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THE EFFECT OF *Hibiscus.Sabdariffa*. L ON BLOOD PRESSURE AND ARTERIAL
STIFFNESS IN HUMANS

By

Basirat T. Shittu

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In Biological Sciences

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This thesis has been approved in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE in Biological Sciences.

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List of abbreviations

ACE	Angiotensin Converting Enzyme
ACC	American College of Cardiology
AIx	Aortic Augmentation Index
AHA	American Heart Association
CAD	Coronary Artery Disease
CPT	Cold Pressor Test
cfPWV	Carotid Femoral Pulse Wave Velocity
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
ECG	Electrocardiogram
ECM	Extracellular Matrix
ESH	European Society of Hypertension
GRA	Glucocorticoid Remediable Aldosteronism
HBP	High Blood Pressure
HCT	Hydrochlorothiazide

HGE	Handgrip Exercise
HPLC-MS	High-Performance Liquid Chromatography Mass Spectrometry
HS	<i>Hibiscus.sabdariffa.</i>
HTN	Hypertension
IHD	Ischemic Heart Disease
JG	Juxtaglomerular cells
LD ₅₀	Lethal Dose
NF- κ B	Nuclear-Factor Kappa
MAP	Mean Arterial Pressure
MI	Myocardial Infarction
NO	Nitric Oxide
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
OSA	Obstructive Sleep Apnea
PDGF	Platelet-Derived Growth Factor
PNS	Parasympathetic Nervous System
PP	Pulse Pressure

PWA	Pulse Wave Analysis
PWV	Pulse Wave Velocity
RAAS	Renin-Angiotensin Aldosterone System
RCT	Randomized Clinical Trial
SNS	Sympathetic Nervous System
TNF	Tumor Necrosis Factor
VCAM	Vascular Cell Adhesion Molecule
VSMC	Vascular Smooth Muscle Cell

Abstract

Cardiovascular diseases (CVD) are the foremost cause of death worldwide. The main risk factor for CVD is uncontrolled hypertension (HTN). The prescription of only anti-hypertensive regimens in the management of HTN is becoming more challenging due to the high cost and adverse effects linked to the persistent usage of the drugs. Eighteen participants completed the study by consuming 2g of *Hibiscus sabdariffa* or oolong tea twice daily for six weeks. We lost an additional twelve participants in the study due to the COVID-19 pandemic. Central arterial stiffness was analyzed as cfPWV using applanation tonometry technique. The HS tea intervention (n=11) had a significant positive effect on SBP (P =0.02), DBP (P =0.001) and HR (P =0.03). Also, the HS tea consumption led to a non-significant (p=0.44) reduction in cfPWV (-0.5m/s) when compared to control tea (+0.3m/s). Although, the decrease in cfPWV could be clinically significant, but will need to be verified with larger sample size.

1 Introduction

Recently, there have been drastic changes in the approach used in the treatment of various diseases, including high blood pressure and other cardiovascular diseases. The advent of several complementary alternative therapies has been proven to be very effective in lowering blood pressure, as well as reducing the potential risks of developing cardiovascular diseases (CVDs) (Whelton et al., 2018). According to the recent trends in the use of herbal tea as an alternative therapy in the treatment of hypertension (Mittal & Singh, 2010; Tabassum & Ahmad, 2011), the use of *Hibiscus sabdariffa* (Hs) tea might offer a cost-effective alternative to reduce the socio-economic burden associated with the treatment of hypertension, especially in the poorer states of the US, and in developing and low-income countries (where insufficient treatment has been a major contributing factor to the high prevalence of hypertension and other CVDs). The treatment of hypertensive individuals with only the available conventional anti-hypertensive regimens is becoming more challenging due to the high cost of the drugs, incomplete adherence to daily intake of pills, combination therapy to achieve maximum drug effect, and undesirable adverse effects associated with prolonged usage of different drug regimens (August, 2004; Susalit et al., 2011; Wang & Xiong, 2012).

1.1 Hypertension

The World Health Organization (WHO, 2013) defined hypertension as a chronic condition that is characterized by a continual rise in the pressure at which blood flows in

the blood vessels, which makes it hard for the heart to pump blood into the circulation. Diagnosis of hypertension is established when an individual has systolic blood pressure (SBP) of $\geq 130\text{mmHg}$ and diastolic blood pressure (DBP) of $\geq 80\text{mmHg}$, this is in accordance with the guidelines formulated by the American Heart Association (AHA). (Whelton et al., 2018). High blood pressure is one of the leading risk factors that contribute to a high prevalence of cardiovascular diseases (CVDs) worldwide. Cardiovascular diseases encompass several groups of other heart diseases such as ischemic heart disease (IHD), coronary artery disease (CAD), congestive heart failure, cerebrovascular disease, and myocardial infarction (MI) (Mendis et al., 2011). Uncontrolled high blood pressure has also been attributed to be the leading cause of chronic renal failure in most of the countries with a high prevalence of hypertension (Pierdomenico et al., 2009). Implementing lifestyle changes, such as dietary intervention with conventional therapy, can help delay or mitigates increases in blood pressure, thereby decreasing the risk of developing CVD and organ damage associated with poorly controlled high blood pressure.

1.1.1 Causes of hypertension

Hypertension (HTN) can be classified as either; primary/essential or secondary HTN based on the causative factors. Currently, about 90-95% of cases of hypertension in adults are primary HTN, while only 5% of cases are classified as secondary HTN. Primary or essential type HTN is usually of unknown etiology and can sometimes be referred to as idiopathic. However, several associated risk factors have been implicated in the development of HTN, and these factors have been broadly categorized as either genetic or

environmental factors. Genetic alterations: overexpression or underexpression of specific genes have been reported as an unchangeable factor associated with HTN. High blood pressure can be inherited and run in families, with an incidence of heritability of about 15-30% for DBP and 15-40% for SBP ((Padmanabhan, Aman, & Dominiczak, 2019). Similarly, certain genetic disorders such as Liddle's syndrome (autosomal dominant mutation in sodium channel), a mutation in the gene that results in excess production of cortisol, glucocorticoid-remediable aldosteronism (GRA) have been identified in the development of genetic HTN (Levanovich, Diaczok, & Rossi, 2019).

Environmental factors linked to HTN include aging, obesity, high consumption of alcohol, excess dietary salt intake, stressful lifestyle, potassium deficiency sedentary lifestyle, chronic intake of caffeine and other sympathomimetic agents (i.e., exciting the sympathetic nervous system) which are all classified as hypertensinogenic factors. An increase in body weight, specifically excess fat in the abdomen is one of the most common hypertensinogenic factors. Several studies have reported a positive correlation between weight gain and increased BP while controlling body weight in healthy individuals can significantly reduce both SBP and DBP (Rafiee, Khaledi, Madmoli, Zafari, & Lotfizadeh, 2019). Obesity has been reportedly linked to type II diabetes mellitus (insulin resistance), hyperlipidemia, deposition of fat droplets in the arterial wall (atherosclerosis), and left ventricular hypertrophy; which can all result in the development of HTN and other CVDs. Although, the mechanism of obesity-induced HTN has not been clearly proven; however, an increase in plasma volume in overweight individuals can subsequently increase cardiac

output and BP. Similarly, possible vasoconstriction in obese individuals and enhanced renin release from the kidney can both increase sympathetic nerve activity (Tesauro & Cardillo, 2011).

Secondary HTN, which accounts for only about 5-10% cases of global HTN, occurs due to an underlying medical condition that is usually reversible once the triggering medical condition is treated promptly. The diagnosis and onset of secondary HTN vary by age and clinical symptoms; however, secondary HTN has been reported to be more prevalent among younger adults within the age of 18-40 years (Charles, Triscott, & Dobbs, 2017). The associated causes of secondary HTN usually vary between adults and preadolescent children; although, medical conditions such as kidney disease, renovascular disease, hyperlipidemia, diabetes mellitus, pheochromocytoma (tumor producing excess catecholamines), obstructive sleep apnea, and pregnancy are linked to secondary HTN across all adult ages. Other uncommon etiology of secondary HTN includes medications such as oral contraceptives, non-steroidal anti-inflammatory drugs (NSAIDs), hormonal therapy, several chemotherapeutic drugs, and some recreational drugs (Grossman & Messerli, 2012).

1.1.2 Prevalence of hypertension

The recent redefinition of HTN in 2017 and 2018 by ACC/AHA and European Society of Hypertension (ESH), respectively, from 140/80mmHg to 130/80 mmHg, which was formerly classified as prehypertensive, has greatly increased the prevalence of HTN

in the US and other countries in the world. The current global prevalence of HTN among young (>45 years) and older adults before the reclassification by AHA/ESH is about 30-35%; however, while applying the updated definition of HTN, the global prevalence in adults is now > 60% (Touyz, 2019). The current guideline suggested prompt and intense implementation of treatment to lower the blood pressure, especially SBP below 140 mmHg, and this has been associated with a significant reduction in mortality rate from stroke and other CVDs (Salam et al., 2019).

