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*Full-Length Research Article*

# The Hypotensive Effect of the Aqueous Calyx Extract of *Hibiscus Sabdariffa* may occur through the Attenuation of Autonomic Nervous System Activity

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**Summary:** This study tested the hypothesis that the hypotensive effect of the aqueous calyx extract of *Hibiscus sabdariffa* (HS) occurs through autonomic mechanisms that may be associated with a reduction in the double product (DP) of the heart. Following ethical approval and informed consent, the Harvard step test (HST) was performed in healthy subjects (n=14) to activate the autonomic nervous system before and after the oral administration of 15mg/kg HS. The blood pressure (BP) and heart rate (HR) responses were measured and DPs and the mean arterial pressure (MAP) were calculated. Results were expressed as mean ±SEM. Paired t-test and one-way ANOVA with a posthoc Bonferroni test were used for statistical analyses. $P<0.05$ was considered significant. HST without HS resulted in a significant rise in MAP, HR and DP (112.6±2.7mmHg, 97.7±2.5/min and 12630.0±642 mmHg.bpm) from the basal values (98.5±2.3mmHg, 76.5±2.0/min and 8730.7±354.9 mmHg.bpm, $P<0.001$, $P<0.01$ and $P<0.001$ respectively). In the presence of HS, HST-induced changes (ΔMAP=7.8±1.6mmHg; ΔHR=8.1±1.6/min; ΔDP= 1113.6±103.4 mmHg.bpm) were significantly dampened compared to its absence (ΔMAP= 13.3±2.6mmHg; ΔHR=17.0±3.7/min; ΔDP= 3899.3±287.2 mmHg.bpm; $P<0.001$, $P<0.01$ and $P<0.0001$ respectively). The HST-induced increase in BP, HR and DP suggest sympathetic nervous system (SNS) activation and parasympathetic nervous system (PNS) withdrawal associated with an increased cardiac $O_2$ consumption and workload. These were dampened by HS suggesting that its hypotensive effect occurs through the inhibition of SNS activation, PNS withdrawal and an associated reduction in cardiac $O_2$ demand and workload.

**Keywords:** *Autonomic nervous system, blood pressure, double product, Harvard step test, heart rate, Hibiscus sabdariffa calyces*



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## INTRODUCTION

*Hibiscus Sabdariffa* linn (Malvaceae) is planted in most parts of the world and cultivated for its leaf, fleshy calyx, seed or fibre (Dalziel, 1937). *Hibiscus sabdariffa* calyces extract (HS) is used in many parts of the world to make a red-coloured beverage which is called *sobo, soborodo or zobo* in Northern Nigeria. Its red hue and that of French wine appear indistinguishable. Indeed huge bales of HS calyces are exported from Senegal to France to service the wine industry. Hence, HS may help explain, at least in part, the so called "French paradox". It also serves as an antihypertensive agent in Nigerian folk medicine. This has been validated in animal (Onyenekwe *et al.*, 1999; Odigie, Ettarh and Adigun, 2003; Mojiminiyi *et al.*, 2007; Mojiminiyi *et al.*, 2012) and human studies (Haji-Faraji and Haji-Tarkani, 1999; Herrera-Arellano et al., 2004; Herrera-Arellano et al., 2007; Mckay *et al.*, 2010).

HS contains organic acids such as citric and malic acids (Ali *et al.*, 2005). In addition, it is rich in anthocyanins (Ali *et al.*, 2005). Anthocyanins are thought to be the compounds responsible for producing its anti-hypertensive effect (Herrera-Arellano *et al.*, 2007; Mckay *et al.*, 2010). There is also evidence that its polyphenol and hibiscus acid contents are important in carrying out its antihypertensive action (Carvajal-Zarrabal *et al.*, 2005; Hopkins *et al.*, 2013). The mode of action of HS is gradually being delineated. Some of its known actions include antioxidant (Wang, Cao and Prior, 1997; Hopkins *et al.*, 2013), diuretic (Mojiminiyi *et al.*, 2000), cholesterol-lowering (Ochani and D'Mello, 2009), acetylcholine-like, and histamine-like relaxant effects, and direct vasorelaxant action (Adegunloye *et al.*, 1996), inhibition of calcium influx (Ali *et al.*, 2005; Ajay *et al.*, 2007) and angiotensin-converting enzyme inhibitory actions (Ojeda *et al.*, 2010). In addition, HS has antimicrobial (Portillo-Torres *et al.*, 2019), hepatoprotective, immunomodulatory, antiparasitic and anti-cancer effects (Izquierdo-Vega *et al.*, 2020).

