 1 shows the changes between the peak value of each parameter and the basal value

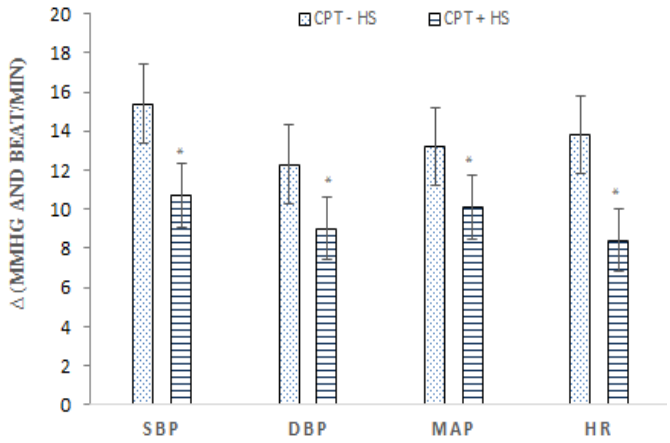

Figure 1: Change (Δ) between peak and basal values of each parameter during CPT with and without HS, *P<0.0001

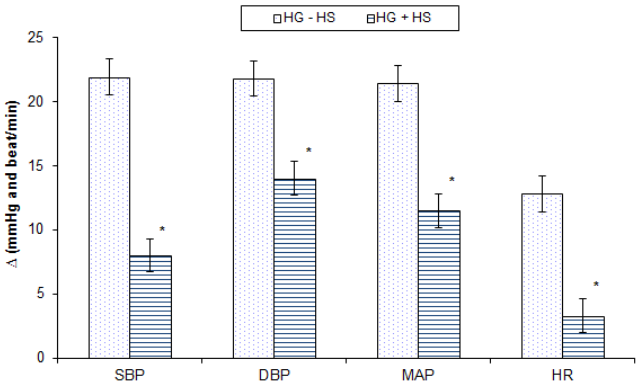

Figure 2: Change (Δ) between the peak and basal values of each parameter during HGE with and without HS, *p<0.0001

during the cold pressor test in the presence and absence of HS. The changes were all significantly (P<0.0001) lower in the presence of HS compared to its absence. Table 4 shows the blood pressure and heart rate values obtained during the static hand grip exercise in the absence of HS. All parameters rose significantly (P<0.0001) compared to the basal values. The parameters obtained during the static hand grip exercise in the presence of HS are presented in table 5. These parameters were all significantly (P<0.0001) higher than the basal values. Figure 2 shows the difference between the peak value of each parameter and the basal values during the static hand grip exercise in the presence and absence of HS. These changes were all significantly (P<0.0001) lower in the presence of HS compared to its absence.

DISCUSSION

The major finding of this study is that HS lowers blood pressure and heart rate through mechanisms associated with the sympathetic nervous system. These findings were obtained using protocols that are known to activate the sympathetic nervous system

such as the cold pressor test (Wood *et al.,* 1984; Halter *et al.,* 1984; Victor *et al.,* 1987; Seals, 1990) and the hand grip exercise (McCraw *et al.,* 1972; Chirinos *et al.,* 2009) The present study might be the first investigation to report that the BP lowering effect of HS may occur through the attenuation of sympathetic nervous system activities.

The blood pressure parameters (systolic, diastolic and mean arterial blood pressure) and heart rate were all raised significantly from the basal values during the cold pressor test. This suggests that the sympathetic nervous system was activated. Earlier studies have established that the pressor response to cold is sympathetically mediated (Wood *et al,* 1984; Victor *et al.,* 1987). Indeed, exposure to cold during CPT leads to stimulation of cold and pain receptors causing reflex vasoconstriction and increase in blood pressure and heart rate (Wood *et al.,* 1984). In the presence of HS the rise in these parameters were dampened. The difference between the peak values of these parameters and their basal values (change or Δ) were significantly lower in the presence of HS compared to its absence. This difference or change is a measure of the vascular reactivity to catecholamines released during the activation of the sympathetic nervous system by the cold stimulus (Wood *et al.,* 1984; Victor *et al.,* 1987). The significant dampening or reduction of these changes by HS suggests that its hypotensive effect may occur through the reduction of vascular reactivity during sympathetic nervous system activation.