The influence of geographical regions classified as developed or developing countries on the global prevalence of HTN cannot be underestimated, although factors such as genetics and environmental factors have a significant influence on the geographical distribution of HTN. According to a recent report of the world health organization (WHO, 2013), prevalence rate of HTN is high (about 46%) in Africa and other developing countries, while low prevalence rate of (35%) was reported by WHO in America region (Al Kibria, 2019). Ethnicity or race has also been reportedly shown to influence the global prevalence of HTN. The high incidence rate of HTN among Latinos, Asians, and African Americans living in the US has been reported when compared with Caucasians. The high blood pressure among non-Caucasians might be related to several factors such as socioeconomic status, high dietary salt intake, heredity, and low level of renin-angiotensin has been detected in the plasma of African American.

Gender and age have been reportedly shown to affect the awareness and management of HTN especially among the young adults within the age of 18 to 54 years

when compared with older adults of age >55 years, thus influencing the global prevalence of HTN (Tadic, Cuspidi, Grassi, & Ivanovic, 2019). The pathophysiology differences associated with gender are related to variation in sex hormones, components of the renin-angiotensin-aldosterone system (RAAS), and the sympathetic nervous and nitric oxide systems (EUGenMed et al., 2016). The cardioprotective effect of estrogen, most notably in premenopausal women, can be related to the low incidence of HTN and reduce the risk of developing CVDs when compared with men within the same age range. Estrogen plays a vital role in the activation of nitric oxide (NO), which is a potent vasodilator, inhibits the activation sympathetic nervous system and decreasing the activity of RAAS by blunting the production of angiotensin-converting enzyme (ACE) which blocks the formation of angiotensin II (Ang II). Blocking Ang II, which is a potent vasoconstrictor, is highly essential in the regulation of blood pressure. However, male sex hormone androgens can increase the synthesis of angiotensinogen II from the liver cells, which subsequently result in increased production of Ang II. Similarly, androgens can also enhance the reabsorption of sodium ion from the proximal convoluted tubules of the kidney via the androgen receptors, resulting in increased vasoconstriction and high blood pressure (Song, Ma, Wang, Chen, & Zhong, 2019).

Several contributing factors, such as the high incidence of smoking and hyperlipidemia, which are more prevalent in men, can account for gender differences in the prevalence of hypertension. Variations in the pharmacokinetics and pharmacodynamics response to antihypertensive drugs between men and women could also account for gender

differences in the prevalence of HTN. Women are more likely to develop adverse drug effects than men, alteration in drug metabolism by female sex hormones could reduce the clinical response of women to antihypertensives (Ueno & Sato, 2012).

1.1.3 Treatment of Hypertension

According to the recent guidelines in the diagnosis and treatment of HTN by ACC/AHA, recommendations for treatment have been revised based on the new directions that redefined HTN as SBP ≥ 130 mmHg and DBP ≥ 80 mmHg. However, not all individuals who are classified as being hypertensive based on recent ACC/AHA definition are recommended to commence treatment with antihypertensive drugs. Eligibility for antihypertensive recommendation is based on age (>65 years) and the presence of preexisting clinical medical conditions, such as diabetes mellitus, chronic renal disease, atherosclerosis, and other CVDs. At the same time, dietary/lifestyle modifications are recommended to hypertensive patients who do not meet the eligibility criteria (Khera et al., 2018).

Several antihypertensive therapies, ranging from ACE inhibitors, calcium channel blockers, beta-blockers, α -blockers, vasodilators, and diuretics have helped to normalize blood pressure in individuals with high blood pressure. However, despite the availability of different types of antihypertensive drugs, only about 36 million hypertensive individuals out of 1.4 billion of affected people globally, had their blood pressure controlled (Control & Prevention, 2012). Studies have shown that about 40-60% of individuals with

uncontrolled BP were due to incomplete adherence or non-compliance to drugs, while others were due to lack of awareness (Control & Prevention, 2012). High prevalence of uncontrolled BP among hypertensive individuals has contributed to increased risk of developing cardiovascular disease, most notably in the developing countries where socio-economic burden has affected the affordability of high costs of antihypertensive drugs. Similarly, adverse effects associated with prolonged use of a single drug type, or combination regimen, have been a great challenge in the management of HTN. Recently, the use of specific antihypertensive drugs has been linked with the development of cancer, although many of the links are still controversial. For instance, treatment with some calcium-calcium channel blockers has been associated with the incidence of breast cancer (Wright et al., 2017), while the use of common diuretics; hydrochlorothiazide (HCT) and thiazide have been linked with skin cancers and ovarian cancers respectively (Huang et al., 2016; Pedersen et al., 2018).

1.2 Pathophysiology of hypertension.

Interaction between several mechanisms that affect the vascular system, arterial system, sodium and fluid balance in the body are all implicated in the pathogenesis of HTN. The role of an arterial system comprising of the aorta (largest arteries), small arteries, and the microcirculation (arterioles and capillaries) in the development of HTN cannot be underestimated. Small arteries and arterioles with a diameter ($<300\mu\text{m}$) are highly resistant vessels that play a significant role in the regulation of BP. According to Poiseuille's law,

out of the three major factors that determine the resistance to blood flow in the vessels, the diameter of vessels is the essential factor. The primary mechanisms that are involved in the pathogenesis of HTN are discussed below.

1.2.1 Autonomic nervous system

Alteration in the autonomic nervous system, which consists of both the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS), plays a crucial role in the regulation of BP. Stimulation of either SNS or PNS, which depends on the type of receptors (α or β) that binds the neurotransmitters (either catecholamines or acetylcholine), can cause alteration in heart rate and blood vessel tone. Activation of SNS is usually excitatory, causing both increases in heart rate and vasoconstriction, which are essential factors that precipitate an increase in BP, while PNS has an inhibitory effect on heart rate.

Activation of sympathetic nervous activity to the heart, skeletal muscles, blood vessels, and kidney contributes to a rapid increase in BP and may lead to subsequent development of HTN, cardiac arrhythmias, atherosclerosis, left ventricular hypertrophy and other CVDs. The SNS is activated by several factors such as obstructive sleep apnea (OSA), genetics (about 50% of SNS over-reactivity can be inherited), stress, increase dietary calorie intake, obesity, chronic exposure to caffeine, and some other sympathomimetic agents (Grassi & Ram, 2016). Similarly, degrees of fat distribution in the body and general obesity has been associated with enhanced SNS activity. Microneurography analysis has confirmed the evidence of epinephrine spillover in the

plasma with the high concentration deposited in the kidney, thus justifying the correlation between the obesity, SNA activation, and HTN (Seravalle & Grassi, 2017).

1.2.2 Alteration in Vascular smooth muscle cell

All the blood vessels composed of vascular smooth muscle cells (VSMC), except for the capillaries. The VSMCs are located in the middle part (tunica media) in the structure of the blood vessel. The layers of VSMC are usually thicker in arteries when compared to the veins due to the pressure of blood flowing through the venous bed. The VSMC cells are differentiated and formed from the neural crest cells of main aorta and arteries; however, alterations in the synthesis of the VSMC, such as immature proliferation, growth, and migration have been implicated in the pathogenesis of HTN (Fisher, 2010; Kietadisorn, Juni, & Moens, 2012). The proliferation of VSMCs, which can either be an abnormal increase in the size of the cells (hypertrophy) or increase in the number of cells (hyperplasia), or presence of both pathological changes have been linked to abnormal growth in VSMC (Marx, Totary-Jain, & Marks, 2011).

Several stimulating growth factors, which are called mitogens, such as endothelin, thrombin, interleukin-1, platelet-derived growth factor (PDGF), vasopressin, serotonin, bradykinin, and angiotensin II are responsible for contraction of VSMC (Bacakova et al., 2018). The constriction of VSMCs, mediated by the contractile agonists listed above, contributes significantly to a decrease in the diameter of blood vessels. Considering Poiseuille's law, three factors, which are vessel diameter, vessel length, and blood

viscosity, determines the resistance to blood flow in the vessel. Decreases in diameter contribute majorly to increased peripheral resistance in blood vessels and subsequent increase in arterial blood pressure.