Furthermore, HS has been reported to lower both systolic blood pressure (BP) and heart rate (HR) in normotensive and hypertensive rats (Mojiminiyi *et al.*, 2007) and normotensive humans (Aliyu *et al.*, 2014). This suggests that its hypotensive effect may be associated with a reduction in the cardiac rate-pressure product or double product (DP) which is a product of systolic BP (SBP) and HR (i.e. SBP x HR). However, this is yet to be investigated. The cardiac DP is a surrogate measure of cardiac $O_2$ demand and workload (Katz and Feinberg, 1958; Kitamura *et al.*,

1972). It also correlates strongly with left ventricular mass (Hermida, Fernández and Ayala, 2001).

The autonomic nervous system is very important in the control of BP in health and disease (Guyenet, 2006). In spite of this, its role in the BP lowering effect of HS is yet to be well studied. In an earlier study, we reported that the hypotensive effect of HS was brought about through the attenuation of the discharge of the sympathetic nervous system (SNS) (Aliyu *et al.,* 2014). In that study, two models were used to activate the autonomic nervous system with and without HS. The methods used were the cold pressor test and hand grip or isometric exercise (Aliyu *et al.,* 2014). In the present study we have used a form of dynamic exercise, the Harvard step test, to activate the autonomic nervous system in the presence and absence of HS in order to further investigate the role of autonomic mechanisms in the hypotensive effect of HS. In addition, we have calculated the cardiac DP in order to delineate its role in the hypotensive effect of HS.

## MATERIALS AND METHODS

**Plant materials:** The dried red calyces of HS were purchased from the Talata Mafara Central Market, Zamfara State, Nigeria. These have been previously identified and a voucher specimen (voucher number PCG/UDUS/MLV 001) deposited in the herbarium of the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria (Aliyu *et al.,* 2014).

**Extraction procedure:** The dried calyces of HS were pounded into powdery form and 500g of it was mixed with 2.7 Litres of hot water (50 $^{o}$C) in a conical flask. It was then mixed thoroughly using a magnetic stirrer overnight and filtered. The filtrate was decanted and evaporated to dryness in a water bath at 60 $^{o}$C leaving a powdery extract**.**

**Tableting of HS powdery extract:** The extract was prepared into tablets containing 500mg of extract per tablet using the wet granulation method as described earlier (Aliyu *et al.,* 2014). The granules were tableted using a single pouch machine type (ART 400 Eureka Gmbh, Germany)

**Ethical clearance:** Before the commencement of the study, ethical approval was obtained from the ethical committee of Specialist Hospital, Sokoto, Nigeria and informed consent was obtained from the subjects. The protocol number of the ethical committee approval was: SHS/SUB/133/1. Indeed all experiments were performed in accordance with the Principles of the Declaration of Helsinki.

**Subjects:** 14 apparently healthy male subjects aged 26.6±1.0 years weighing 62.3±2.0 kg volunteered for the study. Their apparently healthy status was deduced from a questionnaire administered to the subjects.

**Inclusion criteria:** Subjects who were healthy and indicated no history of cardiovascular, renal, endocrine and other diseases were included.

**Exclusion criteria:** Subjects who were not on any medication that affects BP and HR and were neither consuming alcohol, tobacco, nor caffeine-containing beverages, nor involved in strenuous exercise 24hrs before the test were recruited for the study.

**Harvard step test (HST):** Brouha's protocol for Harvard step test (Brouha, Graybiel and Heath, 1943) was used in this study with slight modifications. The nature of the HST was first explained to the subjects. They were then made to rest for at least 30 minutes after which their basal blood pressure (BP) and Heart rate (HR) were measured as follows. The BP and HR were measured using the HuBDIC EchoMax plus BP-400 digital sphygmomanometer (HuBDIC Co. Ltd., Gyeonggi-do, Korea) with the cuff at the same level with the heart. These were regarded as casual BP and HR. Serial BP and HR recordings were then measured at 10- minute intervals until three almost identical readings were obtained. The last of these measurements were taken as the basal BP and HR (Wood *et al.,* 1984). The basal DP was calculated from the systolic BP and HR by multiplying these parameters together (Katz and Feinberg, 1958; Kitamura *et al.,* 1972). The HST was then performed on the subjects. The subjects stepped up onto and back down from a platform at a rate of thirty completed steps per minute (1s up, 1s down) for 5 minutes or until exhaustion and the BP and HR were measured immediately after the exercise. The highest of these were regarded as the peak BP and HR. The peak DP was also calculated. The change (Δ) in BP, HR and DP was calculated for each subject by subtracting the basal value of each parameter from the peak value as follows:

Change (Δ) in BP=peak BP-basal BP,
Change (Δ) in HR=peak HR-basal HR and
Change (Δ) in DP=peak DP-basal DP.

The subjects then rested for 1hour by which time their BP and HR had returned to basal levels. They were then given HS tablets at a dose of 15mg/kg orally and the procedure repeated 1hour post HS consumption. The mean arterial pressure (MAP) was calculated using the formular (Jaja *et al.,* 2000):

Mean arterial pressure=diastolic BP+1/3 pulse pressure
Where pulse pressure=systolic-diastolic BP (i.e. systolic minus diastolic BP)

**Data Analyses**: The data were presented as Mean±SEM. Paired student t-test was used to analyse the data except when three groups were compared. In the latter case one way ANOVA and a posthoc Bonferoni test was used. $P< 0.05$ was considered statistically significant.