During static hand grip exercise blood pressure parameters (systolic, diastolic and mean arterial blood pressure) and pulse rate were all raised significantly from the basal values. Again, this is indicative of sympathetic nervous system activation (Bruce *et al.,* 1972). These parameters were reduced in the presence of HS. Furthermore the difference between the peak values of these parameters and their basal values (change or Δ) were significantly lower in the presence of HS compared to its absence. This difference or change is due to significant increase in systemic vascular resistance during the activation of the sympathetic nervous system by the hand grip (Bruce *et al.,* 1972; Chirinos *et al.,* 2009). These cardiovascular adjustments to hand grip exercise are known to be regulated by the exercise pressor reflex which may be activated by the mechanically and metabolically sensitive receptors located in the nerve endings of group III and IV afferent sensory neurons respectively (Murphy *et al.,* 2011). Activation of this reflex leads to transmission of sensory impulses to cardiovascular control centres in the brain stem with subsequent activation of the exercise reflex resulting in an increase sympathetic nerve activity and withdrawal of parasympathetic nerve activity leading to exaggerated rises in sympathetic nerve activity, BP, HR and vascular resistance (Murphy *et al.,* 2011). Hence the attenuation of the BP and HR increases during the hand grip exercise in the presence of HS suggests that HS may be acting by dampening the sympathetic nerve activation or reducing the parasympathetic withdrawal that occur during handgrip exercise.

A comparison of the effect of HS on changes (Δ) in the measured parameters during CPT and HGE shows that the reduction in the change appears more marked in HGE compared to CPT (Figure 1 compared to Figure 2). This suggests that HS may be more effective in dampening sympathetic activation due to HGE than that due to CPT. This may be related to the fact that during HGE the arterioles are squeezed resulting in significant increase in systemic vascular resistance (Chirinos *et al.,* 2009). Since parasympathetic withdrawal is part of the mechanism for the pressor effect of HGE, it may also be speculated that the greater reduction in changes in the cardiovascular parameters may be due to a reduction in parasympathetic withdrawal by HS.

The results of this study are consistent with the earlier findings of Adegunloye *et al.,* (1996) which showed that HS lowers blood pressure via a vasodilatory effect. Such vasodilation may be achieved through acetylcholine-like and histamine-like mechanisms (Adegunloye *et al.,* 1996).

So far the only drug acting acutely to lower BP is sublingual Nifedipine (Furberg *et al.,* 1995). The present result suggests that, HS also lowers BP acutely. However, more studies are required to confirm this observation.

However, the present results are inconsistent with a finding by Adegunloye *et al.* (1996) which suggested that HS may not lower blood pressure by inhibiting the sympathetic nervous system. They used bilateral carotid occlusion (BCO) test to activate the sympathetic nervous system in rats. The blood pressure and heart rate responses to BCO in the presence of HS did not differ from the responses in its absence (Adegunloye *et al.*, 1996) making them to arrive at their conclusion. The differences in their results and the present one may be due to differences in techniques used to activate the sympathetic nervous system. Although BCO may be permissible in rats as a technique for activating the sympathetic nervous system, it is unethical in humans. The present study settled for CPT and HGE which are ethical in humans and have been used consistently by several authors in human subjects (Hines & Brown 1936; Bruce *et al.,* 1972; Wood et al., 1984 Chen *et al.,* 2008; Cui *et al.,* 2011). The difference between the result of Adegunloye *et al*. and the present one may also be due to species differences. Unpublished data from our laboratory suggest that responses to

BCO with and without HS are similar in rats thereby confirming the latter notion.

A dose of 15mg/kg body weight was used on account of its safety. A recent review by Hopkins *et al.*, 2013 reported that, HS extracts have a low degree of toxicity with LD50 between 2,000 to 5,000mg/kg/day.

The present study has some flaws. These include the lack of blinding of the BP and HR measurements as well as the use of a plier for the handgrip experiment instead of a dynamometer. The latter was not available and so we had to improvise. These flaws will be addressed in future.

In summary, the present study showed that the CPT and HGE induced increases in BP and HR were reduced in the presence of HS. It is concluded that the hypotensive effect of HS may be achieved through the inhibition of systemic vascular resistance mediated by the sympathetic nervous system.

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