1.2.3 Endothelial dysfunction

The endothelial cells, which is a semi-permeable layer (located in the luminal parts of the blood vessel), often referred to as “tunica intima or tunica interna,” form direct contact with blood. Mechanical or biochemical damage to endothelial cells contributes to endothelial dysfunction and the pathogenesis of HTN. Increased production of harmful substances, such as free radicals (superoxide anions, hydroxyl ions, and other oxidants), in the blood and their attachment to the endothelium constitutes biochemical damage to the endothelium (Drummond, Selemidis, Griendling, & Sobey, 2011; Zeng, Villar, Yu, Zhou, & Jose, 2009). Damaged endothelium stimulates the activation of inflammatory cascades that leads to the production of certain substances such as platelet-derived growth factor (PDGF), nuclear factor kappa B (NF- κ B), tumor necrosis factor (TNF), vascular cell adhesion molecules (VCAM), interleukins and other inflammatory cells that stimulate proliferation of VSMC, vasoconstriction and eventually HTN (Masi, Uliana, & Virdis, 2019). Endothelial cells also produce substances that mediate the dilation of blood vessels and vascular tone. Nitric oxide (NO), endothelial-derived hyperpolarizing factors, and prostacyclin are potent vasodilators, and decreased production or release of vasodilators from the endothelial cells can also cause endothelial dysfunction and thus contribute to the development of HTN (Daiber et al., 2019).

1.2.4 Renin-angiotensin-aldosterone system (RAAS).

The RAAS system, which is considered both an endocrine and paracrine system, entails a series of hormonal cascades that exert its effect on renal function, maintenance of sodium/water balance, and physiological regulation of blood pressure. Pathological activation of RAAS in certain disease conditions plays a vital role in the pathogenesis of HTN. The release of renin initiates the activation of RAAS, a glycoprotein secreted and stored in the juxtaglomerular cells (JG) of the nephron. It has been reported that JG cells also contain β_1 adrenergic receptors, which can be stimulated by the sympathetic nervous system, thus inducing the release of renin from the JG cells. Signaling from macula densa cells due to deficiency of sodium ions and water imbalance can also stimulate the release of renin. Renin acts on its substrate called angiotensinogen, which is secreted mainly in the liver, converting it into an inactive peptide named angiotensin I (Ang I). Hydrolysis of Ang I by angiotensin-converting enzyme (ACE) forms angiotensin II (Ang II), which is a potent vasoconstrictor that mediates increased BP and organ damage associated with uncontrolled HTN (Mannelli, Rossi, Vanderriele, & Parenti, 2018).

The Ang II acts by binding to its specific receptors to mediate its effect, two different types of receptors, angiotensin type I (AT_1) and type II (AT_2), have been identified. The binding of Ang II to the AT_1 receptor mediates most of the effects of Ang II. Also, apart from the vasoconstriction effect of Ang II, it stimulates the release of aldosterone from the adrenal cortex. Aldosterone plays a crucial role in maintaining normal homeostasis by controlling sodium, potassium, and water balance in the body. Increased production of

aldosterone enhances the reabsorption and retention of sodium and water in the body, thus causing an increase in blood volume, blood pressure, and development of HTN (Provenzano & Sparks, 2019).

1.3 Arterial stiffness

1.3.1 The physiological role of Arteries

The arterial system, especially the large arteries, perform a conduit function. The arteries in a healthy young adult individual deliver adequate oxygenated blood from the left ventricle of the heart to the systemic circulation. The conduit function of the arteries ensures the proper supply of blood to the peripheral organs and tissues in the body, and the efficiency of the conduit function depends on the distensibility and stretching ability of the large artery (aorta) to accommodate an increase in blood flow. This physiological stretching ability of the arteries when there is an increase in blood flow widens the diameter of arteries, decreases peripheral resistance, and thus controls mean arterial blood pressure (London & Guerin, 1999).

Similarly, another interrelated function of the arteries is the ‘dampen function or cushion function’(Sun, 2015). During systole, only about 40% of the blood pumped out of the left ventricle is supplied to the organs and tissues. In contrast, the remaining blood is stored in the elastic arteries. The aorta recoils during diastole, squeezing the preserved blood into the peripheral tissues, thus ensuring continuous organ and tissue perfusion. This

recoil during diastole is also essential to perfuse cardiac muscle with oxygen-rich blood (W. W. Nichols et al., 2008).

1.3.2 Pathophysiology of Arterial stiffness

The Stiffening of the arteries termed “arteriosclerosis” occurs because of the alteration in the structure of the arterial wall. The arterial wall consists of smooth muscle cells, connective tissues (mainly elastin and collagen fibers), and fibrous tissue. The arrangement of elastin and collagen fibers varies according to the size of the arteries; the ratio of elastin to collagen is usually high in the aorta/large arteries when compared to peripheral arteries and arterioles (Wagenseil & Mecham, 2012). The Degenerative changes observed in the ECM of the tunica media part of the arteries, which is characterized by a reduction in the elastin/collagen ratio or viscoelastic properties of the artery is the primary pathophysiology that reflects arterial stiffness (London, Marchais, Guerin, & Pannier, 2004).

Contraction of the left ventricle during systole generates a wave called a pulse wave, which is palpable at the wrist or at the carotid artery of the neck (and along many other arteries that are near the skin’s surface). This wave produced during ventricular ejection is termed an incident wave, and the speed at which the waves travel away from the heart is called pulse wave velocity. The incident wave can be reflected at any point during the cardiac cycle, and the timing for the reflected waves depends significantly on the viscoelastic properties of the arteries (W. Nichols, 1998). Arterial stiffness can cause a

significant increase in the pulse wave velocity and untimely return of reflected wave during systole instead of diastole. In a healthy artery, the reflected wave should return to the heart during diastole to ensure a continuous supply of blood to coronary arteries, while the early return of the reflection wave during the systolic phase can augment the incident wave causing an increase in both pulse pressure and systolic blood pressure. Figure 1.1 illustrates the incident and reflection waves, as well as hemodynamic changes in both normal and stiffened arteries.

Figure 1.1. (A) Aortic pressure waveform showing both forward traveling wave (incident wave) and reflected wave of an individual with a normal artery. (B) Aortic pressure

waveform in an individual with a stiffen artery where the summation of the incident wave and reflected wave had augmented the pulse pressure (Van Varik et al., 2012).

1.3.2.1 Arterial Stiffness and Hypertension

Arterial stiffness is commonly one of the first contributing mechanisms to the development of essential hypertension. Disruption in elastin, which is the major ECM component in the arterial wall, contributes mainly to arterial stiffness and HTN. The large arteries and aorta serve as capacitance vessels that distend and recoil during systole and diastole, respectively. However, a stiffened artery requires more pressure to stretch the arterial wall to pump blood to distal vessels and systemic circulation. The early return of reflected waves back to the aorta during the systolic phase increases the systolic pressure, especially in older individuals. In young individuals, PWV is relatively slow due to the late return of reflected wave at the diastolic phase of the cardiac cycle, thereby increasing the diastolic pressure.

The importance of altered elastin in the development of arterial stiffness and HTN was confirmed by an animal model study where elastin was genetically knocked down in mice. Increased left ventricular pressure, and systolic pressure was observed in modified mice when compared with wild type mice (Wagenseil & Mecham, 2012). Arterial stiffness was also significantly increased in elastin-knocked out mice, and this preceded the observed blood pressure increase. Thus, early detection of increased PWV even before the development of high blood pressure can be a new clinical marker in the diagnosis of HTN

and CVDs. While developing a new therapeutic approach targeting the reduction of PWV and arterial stiffness can significantly reduce the high prevalence rate of HTN and CVDs.

1.3.3 Measurements of Arterial Stiffness

Evaluation of arterial blood pressure and pulse pressure at the brachial artery has been used as a predictor for the diagnosis of HTN and other cardiovascular diseases. Although brachial arterial blood pressure does not usually provide accurate analysis of the pressure in the aorta and large elastic arteries; thus measurements at the brachial artery are an indirect evaluation of arterial stiffness (Wagenseil & Mecham, 2012). In healthy individuals with normal arterial distensibility, their aortic blood pressure is usually lower than the systolic brachial pressure, because the aorta has higher distensibility than other large arteries. However, in certain circumstances, the brachial arterial blood pressure might be of the same value as the aortic blood pressure, thus providing an inconclusive marker of cardiovascular events. The rough and inaccurate measurement of arterial stiffness with the brachial artery has led to the discovery of several non-invasive methods, such as Doppler ultrasound, pressure transducer, photoplethysmography and magnetic resonance imaging (MRI), which can provide a more specific indication of arterial stiffness (Urbina et al., 2009).

In-vivo measurement of arterial stiffness humans depends on the ratio of pressure changes in the arteries to the alteration in diameter at both diastolic and systolic phase of the cardiac cycle. Measuring aortic PWV by recording the pulse wave in the common

carotid arterial and femoral artery (cfPWV) has been reported as the standard method for in-vivo measurement of arterial stiffness in humans. The distance between the carotid and femoral arteries can be measured superficially with a measuring tape, while the pulse wave can be detected using applanation tonometry. The cfPWV is obtained from a distance/per unit time of the traveling wave between the two locations. Applanation tonometry, which makes use of computer software to record both the incident and reflected waves, has been proven to be a reliable diagnostic tool used in estimating arterial stiffness in clinical and research cardiovascular laboratories (Crilly, Coch, Bruce, Clark, & Williams, 2007).