## RESULTS

Table 1 shows the demographic and physiological characteristics of the subjects who volunteered for the study. The basal BP parameters and HR compared to the peak parameters during the Harvard step test in the subjects are presented in table 2. There was a significant rise in the BP ($P<0.001$) parameters and HR ($P<0.01$) during the HST compared to the corresponding basal values.

Table 3 shows the peak BP parameters and HR of the volunteers during the Harvard step test with and without HS. The BP parameters and HR fell significantly ($P<0.001$ and $P<0.01$ respectively) in the presence of HS compared to its absence.

**Table 1:**
The demographic and physiological characteristics of apparently healthy human subjects who volunteered for the study. Values are presented as Mean ± SEM. n=14.

| Parameter | Values |
|---|---|
| Age (years) | 26.6±1.0 |
| Sex | Male |
| Height (meters) | 1.71±1.0 |
| Weight (kg) | 62.3±2.0 |
| BMI (kg/m$^2$) | 21.1±2.1 |

*BMI = Body mass index*

**Table 2:**
The basal systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure (PP) and heart rate (HR) in apparently healthy human subjects compared to the peak parameters during the Harvard step test (HST). Values are presented as Mean ± SEM. n=14.

| Parameter | Basal | Peak (HST) |
|---|---|---|
| SBP (mmHg) | 127.8±2.6 | 147.8±3.0[a] |
| DBP (mmHg) | 83.9±2.2 | 95.8±3.0[a] |
| MAP (mmHg) | 98.5±2.3 | 112.6±2.7[a] |
| PP (mmHg) | 38.9±2.0 | 52.0±3.2[a] |
| HR (beats/min) | 76.6±2.0 | 97.7±3.9[b] |

[a]*= P<0.001 peak blood pressure values during HST vs. basal blood pressure values.*
[b]*= P<0.01 peak heart rate values during HST vs. basal heart rate values.*

**Table 3:**
The peak SBP, DBP, MAP, PP and HR in apparently healthy human subjects during the HST with and without administration of HS extract. Values are mean ± SEM. n=14.

| Parameter | HST | HST+HS |
|---|---|---|
| SBP (mmHg) | 147.8±3.0 | 128.6±2.7[a] |
| DBP (mmHg) | 95.8±3.0 | 84.0±3.0[a] |
| MAP (mmHg) | 112.6±2.7 | 98.4±2.5[a] |
| PP (mmHg) | 52.0±2.8 | 39.6±2.6[a] |
| HR (beats/min) | 97.7±3.9 | 86.3±3.0[b] |

[a] *= P<0.001 peak blood pressure values during HST vs. corresponding HST + HS values.*
[b]*= P<0.01 peak heart rate values during HST vs. corresponding HST + HS values.*
*SBP= Systolic Blood Pressure; DBP= Diastolic blood pressure; MAP= Mean arterial pressure; PP = Pulse pressure; HR= Heart rate.*

Figures 1A-E show the changes (Δ) between the peak and basal values of each parameter during the Harvard step test with and without HS. Taken together, the changes in the blood pressure parameters and HR were significantly (P<0.001 and P<0.01 respectively) lower in the presence of HS compared to its absence.

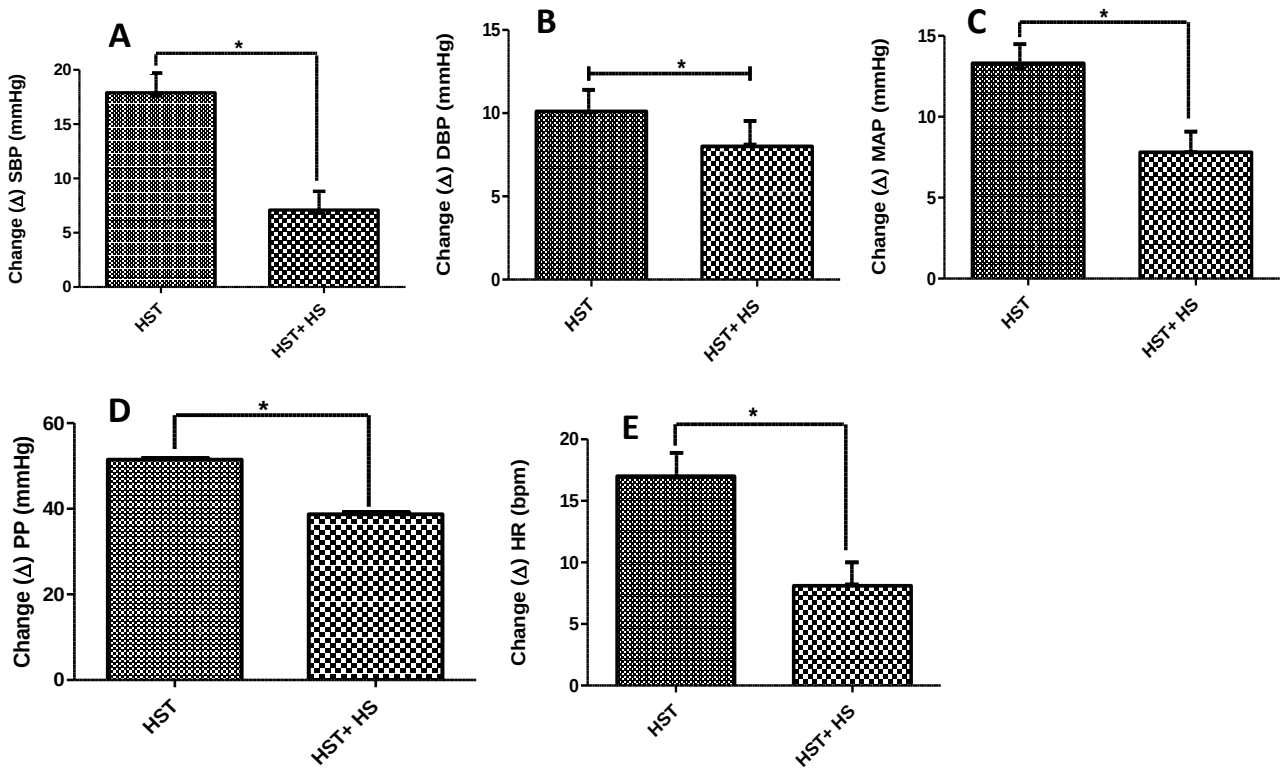


**Figure 1**
Change (Δ) between peak SBP and basal SBP during Harvard step test with and without HS (A). *=P<0.001 HST+HS vs. HST. SBP= systolic blood pressure; HS = *Hibiscus sabdariffa* calyx extract; HST = Harvard step test without HS and HST+HS = Harvard step test with HS. Change (Δ) between peak DBP and basal DBP during Harvard step test with and without HS (B). *=P<0.001 HST+HS vs. HST; DBP=diastolic blood pressure. Change (Δ) between peak MAP and basal MAP during Harvard step test with and without HS (C). *=P<0.001 HST+HS vs. HST; MAP=mean arterial pressure. Change (Δ) between peak PP and basal PP during Harvard step test with and without HS (D). *=P<0.001 HST+HS vs. HST. PP = pulse pressure. Change (Δ) between peak HR and basal HR during Harvard step test with and without HS (E). *=P<0.01 HST+HS vs. HST; HR = Heart rate.

Figure 2 shows the effect of HS on the DP of the volunteers. The DP rose significantly ($P<0.001$) during the Harvard step test compared to the basal value. However in the presence of HS (i.e. HST+HS), it fell significantly ($P<0.001$) compared to the Harvard step test alone. Also, the DP during the Harvard step test in the presence of HS (HST+HS) did not differ significantly from the basal value (Figure 2).

Figure 3 shows the change (Δ) in DP between the peak DP and basal DP during the Harvard step test with and without HS. The change fell significantly ($P<0.0001$) in the presence of HS compared to its absence.

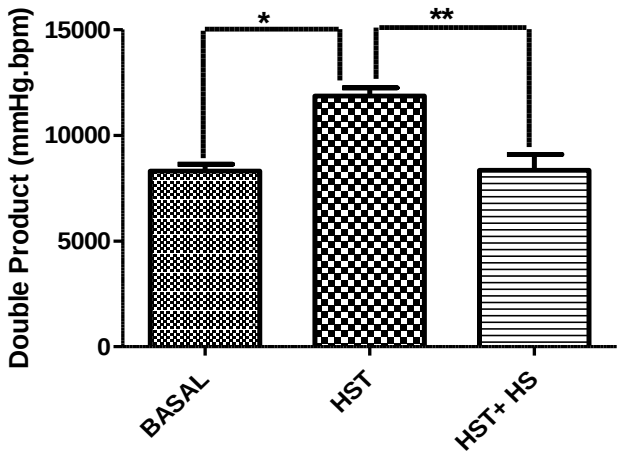


**Figure 2:**
Basal DP and peak DP during Harvard step test with and without HS. *=P<0.001 HST vs. BASAL; **=P<0.001 HST+HS vs. HST. DP = double product, HS = *Hibiscus sabdariffa* calyx extract; HST = Harvard step test without HS; HST+HS = Harvard step test with HS.