1.3.3.1 Applanation Tonometry

Evaluating arterial stiffness by applanation tonometry involves two main techniques: pulse wave analysis (PWA) and pulse wave velocity (PWV). Mohamed first invented the use of the PWA technique to assess arterial stiffness in 1872, where the pressure waveform differences between central and peripheral arteries were analyzed (Mohamed, 1872). However, the current PWA involves the use of a tonometer to record the pulse waves of a specific artery. Most commonly, the radial artery and the waves are automatically analyzed by SphygmoCor computer software. This PWA gives an estimate of aortic blood pressure waveform when calibrated with individual brachial blood pressure, thus providing the estimates of central SBP, DBP, PP, and MAP. Apart from the aortic blood pressure, aortic augmentation index (AIx) can also be obtained from the PWA. Figure 1.2 shows a sample report of PWV measured at the radial artery indicating the aortic blood pressure and AIx. The AIx means the augmented aortic systolic blood pressure

caused by the return of reflected waves at the aorta either at the systolic or diastolic phase, depending on the mechanical structure of the large arteries (Papaioannou et al., 2004). Also, the AIx has been reportedly linked to an individual's heart rate, blood ejection time, and height. Monitoring arterial stiffness using PWA can be used to provide useful information about cardiovascular events (Townsend et al., 2015).

Figure 1.2. Sample recordings of PWA of radial artery obtained from SphygmoCor applanation tonometry showing the aortic blood pressure (SBP, DBP, MAP, and PP) and the aortic augmentation indices.

Pulse wave velocity (PWV) involves the evaluation of the speed of pulse wave, which is automatically calculated based on the distance traveled by the wave from one point to another per unit time. Unlike the PWA, measurement of PWV by the SphygmoCor usually corresponds with the electrocardiogram (ECG) reading, specifically the R-wave of the ECG, which indicates the start of ejection of blood from the ventricles. The carotid and femoral arteries are the two points commonly used to estimate pulse wave velocity (cfPWV). Measurement involves placing a measuring tape to measure the superficial distance from the aorta (located at the suprasternal notch) to the carotid and femoral arteries, while time delay from the R-wave to the pulse arriving at the given palpated arterial site is measured by the tonometer placed on the respective arteries. The SphygmoCor computer software automatically utilizes the measured distance and time to evaluate the cfPWV (m/s). Figure 1.3 shows the sample report indicating the carotid-femoral pressure pulse waves, ECG, and cfPWV.

Figure 1.3. Sample recording of cfPWV obtained from the automatic calculation of the distance from carotid and femoral sites for a delay time of pulse arrival after the respective R-waves.

1.3.4 Factors Influencing Arterial Stiffness.

The extent of arterial distensibility depends greatly upon the composition of the blood vessels, most especially aorta and large arteries. Any factors either modifiable or

non-modifiable that alter the fundamental structure of blood vessels can result in stiffening of the arteries. The most important structural components include VSMC, collagen, and elastin. Aging is one of the essential non-modifiable independent risk factors that induce the development of arterial stiffness and HTN. An increase in age has been associated with altered ECM proteins in the blood vessel, such as calcification and fragmentation of elastin (decrease elastin) and accumulation of collagen, thus reducing the viscoelastic properties of the vessel wall. This structural disruption in ECM protein would reduce the dampening function of the arteries and increase PWV with a resultant increase in systolic blood pressure and pulse pressure.

Several reports have shown the relationship between age and arterial stiffness (Franklin et al., 1997; Mitchell et al., 2004; Wen et al., 2015). The stiffness is usually more noticeable in the central artery and aorta when compared to muscular or peripheral arteries. Evidence has shown a strong correlation between age and carotid artery stiffness, while there was no correlation between femoral artery stiffness and age (W. Nichols, 2005; van der Heijden-Spek et al., 2000). Aortic and carotid PWV increases with age with an approximate value of about 0.1m/s every year with an estimate of almost 10% to 15% increase during ten years (W. Nichols, 2005). In addition to the alteration in viscoelastic properties of the vascular wall, the presence of metabolic syndrome and inflammation could also contribute to age-related arterial stiffness and HTN. The presence of diseases such as type II diabetes, obesity, dyslipidemia, hyperglycemia, and end-stage renal disease

could promote vascular aging, stiffening of the arteries, and development of HTN (Ferreira, Schouten, Smulders, Twisk, & Stehouwer, 2012).

Dietary modifications are one of the modifiable factors that can influence arterial stiffness. Consumption of alcoholic wine can induce a small but significant decrease in BP, AIx, PP, and PWV measured at the radial artery, while no significant hemodynamic changes were observed in participants exposed to non-alcoholic wine (Mahmud & Feely, 2002). Similarly, chronic intake of caffeine, either in the form of a tablet or consumed in coffee can induce have been associated with an acute increase in AIx and SBP when compared with participants exposed to placebo or decaffeinated coffee (Waring, Goudsmit, Marwick, Webb, & Maxwell, 2003). Other dietary modifications, such as high consumption of fish (Del Brutto, Mera, Peñaherrera, Peñaherrera, & Costa, 2018) and high intake of antioxidant vitamins most especially, vitamins A, C, D, E and K (Mozos, Stoian, & Luca, 2017), have been shown to reduce arterial stiffness.

Lifestyle medications is another modifiable factor that influences changes in arterial stiffness. Regular exercise either, aerobic endurance or resistance exercise, can induce a significant decrease in aortic PWV and carotid AIx when compared to individuals with a sedentary lifestyle (Mutter, Cooke, Saleh, Gomez, & Daskalopoulou, 2017). Cigarette smoking has also been associated with the development of CVDs. Both short- and long-term smoking can induce endothelial dysfunction, thereby increasing arterial stiffness. Numerous studies have confirmed the effect of long and short-term smoking on arterial compliance among active smokers; however, in cases of short smoking, most significant

changes in arterial compliance are usually observed within the first five minutes after acute smoking (Kim et al., 2005; Xue et al., 2019). Besides, passive smoking involving long term exposure to environmental air polluted with tobacco smoke has been associated with carotid arterial stiffness. However, this depends on the dose of exposed pollutant mixtures (Ljungman et al., 2018).

1.4 *Hibiscus sabdariffa*. L

Hibiscus sabdariffa. L (Hs) is an annual herb that belongs to family Malvaceae, and it is widely known in English as a roselle or red sorrel. The HS plant (figure 1.4) is a tropical shrub that is commonly grown in many tropical regions of the world, especially in South East Asia, India, America, Central and West Africa (Ngamjarus et al., 2010). Different parts of the hibiscus plant, leaves, flowers, and calyx are used medicinally for treatment of various health issues; however, fresh, or dried calyces of the flower are popularly used to produce hibiscus tea or sour tea. The phytochemical and pharmacological analysis of Hs reviewed by several studies has shown the presence of bioactive compounds, which are mainly: polyphenols.

Phytochemical analysis indicates the presence of five essential polyphenols in HS and other plant extracts: anthocyanins, flavonoids, anthocyanidins, phenolic and organic acids. Anthocyanins have been identified as the significant polyphenols compound in the hibiscus plant, and they confer the red pigment to the calyces of the HS flower (Grajeda-Iglesias et al., 2016). Four types of anthocyanins have been unearthed and are described

based on differences in the make-up of their chemical structure, these include Delphinidin (anthocyanidin), Delphinidin-3-sambubioside (hibiscin), Cyanidin -3-sambubioside, and Cyanidin-3,5- diglucoside. Both delphinidin-3-sambubioside and cyanidin-3-sambubioside are the most effective anthocyanins in HS extract (Yang et al., 2009).

Figure 1.4. *Hibiscus sabdariffa* L. flower showing the petals, stigma, and stamen.

(Picture was taken at MTU greenhouse; photo credit: John Durocher).

1.4.1 *Hibiscus sabdariffa*. L and Blood Pressure.

The beneficial effect of *Hibiscus sabdariffa* extracts on lowering blood pressure both in normotensives and hypertensives have been extensively reported in both human and animal studies. According to Inuwa et al, there was a significant reduction in SDP, DBP, mass of the left ventricle of the heart when the effects of HS extract on hypertension was observed on rats for about ten weeks, although they show no effect on heart rate (Inuwa et al., 2012). In another comprehensive report by Hopkins et al. (Hopkins, Lamm, Funk, & Ritenbaugh, 2013), HS extract showed an effective reduction in blood pressure in both hypertensive and normotensive animals. Several clinical randomized human trials where dried calyxes of HS were brewed as tea, used as a juice, or directly as an extract on different populations have confirmed the hypotensive effect of HS. A randomized controlled trial (RCT), where hypertensive patients were exposed to 10g of dried HS extract per day, reported a substantial reduction in SBP and DBP after four weeks intervention period (McKay, Chen, Saltzman, & Blumberg, 2009). Similarly, the hypotensive effect of HS was compared with Lisinopril (a potent antihypertensive drug) in a double-blind controlled clinical trial. After four weeks intervention period, a similar reduction in BP was observed among participants exposed to Lisinopril and HS tea, thus confirming the effectiveness of HS extract as other pharmaceutical antihypertensive therapies (Herrera-Arellano et al., 2007).

1.4.2 Antihypertensive mechanisms of Hibiscus sabdariffa. L

The exact mechanism by which HS mediates a reduction in BP in humans is still unknown. Studies showing the three main antihypertensive mechanisms HS have been reported and are compared with the mechanisms of action of most of the generally used antihypertensive drugs. The identified mechanisms of action include; angiotensin-converting enzyme inhibitors (ACE inhibitor), vasodilator, and diuretic properties (Alarcón-Alonso et al., 2012; Ojeda et al., 2010; Sarr et al., 2009). The anthocyanins, specifically delphinidin-3-sambubioside and cyanidin-3-sambubioside, are responsible for most of the mechanisms involved in lowering BP.

1.4.2.1 Diuretic mechanism

The HS can stimulate the release of nitric oxide which is a vasorelaxant from the endothelial lining of the blood vessels. The nitric oxide (NO) regulates the rate of blood flow to the kidney and glomerular filtration rate. The role of NO in normal function of the kidney has been linked with its ability to increase the reabsorption of sodium from the kidney tubules. The diuretic property of HS was assessed in an animal model and a randomized clinical trial, where a decrease in plasma concentration of sodium due to increased urinary excretion was observed in both hypertensive rats and patients with HBP after four weeks of exposure to HS extracts. (Kvam, Ofstad, & Iversen, 2000). The diuretic action HS extract is comparable to the most frequently used diuretics such as

hydrochlorothiazide (HCT) and furosemide used in the treatment of hypertensive individuals (Alarcón-Alonso et al., 2012).

1.4.2.2 Hibiscus sabdariffa. L as a vasodilator.

The vasodilating effect of HS extract is induced by the presence of a high concentration of anthocyanin bioactive compounds in the extract compared to other polyphenols. (Adegunloye et al., 1996) . The existence of anthocyanin in HS extract has been implicated in the activation of potassium ion (K^+) channels, while activation of K^+ causes the rapid efflux of K^+ into the extracellular compartment and can stimulate hyperpolarization of the blood vessel membrane. Hyperpolarization of the membrane inhibits cardiovascular activity which can stimulate a significant reduction in blood pressure (Sarr et al., 2009).

Similarly, HS has been shown to reduce BP and HR by inhibiting the SNS. It was investigated by a study in humans where an increased in sympathetic nervous activity and BP induced by cold pressor test (CPT) was subsequently inhibited after the administration of HS tablet (Aliyu, Oyeniyi, Mojiminiyi, Isezuo, & Alada, 2014). Another study confirmed the vasorelaxant effect of HS by its ability to induce the release of endothelium-derived nitric oxide, which is a potent vasodilator and block the influx of calcium ions. This was confirmed in an animal model, where hypertensive rats were exposed to 0.3mg/ml of Hs extract daily for six weeks intervention period, and the vasodilating effect was observed in the aorta of the rats (Ajay, Chai, Mustafa, Gilani, & Mustafa, 2007).

1.4.2.3 Angiotensin-converting enzyme (ACE) inhibitor

The capability of HS extract to block the action of ACE is another hypotensive mechanism. The ACE is vital enzyme that facilitates the conversion of angiotensin-I to angiotensin-II in the RAAS. The anthocyanins in HS extract compete with the ACE binding site, thus preventing the formation of Ang II, which is a potent vasoconstrictor. The ability of HS extract to hinder the ACE activity was investigated in a randomized clinical trial where hypertensive patients (HTN stage 1 and 2) were exposed to either HS extract or 25mg captopril twice daily (ACE inhibitor drug). The decreased in BP observed among patients exposed to anthocyanin-rich HS extract was similar to the BP reduction rates reported among patients on captopril (Ojeda et al., 2010).

1.4.3 Toxicity of *Hibiscus sabdariffa*. L

Despite the promising hypotensive benefit of HS and its outcome in minimizing the risks of developing cardiovascular diseases, its safety and contradictions among specific individuals should be clearly stated. Apart from consuming the pure HS extract, there are lots of commercially prepared herbal tea, where more than half of the total constituents consist of HS extract. While HS extract is considered to be safe and natural, the ingestion of HS extract can as well be harmful to individuals who are taken certain medications such as hydrochlorothiazide (frequently used diuretics), acetaminophen, diclofenac, and other high risk groups (gravid ,lactating women and children). Some pre-clinical trials have reported a very low degree of acute toxicity when a high dose of HS extract is being

consumed. At the same time, exposure to more than 300mg/kg daily for a prolonged period of three months can result in significant hepatotoxic effects. The lethal dose (LD₅₀) of HS extract was confirmed in an in-vivo experimental animal model, where Wister albino rats exposed to high dosage of 300-2000mg/kg HS extract per day for 3 months showed a significant increase in liver enzymes and plasma creatinine, thus confirming the damaging effect on the liver (Fakeye, Pal, Bawankule, Yadav, & Khanuja, 2009). Similarly, in another animal study, rats exposed to a daily dose of 250-1000 mg/kg revealed no organ damage (Prommetta, Phivthong-ngam, Chaichantipyuth, Niwattisaiwong, & Lawanprasert, 2006). Based on several studies on HS toxicity dosage, it has been recommended that a maximum dose that is less than 1000mg/kg is considered safe and would not induce any organ damage in humans.

1.5 Aim of the Study

The study aimed to examine the effect of *Hibiscus sabdariffa* tea and oolong tea on blood pressure and arterial stiffness in healthy human subjects after six weeks of the intervention period. We hypothesized that individuals who are exposed to a daily intake of HS tea would have a decrease in blood pressure and arterial stiffness after six weeks of the intervention period when compared to participants exposed to oolong tea.

1.6 Significance of the Study

In the recent survey by the WHO, cardiovascular disease remains the number one cause of death in the United States, as well as low- and middle-income countries in the world. High blood pressure has been considered as the major contributing factor responsible for CVDs. Despite the availability of antihypertensive drugs in many countries in the world today, more than 50% of hypertensive individuals were unable to control their HBP (Muntner et al., 2018). High costs of drugs, insufficient treatment due to non-compliance, and undesirable side effects resulting from prolonged use of drugs have been implicated in poor management of hypertensive individuals. With the growing increase in uncontrolled HTN, the WHO has estimated the global annual death toll resulting from HBP would reach 23.5 million by the year 2030 if alternative treatment approaches are not being implemented (Organization, 2015). Thus, there is a need for urgent intervention to avert the current increasing rate of uncontrolled HBP in hypertensive individuals, hence the emerging use of *Hibiscus sabdariffa* and other complementary alternative therapies in the management of hypertension.

Numerous studies have investigated the effect of HS extract/tea on reducing blood pressure both in humans and animals, while HS tea has also been reported to reduce arterial stiffness in animal and tissue models (GWALA, SIBUOR, Olabu, Pulei, & Julius, 2019). However, the effect of HS tea on arterial stiffness in humans is still unknown, to the best of our knowledge, this is the first study to investigate the impact of HS tea on arterial stiffness in healthy humans.

2. Methods

2.1 Participants Information.

Forty-five healthy volunteers from Michigan Tech and Houghton community were assessed for eligibility into the study. Out of the 45 participants assessed, only 30 (17 males, 13 females) met all the inclusion criteria and were recruited into the study. The inclusion criteria for participation in this study were; men and women age 18-45 years, not pregnant, resting systolic blood pressure of at least 110 mmHg, body mass index (BMI) in the range 18.5-35kg/m², not on any blood pressure medication, non-smokers and free of any acute or chronic metabolic diseases. Throughout the study, a total of 18 participants completed the study, while 12 participants were lost to follow up due to the COVID-19 pandemic (Figure 2.1).

2.2 Study design and Procedures

Initially, an orientation session was held at Michigan Tech's Clinical and Applied Physiology lab (CAHP) following fasting for 5 hours by abstaining from food, caffeinated beverages, alcohol, and strenuous exercise. During the orientation, detailed study procedures and restrictions were explained to the volunteers. Questions to determine the subject's eligibility were obtained using questionnaires; height, weight, and BMI were also measured and recorded. Potential participants were instructed to rest for 5 minutes before resting seated brachial blood pressure was measured in triplicate using an automated sphygmomanometer to screen for participants with low blood pressure (SBP<110mmHg).