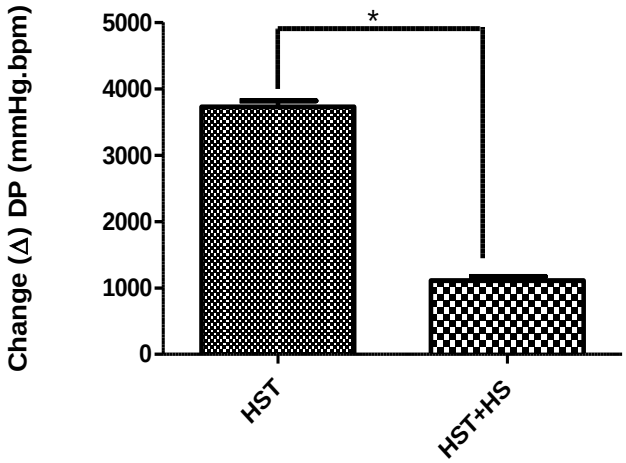


**Figure 3:**
Change (Δ) between peak DP and basal DP during Harvard step test with and without HS. *=P<0.0001 HST+HS vs. HST. HS = *Hibiscus sabdariffa* calyx extract; HST = Harvard step test without HS; HST+HS = Harvard step test with HS; DP = Double product.

## DISCUSSION

The main finding of the present study is that HS reduces BP and HR via autonomic mechanisms associated with the sympathetic and parasympathetic nervous systems. In addition HS significantly lowered the cardiac rate pressure product or double product. These findings suggest that the hypotensive effect of HS may occur through the reduction of the discharge of the sympathetic nervous system (SNS) and inhibition of parasympathetic withdrawal and these are associated with a reduction in cardiac $O_2$ demand and workload.

During dynamic exercise, such as HST, the stroke volume, HR, and consequently cardiac output increase, whilst the peripheral resistance decreases. These haemodynamic changes result in a moderate increase in BP which produces a rise in blood flow to the skeletal muscle. This results in increased $O_2$ and nutrient supply to the contracting muscle (Pardeshi and Kirtikar, 2016). The peak BP, HR and DP obtained during the HST were significantly higher than the basal values of these parameters recorded before the start of the exercise. This is consistent with the rise in BP, HR and DP associated with dynamic exercise. Furthermore, the peak BP, HR and DP during the HST fell significantly in the presence of HS compared to its absence again suggesting that the rise in these parameters were attenuated in the presence of HS. In addition, the changes (Δ) in BP, HR and DP obtained during the administration of HS were significantly smaller compared to its absence. The change (Δ) was obtained by subtracting the basal values of these parameters from their peak values. The change (Δ) is a measure of vascular reactivity (Wood *et al.,* 1984) and is directly proportional to muscle sympathetic nerve activity (Cui *et al.,* 2011). Taken together, these findings suggest that HS may reduce vascular reactivity, attenuate the discharge of the SNS and inhibit the parasympathetic withdrawal associated with dynamic exercise. In addition, the decrease in the change in DP in the presence of HS suggests that it reduces myocardial $O_2$ demand and workload. Hence these may be some of the mechanisms by which HS acts. This is consistent with the earlier finding from our laboratory (Aliyu *et al.,* 2014) suggesting that HS may act through the attenuation of the discharge of the SNS.

The cardiovascular responses accompanying dynamic exercise (HST) described above are mediated by the autonomic nervous system through a rise in the activity of the SNS and inhibition of the activity of parasympathetic nervous system (PNS) (Murphy *et al.,* 2011). This occurs through the following mechanisms namely: the arterial baroreflex, the exercise pressor reflex and the central command (Smith, Mitchell and Garry, 2006; Murphy *et al.,* 2011). While evidence from the current study suggests that HS may act by inhibiting the activity of the SNS and by inhibiting PNS withdrawal, the precise mechanisms by which it does so remain to be seen. In other words, does it work by acting on the arterial baroreflex, or the exercise pressor reflex or the central command or through a combination of these? This suggests that more work is required to answer these questions. However, the extract contains anthocyanins (ACNs) which increase the elaboration of nitric oxide (NO) from the vascular endothelium (Bell and Gochenaur, 2006), as well as regulate the expression and function of endothelial NO synthase

(eNOS) (Vendrame and Klimis-Zacas, 2019). NO is largely responsible for endothelium-dependent relaxation. ACNs also stimulate soluble guanylate cyclase resulting in the relaxation of the smooth muscles in the vasculature. This also results in a rise in cyclic guanosine monophosphate (cGMP) which inhibits the contraction of the vascular smooth muscle by preventing intracellular $Ca^{2+}$ release (Vendrame and Klimis-Zacas, 2019). In addition, ACNs increase the bioavailability of NO by preventing the destruction of NO by reactive oxygen species due to its antioxidant effect (Vendrame and Klimis-Zacas, 2019). This ensures vasodilation and fall in blood pressure. Furthermore ACNs reduce the synthesis of vasoconstrictor molecules such as angiotensin II, endothelin-I and thromboxanes thereby lowering the BP (Parichatikanond, Pinthong and Mangmool 2012; Vendrame and Klimis-Zacas, 2019).