All eligible participants signed informed written consent and information sheet before being officially enrolled in the study, and this study was approved by the Michigan Technological University Institutional Review Board (M1889). Eligible volunteers were scheduled for a full baseline testing session.

Figure 2.1. Schematic illustration of study design and participant enrollment.

The study was a randomized controlled clinical trial (RCT), subjects were randomized into one of two intervention groups: *Hibiscus sabdariffa* tea and oolong tea (control group). Before baseline testing, participants were instructed to fast overnight for a minimum of 12 hours, without food, drinks (alcohol, caffeine, except water), or exercise. Participants reported to SDC sleep research lab, where each was instructed to rest for 5 minutes before measuring three seated blood pressures. They were also told to lay supine for 5 minutes

before supine blood pressure was assessed in triplicate as well. Average values were recorded for both seated and supine blood pressures. Subjects in each group were instructed to consume the tea twice daily (one tea bag in the morning and one in the late evening/night) for six weeks.

Dried calyces of organic *Hibiscus sabdariffa* tea and oolong tea leaves were commercially purchased, and 2g of dried leaves were measured and put in each of the teabags. Also, commercially bagged *Hibiscus sabdariffa* and oolong Alvita brand teabags were purchased and allocated to only the first 4 HS participants and the first three oolong participants. All the participants were instructed to steep 2 g of the teabag in 8 oz. of hot water, advised to abstain from drinking any other tea aside the one provided, but were not restricted from their usual dietary and exercise pattern throughout the study period. Participant's compliance with tea intake at the end of the study was assessed by asking them to return unconsumed tea bags, and only readings of those that consumed more than 90% of the tea bags were included in the final data analysis. Finally, volunteers were informed to report any unusual allergy or abnormal sensations to tea intake and stop drinking the tea immediately; however, none of the participants reported any adverse reaction to the tea.

2.3 Measurements

2.3.1 Blood Pressure

The brachial blood pressure was measured, first in a seated position, and subsequently in a supine position with an automated sphygmomanometer (Omron HEM-907XL, Omron Health Care, Kyoto, Japan). Participants were informed to refrain from talking while recording the blood pressure readings in both positions. Blood pressure readings were assessed three times, and average blood pressure readings for both seated and supine blood were recorded. The blood pressure measurement was standardized by using the same sphygmomanometer and participant's right arm throughout the testing period.

2.3.2 Pulse Wave Analysis

Before performing pulse wave analysis, subjects rested on a bed in the supine position for 5 minutes. The average supine blood pressure reading obtained was used to calibrate the SphygmoCor (SphygmoCor CPVH; AtCor Medical, Sydney, Australia). Radial applanation tonometry was performed non-invasively on each of the participants, which involved placing a tonometer probe on the radial artery and pressing the probe slightly against the carpal bone at the wrist. Ten strong radial pulse waves were captured by pressing/holding the probe for a few seconds on the right spot of the radial artery to provide trusted estimates of aortic blood pressure. This procedure was done twice to ensure

consistency in the radial waves and aortic readings generated. An operator index greater 80 was considered satisfactory throughout the testing period.

The radial pulse wave generated was analyzed by the SphygmoCor software to provide estimates of aortic systolic blood pressure (SAP), diastolic blood pressure (DAP), mean aortic pressure (MAP) and pulse pressure (PP). Also, aortic augmentation index (AIx) was calculated automatically by the SphygmoCor software based on the characteristics of the pulse waves. The procedure was standardized by excluding any participant with aortic pulse pressure greater than 50 mmHg, as these individuals may be more susceptible to plaques that could potentially become dislodged with force (Roman et al., 2007).

2.3.3 Pulse Wave Velocity

The pulse wave velocity was determined by non-invasive applanation tonometry. Using a three-lead electrocardiogram (ECG) involving the use of three surface electrodes, two electrodes were placed at the left and right side of the shoulder region, while the last electrode was placed below the rib cage of the left side of the participants. The distance (mm) between the pulse site in the carotid artery and the suprasternal notch was measured with a tape. At the same time, the second distance measured from the femoral pulse located in the femoral artery and the suprasternal notch was also recorded. Central arterial stiffness (carotid-femoral) was measured by placing the tonometer on the pulse site first on the carotid artery and subsequently on the femoral artery on the first trial, while in the opposite

direction on the second trial. The SphygmoCor software utilizes the measured distance and the time delay from the appearance of the incident wave in the heart and the arrival of the reflected wave back at the pulse site for the estimation of carotid-femoral pulse wave velocity (cfPWV). The test was performed in duplicate to standardize the procedure, and readings with a standard deviation of $\leq 10\%$ were included in the final data analysis.

2.4 Statistical Analyses

Statistical analysis of the data was performed using IBM SPSS statistics software (SPSS version 20.0). Analysis of variance (2 x 2 repeated-measures ANOVA) was used to determine the time and treatment effects on tested variables between the two groups. Welch two-sample t-test was used to compare the baseline variables due to the unequal sample size between the two groups. The cfPWV of two participants was not measured, while one subject did not have recordings for PWA, and was excluded in the final data analysis. The results were expressed as mean values $\pm$ standard deviation (SD), and P-value < 0.05 was considered significant.

3 Results

3.1 Participants Characteristics

The baseline demographic and clinical characteristics of the study participants are shown in Table 3.1. The mean age of the participants in the HS group and control group was 32 ± 10 years and 27 ± 5 , respectively. Generally, there was no significant difference in the demographic data and tested clinical variables between the two groups at baseline ($P > 0.05$).

3.2 Tested Clinical Variables

The within-group and between-group comparisons were analyzed on the following clinical variables: SBP, DBP, HR, AIX, and cfPWV before and after the intervention. A reduction in the brachial seated blood pressure was observed within the HS group, with a decrease in post-intervention mean SBP (-4 mmHg) and DBP (-3 mmHg) when compared with pre-intervention values. Also, the oolong tea (control) induces reductions in SBP (-4mmHg) and DBP (-9.06 mmHg) (Figure 3.1A and B). A 2 x2 repeated-measures ANOVA between the group revealed a significant time effect for both SBP ($P = 0.02$) and DBP ($P = 0.001$). However, there was no significant time by group effect; a reduction in blood pressure was also observed in the control group.

Table 3.1. Baseline Demographic and Clinical Characteristics of the Participants

Variable	HS tea n=11	Oolong tea n=7	P-value
Age (yrs.)	32 ± 10	27 ± 5	0.17
Sex (men/women), n	8/3	5/2	
Weight (kg)	82 ± 18	84 ± 16	0.79
Height (cm)	176 ± 10	172 ± 10	0.58
BMI (kg/m ²)	26 ± 3	28 ± 4	0.45
Seated SBP (mmHg)	123 ± 7	122 ± 9	0.81
Seated DBP (mmHg)	74 ± 7	70 ± 9	0.36
Supine SBP (mmHg)	121 ± 7	118 ± 5	0.54
Supine DBP (mmHg)	70 ± 7	64 ± 5	0.06
HR (beats/mins)	64 ± 11	59 ± 10	0.32
AIx@HR75 (%)	3.1 ± 19.0	-0.6 ± 10	0.35
cfPWV (m/s)	6.2 ± 0.9	5.5 ± 0.4	0.05

Values are means ± standard deviation (SD); n: number of participants; HS: *Hibiscus sabdariffa*; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HR: Heart rate; AIx@HR75: Aortic augmentation index normalized to 75 heartbeats; cfPWV: Carotid-femoral pulse wave velocity. P-value obtained from Welch two Sample t-test for baseline comparison between the two groups.

Figure 3.1. **A:** comparing mean SBP (systolic blood pressure) pre- vs. 6weeks post-intervention; **B:** mean DBP (diastolic blood pressure) for pre -vs post intervention. A 2 x2 repeated-measures ANOVA between groups revealed significant time effect ($P = 0.02$; $P = 0.001$) for SBP and DBP respectively, and no significant time by group effect ($P > 0.05$). Results are mean $\pm$ standard deviation (SD).

Figure 3.2: Comparing the heart rate (HR) before and after intervention between the two groups. * Significant difference within HS group ($P < 0.05$). No significant difference within control ($P = 0.69$). Repeated ANOVA between the groups revealed a significant time effect on HR ($P = 0.028$). Results are mean $\pm$ standard deviation (SD).

Figure 3.3: Carotid-femoral pulse wave velocity (cfPWV) before and after intervention between HS and control groups. Repeated ANOVA revealed no significant time effect ($P=0.44$) and time by treatment effect between groups ($P=0.35$). All values are mean $\pm$ standard deviation (SD)

Also, there was a reduction in HR in both groups when compared with pre-intervention values (Figure 3.2). However, only the HS tea intervention induced a significant decrease in HR ($P=0.01$) when compared with oolong tea ($P=0.149$), as depicted in figure 3.2. Furthermore, the carotid-femoral pulse wave velocity (cfPWV) comparison before and after tea intervention showed a slight reduction in cfPWV (-0.5m/s) in the HS group, while an increase of ($+0.3\text{m/s}$) was observed in the control group (Figure 3.3). Although, we did not find any significant time ($P=0.44$) and time* treatment group ($P=0.35$) effect in both case and the control groups.

4 Discussion

In this study, we investigated the effects of *Hibiscus sabdariffa* and Oolong tea on blood pressure and arterial stiffness in healthy adults. The results showed that the six weeks tea intervention had a significant positive impact on SBP, DBP, and heart rate. However, there was no significant time by treatment group effect on the blood pressure, as both SBP and DBP were also decreased in the Oolong tea group after six weeks of intervention. The HS tea consumption resulted in a significant decrease in HR, while the observed reduction in the control group was not statistically significant. To our knowledge, this is the first study to investigate the effect of HS tea consumption on central arterial stiffness in humans. There was a slight decrease in the mean cfPWV value within the HS group when compared with the control, but the reduction was not statistically significant.

The partial oxidation and fermentation of *Camellia sinensis* plant leaves results in the production of Oolong tea. The concentration of caffeine in the tea derived from *Camellia sinensis* depends on the method of cultivation and intensity of oxidation during processing. While the caffeine content in oolong tea is considerably small when compared to black tea, although excessive consumption could also stimulate an acute rise in blood pressure. Several clinical trials on the antihypertensive effect of oolong tea and other tea derived from *Camellia sinensis* (green and black tea) reported convincing data that need to be verified and confirmed. In this current study, both the HS and Oolong tea (control) lowered blood pressure. The results from this study are in agreement with most of the previous

clinical and experimental studies on antihypertensive effects of HS tea. In a clinical trial, where the impact of HS tea on stage 1 hypertensive patients was investigated, a decrease in blood pressure was reported in both the case and the control groups (Jalalyazdi et al., 2019). In another trial, the effect of HS tea consumption on patients with mild hypertension resulted in significant reductions in SBP, DBP, and MAP when consumed thrice daily for six weeks (McKay et al., 2009). A meta-analysis of 13 RCTs on the effect of green tea consumption on blood pressure showed a significant decrease in SBP and DBP by 1.98 mmHg and 1.92 mmHg, respectively, when compared to the control group (Peng et al., 2014).

Contrary to our study, is a RCT that compared the antihypertensive effect of HS tea with green tea in healthy adult men. The study concluded that the daily administration of 450mg of HS could induce a significant reduction in only SBP when compared with green tea, the study did not report any significant difference in DBP in either the HS and green tea intervention (Kafeshani et al., 2017). While, another meta-analysis of 5 RCTs showed no significant changes in the blood pressure after about four weeks of oolong tea consumption (Liu et al., 2014).

The significant decrease in HR within the participants exposed to HS tea when compared to control in this study is similar to the reports obtained from another randomized clinical trial. In the previous study, a significant reduction in HR was observed after the administration of 15mg/kg of HS extract to individuals with increased HR-induced by hand-grip exercise (HE) and cold pressor test (Aliyu et al., 2014). Also, reports from an

animal study confirmed the HR reduction induced by HS extract. Chronic ingestion of HS extract (250mg/kg/day) into renovascular hypertensive-induced rats for eight weeks resulted in a significant decrease in HR (Odigie, Ettarh, & Adigun, 2003). The probable mechanism of reducing HR has been associated with HS's ability to inhibit sympathetic nerve activity. It was reported that the activation of SNS induced by CPT, which led to the stimulation of pain, cold receptors, constriction of blood vessels, caused an increase in HR. The subsequent administration of HS extract counteracted the activated SN activity, thereby mediating the reduction in HR back to normal (Aliyu et al., 2014).

Despite the numerous cardioprotective effects of HS reported in previous studies, the specific antihypertensive mechanisms of HS are not fully understood. Different antihypertensive mechanisms have been reported; regulating serum electrolyte due to its diuretic effect (Herrera-Arellano et al., 2007), ACE inhibitory activity (Ojeda et al., 2010), inhibition of SNS and regulation of blood lipids (Morales-Luna et al., 2019), which are all involved in the pathogenesis of hypertension. Recently, the anthocyanin which is the bioactive compound in HS extract has been reportedly linked to most of the antihypertensive mechanisms of HS. This was confirmed in an animal study where the effect of dried HS calyx was compared with HS anthocyanin (HSA) extract on spontaneous hypertensive rats. It was indicated that long-term ingestion (10 weeks) of HSA resulted in a significant reduction in SBP, DBP, and left ventricular mass when compared to the whole HS calyx (Inuwa et al., 2012). Similarly, the probable antihypertensive mechanism of oolong tea can be linked to the concentration of "catechins," which is the bioactive

compound in tea. Catechins have been shown to induce the release of NO from the endothelium, which can inhibit the constriction of blood vessels. Extracts from *Camellia sinensis* lowers blood pressure by enhancing the production of antioxidant enzymes that inactivate reactive oxygen species (ROS). The antioxidant enzymes includes; catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase (Thomson, Al-Qattan, Mansour, & Ali, 2012)

As previously stated, arterial stiffness is one of the most common mechanisms that could contribute to the development of hypertension, increased arterial stiffness has been associated with aging, high blood pressure and increased risk of cardiovascular diseases. In this study, we investigated the effect of HS tea on arterial stiffness as a probable mechanism by which HS tea could decrease blood pressure. While the observed slight decrease in cfPWV (-0.5m/s) was not statistically significant with our limited sample-size, the -0.5m/s reduction could be clinically significant as 1m/s reduction in cfPWV could all reduce CVD-induced mortality by 15% (Vlachopoulos, Aznaouridis, & Stefanadis, 2010). Thus, the 0.5 m/s reduction after HS could indicate the risk for mortality is reduced by approximately 7.5%.

4.1 Limitations

Generally, the variability in response reported in this study and some previous HS clinical trials have been linked to lack of standardization in the amount of HS consumed, different methods/conditions used in the preparation of HS (infusion, extraction or decoctions) and the methods of use by the trial participants (a varying amount of water and

temperature condition). All those factors listed above would affect the dose of the bioactive compound (anthocyanins) in HS given to trial participants.

One major limitation of this study was our small size. The sample size was considerably small due to participants who were lost to follow-up because of the COVID-19 pandemic. The imbalance in the number of participants between the HS and the control groups was a concern, as more of the participants lost to follow-up were in the control group. Also, the true blinding of our treatments was not feasible because of the distinct tastes of HS and the oolong tea used as control. Furthermore, in this trial, we used dried calyx of HS, measured, and packed in the teabags, we were unable to extract and standardize the amount of the active anthocyanins in the HS tea consumed by the participants, and thus could lead to some variability in the doses of bioactive compound. Lastly, most of the participants recruited for this study were volunteers studying at the university, so we were unable to completely exclude other confounding factors such as stress, diets, and exercise conditions that could potentially influence the cardiovascular measurements.

4.2 Future Directions

Future studies on the antihypertensive effects of HS on humans should be well standardized by extracting the anthocyanin bioactive compound from the dried calyx. The quantification of anthocyanin doses by high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) before administering it to trial participants is highly

recommended to achieve the desired hypotensive effects and at the same time preventing HS overdose that could potentially be toxic to some organs in the body. Previous studies on the HPLC analysis of HS have shown the presence of four different types of anthocyanins in HS extract; however, a large amount of delphinidin-3-O- sambubioside (Dp-samb) has been reported. As such, we recommend further fractionation of HS to separate the Dp-samb from other anthocyanins in the HS extract before consumption to achieve maximum benefits. Also, more robust, randomized controlled clinical trials with a high dosage of Dp-samb targeted for a longer duration of intervention would be desirable to achieve the beneficial effects of HS extract in the treatment of hypertension.

The selection of the most desirable control tea that would not have any effect on the cardiovascular system was a challenge to our study and most of the previous RCTs. While trying to select a tea that would have the same color and similar sour taste like HS tea to ensure blinding of the participants, it is also imperative to choose a tea that lacks the bioactive anthocyanin compounds found in HS tea. We conducted a small preliminary study, by randomizing four participants to a peach control tea, which has a light red color and somewhat sour taste. It was observed that all the tested cardiovascular variables remain unchanged after six weeks of peach tea consumption (Figure 3.4 A-D).

Figure 3.4 A: Comparing systolic blood pressure (SBP), B: Diastolic blood pressure (DBP), C: Aortic augmentation index (AIX), and D: Carotid-femoral pulse wave velocity (cfPWV) before and after peach tea consumption. Results are mean $\pm$ SD.