Another interesting finding in this study is that HS acts acutely, within 1 hour of its administration, to carry out its effect. A similar finding was observed in the earlier work from our laboratory (Aliyu *et al.,* 2014). However, this finding needs to be confirmed by additional studies. To the best of our knowledge, the only drug known to lower BP acutely is sublingual nifedipine (Furberg, Psaty and Meyer, 1995).

However, the results of the present study are not in agreement with those of Adegunloye *et al*. which indicated that the hypotensive effect of HS may not be mediated by the inhibition of the sympathetic nervous system (Adegunloye *et al.,* 1996). In that study, the bilateral carotid occlusion (BCO) test was used to activate the sympathetic nervous system in rats. The subsequent rise in BP and HR in response to BCO with and without HS was similar making them to conclude that the sympathetic nervous system may not be involved in the action of HS (Adegunloye *et al.,* 1996). The discord between their findings and those of the present study may be due to differences in the methods used to stimulate the sympathetic nervous system. Although BCO may be used in animals as a method for stimulating the sympathetic nervous system, it cannot be used in man for ethical reasons. The present study used the Harvard step test which meets the ethical requirements for human studies and has therefore been used consistently by several authors in man (Leung *et al.,* 2013; Pardeshi and Kirtikar, 2016). The discord between the findings of Adegunloye *et al*. (1996) and the present study may also be attributable to differences in species used. Unpublished work from our laboratory indicates that the rise in BP and HR following the performance of BCO in the presence of HS compared to its absence are not different in rats thereby affirming the latter notion. It may also suggest that, perhaps, HS may not be acting through the baroreceptor mechanism since BCO tests mechanisms that are associated with the arterial baroreflex by occluding the common carotid arteries proximal to the carotid sinus baroreceptors.

The reduction in DP by HS seen in this study is another interesting observation. As far as we know, our study is the first to report this observation. DP is an index of cardiac $O_2$ demand as well as cardiac workload (Katz and Feinberg, 1958; Kitamura *et al.,* 1972). It also correlates highly with the left ventricular weight (Hermida, Fernández and Ayala, 2001). The DP rose significantly from the basal value during the HST. However, HS significantly lowered it back to the basal level. Furthermore, HS significantly reduced the change in DP that occurred during HST. These findings suggest that HS lowers myocardial $O_2$ demand and myocardial workload during dynamic exercise to levels similar to basal conditions. This implies that HS enables the heart to utilize $O_2$ more efficiently as well as reduce the cardiac workload during dynamic exercise. It is not clear why this is so but a plausible reason may be that HS may dilate the coronary arteries, as it dilates arteries elsewhere (Adegunloye *et al.,* 1996; Abubakar *et al.,* 2019), thereby increasing blood flow to the heart. This finding also suggests that HS may be useful in treating cardiac diseases such as angina pectoris as drugs used in the treatment of such diseases act by reducing DP (Jackson *et al.,* 1980; Kambara *et al.,* 1984). However actual experiments need to be done to confirm these speculations.

The present study has some flaws. Firstly, the BP and HR measurements were not blinded. Ideally, the person measuring the BP and HR should not have known what was administered to the subjects in order to make the study more objective. The subjects should also not have known what was administered to them. In other words the study should have been double blinded. Secondly, a placebo should have been administered to the subjects before the administration of HS. Alternatively, it could have been administered to a separate control group. The present design made each subject his own control without the administration of a placebo. It is a truism that a double blind placebo controlled design would have been more appropriate for this study. These flaws will be addressed in future.

Another limitation of the study is the small number of subjects used. In addition, heart rate variability (HRV) would have proved useful in determining the autonomic mechanisms underlying the effects of HS. However, the equipment required to perform HRV was not available to us.

In summary, the blood pressure, heart rate and double product rose during the HST, a form of dynamic exercise. The rise in these parameters were significantly inhibited in the presence of HS compared to its absence. Since the rise in BP, heart rate and double product are mediated by autonomic mechanisms associated with sympathetic activation and parasympathetic withdrawal, it is concluded that HS may be acting by inhibiting sympathetic activation and parasympathetic withdrawal and this action is associated with a reduction in the cardiac demand for oxygen and cardiac workload.

## REFERENCES

Abubakar SM, Ukeyima MT, Spencer JPE, Lovegrove JA (2019). Acute Effects of Hibiscus sabdariffa Calyces on Postprandial Blood Pressure, Vascular Function, Blood Lipids, Biomarkers of Insulin Resistance and Inflammation in Humans. Nutrients. 11: 341 DOI:10.3390/nu11020341

Adegunloye BJ, Omoniyi JO, Owolabi OA, Ajagbonna OP, Sofola OA, Coker HA (1996). Mechanisms of blood pressure lowering effects of the calyx extract of Hibiscus sabdariffa in rats. Afr J Med Med Sci. 25:235-38

Ajay M, Chai HJ, Mustafa AM, Gilani AH, Mustafa MR (2007). Mechanism of `antihypertensive effect of

Hibiscus sabdariffa L. calyces. J Ethnopharmacol. 109:338-93

Ali BH, Wabel NA, Blunden G (2005). Phytochemical, pharmacological and toxicological aspects of Hibiscus sabdariffa L.: a review. Phytother Res. 19:369–75.

Aliyu B, Oyeniyi YJ, Mojiminiyi FBO, Isezuo SA, Alada, ARA (2014). The Aqueous Calyx Extract of Hibiscus sabdariffa Lowers Blood Pressure and Heart Rate via Sympathetic Nervous System Dependent Mechanisms. Niger J Physiol Sci. 29: 131-36

Bell DR, Gochenaur K (2006). Direct vasoactive and vasoprotective properties of anthocyanin-rich extracts. J Appl Physiol. 100: 1164–170

Brouha L, Graybiel A, Heath CW, (1943). The step test: a simple method of measuring physical fitness for hard muscular work in adult man. Rev. Can. Biol. 2:86-92

Carvajal-Zarrabal O, Waliszewski S, Barradas-Dermitz D, Orta-Flores Z, Hayward-Jones P, Nolasco-Hipólito C (2005). The consumption of Hibiscus sabdariffa dried calyx ethanolic extract reduced lipid profile in rats. Plant Foods Hum Nutr. 60:153–159.

Cui J, Leuenberger UA, Blaha C, King NC, Sinoway LI (2011). Effect of P2 receptor blockade with pyridoxine on sympathetic response to exercise pressor reflex in humans. J Physiol. 589:685–95

Dalziel JM (1937). The useful of plants of West Tropical Africa. London: The crown agents for the colonies. 52-560.

Furberg CD, Psaty BM, Meyer JV (1995). Nifedipine. Dose-related increase in mortality in patients with coronary heart disease. Circulation. 92: 1326-31.

Guyenet PG (2006). The sympathetic control of blood pressure. Nat Rev Neurosci. 7:335-46

Haji-Faraji M, Haji-Tarkhani A (1999). The effect of sour tea (Hibiscus sabdariffa) onessential hypertension. J Ethnopharmacol. 65:231-36

Hermida RC, Fernández JR, Ayala DE (2001). Circadian Rhythm of Double (Rate-Pressure) Product in Healthy Normotensive Young Subjects. Chronobiology International. 18: 475–89

Herrera-Arellano A, Flores-Rameo S,Chavez-Soto MA, Tortoriello J (2004). Effectiveness and tolerability of standardized extract from Hibiscus sabdariffa in patients with mild to moderate Hypertension. Phytomedicine. 11:375-82

Herrera-Arellano A, Miranda-Sanchez J, Avica-Castro P (2007). Clinical effects produced by a standardized herbal medicinal product of Hibiscus sabdariffa in patients with hypertension. A randomized, double-blind, lisinopril-controlled clinical trial. Planta Medica. 73: 6-12

Hopkins AL, Lamm MG, Funk J, Retenbaugh C (2013). Hibiscus sabdariffa L. in the treatment of hypertension and hyperlipidemia: a comprehensive review of animal and human studies. Fitoterapia. 85:84-94

Izquierdo-Vega JA, Arteaga-Badillo DA, Sánchez-Gutiérrez M, Morales-González JA, Vargas-Mendoza N, Gómez-Aldapa CA, Castro-Rosas J, Delgado-Olivares L, Madrigal-Bujaidar E, Madrigal-Santillán E (2020). Organic Acids from Roselle (Hibiscus sabdariffa L.)-A Brief Review of Its Pharmacological Effects. Biomedicines.8(5):100. doi: 10.3390/biomedicines8050100. PMID: 32354172; PMCID: PMC7277581.

Jackson G, Schwartz J, Kates RE, Winchester M, Harrison DC (1980). Atenolol: Once-daily Cardioselective Beta Blockade for Angina Pectoris. Circulation. 613:555-60

Jaja SI, Kehinde MO, Gbenebitse S, Mojiminiyi FBO, Ogungbemi A (2000). Effect of Vitamin C on Arterial Blood Pressure, Irreversibly Sickled Cells and Osmotic Fragility in Sickle Cell Anemia Subjects. Niger. J. Physiol. Sci. 16 (1-2): 14-18

Kambara H, Kinoshita M, Hirota Y, Kadota K, Sawamura M, Saito T, Kawai C (1984). Effect on Exercise Tolerance and Pharmacokinetics of Conventional and Sustained Release Preparations of Propranolol in Patients with Angina Pectoris: A Double-blind Cross-over Study. Japanese Circulation Journal. 48: 1066-1073

Katz LN, Feinberg H (1958). The relation of cardiac effort to myocardial oxygen consumption and coronary flow. Circ Res. 6: 656–669.

Kitamura K, Jorgensen CR, Gobel FL, Taylor HL, Wang Y (1972). Hemodynamic correlates of myocardial oxygen consumption during upright exercise. J Appl Physiol. 32: 516–522.

Leung T, Kuo C, Lee C, Kan N, Hou C (2013). Physiological Effects of Bioceramic Material: Harvard Step, Resting Metabolic Rate and treadmill running assessment. Chinese J Physiol. 56: 334-340

Mckay DL, Chen CYO, Saltzman E, Blumberg JB (2010). Hibiscus sabdariffa L tea (tisane) lowers blood pressure in Pre hypertensive and mildly hypertensive adults. J Nutr. 2010; 140: 298-303

Mitchell JH. (1990). Wolffe memorial lecture. Neural control of the circulation during exercise. Med Sci Sports Exerc. 22: 141–154

Mojiminiyi FBO, Adegunloye BJ, Egbeniyi YA, Okolo RU (2000). An investigation of the diuretic effect of an aqueous extract of the petals of Hibiscus sabdariffa. JMedicine Medical Sci. 2:77-80

Mojiminiyi FBO, Audu Z, Etuk EU, Ajagbonna OP (2012). Attenuation of salt-induced hypertension by aqueous calyx extract of Hibiscus Sabdariffa. Niger J Physiol Sci. 27: 195 –200

Mojiminiyi FB, Dikko M, Muhammad BY, Ojobor PD, Ajagbonna OP, Okolo RU, Igbokwe UV, Mojiminiyi UE, Fagbemi MA, Bello SO, Anga TJ(2007). Antihypertensive effect of an aqueous extract of the calyx of Hibiscus sabdariffa. Fitoterapia 78: 292-297.

Murphy MN, Mizuno M, Mitchell JH, Smith SA (2011). Cardiovascular regulation by skeletal muscle reflexes in health and disease. Am J Physiol Heart Circ Physiol. 301: 1191-1204

Ochani PC, D'Mello P (2009). Antioxidant and antihyperlipidemic activity of Hibiscus sabdariffa Linn. leaves and calyces extracts in rats. Indian J Exp Biol. 47:276–282.

Odigie IP, Ettarh RR, Adigun SA (2003). Chronic administration of aqueous extract of Hibiscus sabdariffa attenuates hypertension and reverses cardiac hypertrophy in 2K-1C hypertensive rats. J Ethnopharmacol. 86:181-185

Ojeda D, Jimenez-Ferrer E, Zamilpa A, Herrera-Arellano A, Tortoriello J, Alvarez L (2010). Inhibition of

angiotensin converting enzyme (ACE) activity by the anthocyanins delphinidin and cyanidin-3-O-sambubiosides form Hibiscus sabdariffa. J Ethnopharmacol. 127:7-10

Onyenekwe PC, Ajani EO, Ameh DA, Gamaniel KS (1999). Antihypertensive effect of roselle (Hibiscus sabdariffa) calyx infusion in spontaneously hypertensive rats and comparison of its toxicity with that in Wistar rats. Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agentsor disease, 17: 199-206.

Pardeshi AM, Kirtikar SN (2016). Comparison of Anthropometric Parameters and Blood Pressure Changes in Response to Physical Stress Test in Normotensive Subjects with or Without Family History of Hypertension. Indian J Physiol Pharmacol. 60: 208–212

Parichatikanond W, Pinthong D, Mangmool S (2012). Blockade of the renin-angiotensin system with delphinidin, cyanin, and quercetin. Planta Med. 78:1626–1632.

Portillo-Torres, L.A., Bernardino-Nicanor, A., Gómez-Aldapa, C.A., González-Montiel, S., Rangel-Vargas, E., Villagómez-Ibarra, J.R., González-Cruz, L., Cortés-López, H., Castro-Rosas, J., (2019). Hibiscus acid and chromatographic fractions from Hibiscus sabdariffa calyces: Antimicrobial activity against multidrug-resistant pathogenic bacteria. Antibiotics, 8: 218. DOI:10.3390/antibiotics8040218. PMID: 31718033; PMCID: PMC6963829.

Smith S A, Mitchell J H, Garry M G (2006). The mammalian exercise pressor reflex in health and disease. Exp Physiol. 91: 89–102

Vendrame S, Klimis-Zacas D (2019). Potential Factors Influencing the Effects of Anthocyanins on Blood Pressure Regulation in Humans: A Review. Nutrients. 11: 1431. DOI: 10.3390/nu11061431. PMID: 31242638; PMCID: PMC6628116.

Wang H, Cao G, Prior RL (1997). The oxygen radical absorbing capacity of anthocyanins. J Agric Food Chem. 45:304-309

Wood DL, Sheps SG, Elveback LR, Schirger A (1984). Cold pressor test as a predictor of hypertension. Hypertension. 6:301-306