In figure 3.4 above, there were no changes in all the measured cardiovascular variables after six weeks of drinking peach tea when compared to baseline measurements. Based on these promising preliminary findings, we recommend the use of peach tea as a reliable control in future tea studies. RCTs with an active control protocol would be superior to many of the previous studies on tea that have used a wait-list period in place of randomization into an active control group.

5 Reference Lists

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A. Appendix A- Raw Data

A.1 Participant's Baseline Characteristics

Table A.1: Baseline Demographics and Clinical Characteristics Raw Data

Part.	Age	Sex	Height	Weight	BMI	HR	SBP (seated)	DBP (seated)	SBP (supine)	DBP (supine)
1	26	M	1.7	64.4	22.2	72	124	68	109	63
2	31	M	1.7	78	26.9	71	110	70	116	71
3	35	F	1.6	66.2	26.7	59	130	89	128	87
4	33	F	1.7	75.7	26.2	80	130	80	126	76
5	37	M	1.8	80.2	24.7	59	127	69	129	63
6	39	F	1.7	72.6	25.8	52	123	81	114	68
7	35	M	1.9	93.4	24.4	82	130	75	129	68
8	20	M	1.7	77.1	26.7	57	126	70	126	70
9	55	M	1.82	87.3	26.4	50	118	71	118	71
10	18	M	1.95	130.5	34.3	58	126	66	126	60
11	24	M	1.75	76.4	24.9	67	114	71	114	71
12	27	M	1.9	103	26.9	62	110	68	100	60
13	23	F	1.6	64.9	24.5	58	118	73	128	68
14	27	F	1.7	66.2	22.9	66	137	75	128	64
15	29	M	1.8	110.2	34.9	65	129	80	110	68
16	36	M	1.6	75.7	28.7	70	114	74	114	67
17	22	M	1.73	87.5	29.2	52	122	66	122	66
18	24	M	1.76	82.1	26.4	41	127	53	127	53
19	26	F	1.7	76.2	25.4	76	114	68	117	66

20	26	F	1.7	97.9	33.8	79	127	77	118	68
21	42	F	1.7	98.4	34	88	118	80	122	74
22	25	F	1.7	71.6	24	53	123	71	113	65
23	40	M	1.8	69.3	22.6	45	112	58	103	48
24	20	F	1.7	64.4	21.7	66	129	86	116	76
25	22	F	1.9	63.5	26.4	75	114	73	115	61
26	21	M	1.8	68	20.3	47	111	66	111	56
27	20	M	1.7	68.9	23.8	83	124	73	108	57
28	21	F	1.8	64.5	19.9	65	117	70	110	62
29	22	F	1.7	74.9	25.9	80	124	75	120	71
30	25	F	1.7	76.1	26.3	74	122	71	115	69

Part. -Participants; Age -years; Height (m); Weight (kg); BMI- Body mass index (kg/m^2); SBP- Systolic blood pressure (mmHg); DBP -Diastolic blood pressure (mmHg), HR- Heart rate (beats/minutes)

A.2 Blood Pressure Measurements

Table A.2. Pre and Post Intervention Blood Pressure Readings Raw Data

Participant	Pre- SBP (seated)	Pre- DBP (seated)	Post- SBP (seated)	Post- DBP (seated)	Pre - PP	Post- PP
1	124	68	125	66	56	59
2	110	70	121	67	40	54
3	130	89	121	79	41	42
4	130	80	116	79	51	37
5	127	69	124	68	58	60
6	123	81	120	71	42	49
7	130	75	128	77	55	51
8	126	70	120	70	56	50
9	118	71	112	71	47	41
10	126	66	128	71	60	57
11	114	71	99	62	43	37
12	110	68	116	66	42	50
13	118	73	111	69	45	42
14	137	75	127	67	62	60
15	129	80	126	77	49	49
16	114	74	111	69	40	42
17	122	66	117	58	56	59
18	127	53	120	46	74	74

19	114	68	N/A	N/A	46	N/A
20	127	77	N/A	N/A	50	N/A
21	118	80	N/A	N/A	38	N/A
22	123	71	N/A	N/A	52	N/A
23	112	58	N/A	N/A	54	N/A
24	129	86	N/A	N/A	43	N/A
25	114	73	N/A	N/A	41	N/A
26	111	66	N/A	N/A	45	N/A
27	124	73	N/A	N/A	51	N/A
28	117	70	N/A	N/A	47	N/A
29	124	75	N/A	N/A	49	N/A
30	122	71	N/A	N/A	51	N/A

SBP- Systolic blood pressure (mmHg); DBP- Diastolic blood pressure (mmHg); PP- Pulse pressure; N/A -missing values due to lack of follow-up.

A.3 Central Hemodynamics Measurements

Table A.3. Pre- and Post-intervention Central Hemodynamics Measurements Raw Data

Participant	Pre-SAP	Pre-DAP	Post-SAP	Post-DAP	Pre-AIx@HR75	Post-AIx@HR75	Pre-cfPWV	Post-cfPWV
1	91	64	101	64	2.5	8	5.1	5.2
2	97	65	97	66	11.5	11	5.9	4.6
3	121	88	113	79	23	25	N/A	N/A
4	119	78	114	84	38	31	6.2	6.2
5	105	63	103	65	-13	-10	4.8	6.2
6	N/A	N/A	N/A	N/A	N/A	NA	7.9	6.1
7	104	71	106	67	5.5	6	7.2	6.8
8	102	71	99	71	-	23	5.7	6.6
9	107	72	100	71	13	2	6.2	5.6
10	104	66	104	72	-22	-10	6.5	5.3
11	97	53	86	63	-14	-15	6.2	5.3
12	97	69	91	56	11.5	7	5.2	4.9
13	102	74	88	64	1..0	2	4.8	4.8
14	101	64	110	170	2	5	5.2	5.4
15	95	69	94	66	3.5	0	N/A	NA
16	99	68	100	67	6	9.5	6.1	5.6
17	99	66	92	58	-17	-30	5.6	6.1
18	97	53	95	47	-11	-1	5.8	6.1
19	101	67	NA	NA	NA	NA	NA	NA

20	106	69	NA	NA	NA	NA	NA	NA
21	104	76	NA	NA	NA	NA	NA	NA
22	94	65	NA	NA	NA	NA	NA	NA
23	81	49	NA	NA	NA	NA	NA	NA
24	103	77	NA	NA	NA	NA	NA	NA
25	96	63	NA	NA	NA	NA	NA	NA
26	88	57	NA	NA	NA	NA	NA	NA
27	87	59	NA	NA	NA	NA	NA	NA
28	94	55	NA	NA	NA	NA	NA	NA
29	102	78	NA	NA	NA	NA	NA	NA
30	99	61	NA	NA	NA	NA	NA	NA

SAP -Systolic aortic pressure; DAP- Diastolic aortic pressure; AIX@HR75- Aortic augmentation index in 75beats; cfPWV- Carotid-femoral pulse wave velocity; N/A - missing values due to absence of follow up

A.4. Peach tea Control Raw Data for the Pilot study

Table A.4. Baseline and Post Peach Tea Intervention Raw Data

Participant	Pre-SBP (supine)	Pre-DBP (supine)	Post-SBP (supine)	Post-DBP (supine)	Pre-AIX	Post-AIX	Pre-cfPWV	Post-cfPWV
1	117	66	114	69	13	20	5	5
2	118	68	119	69	24	17	6.6	6.6
3	122	74	127	72	2.5	12.5	5.9	6.2
4	113	65	116	70	8	7	7.1	6.9

SBP-Systolic blood pressure; DBP-Diastolic blood pressure; Aix- Aortic augmentation index, cfPWV- Carotid-femoral pulse wave velocity.

B APPENDIX B- Statistical Analyses

Table B.1 a Descriptive Statistics Of SBP

Treatment	Mean	Std. Deviation	N
Pre_SBP Hibiscus	123.4545	6.74335	11
Camellia_sinensis	122.4286	9.32483	7
Total	123.0556	7.59622	18
Post_SBP Hibiscus	119.4545	8.29896	11
Camellia_sinensis	118.2857	6.47339	7
Total	119.0000	7.45970	18

Table B.1b- Multivariate Tests by 2x2 Repeated-measures ANOVA

Effect	Value	F	Hypothesis df	Error df	Sig.
time Pillai's Trace	.288	6.457b	1.000	16.000	.022
Wilks' Lambda	.712	6.457b	1.000	16.000	.022
Hotelling's Trace	.404	6.457b	1.000	16.000	.022
Roy's Largest Root	.404	6.457b	1.000	16.000	.022
time * Treatment Pillai's Trace	.000	.002b	1.000	16.000	.965
Wilks' Lambda	1.000	.002b	1.000	16.000	.965

Hotelling's Trace	.000	.002b	1.000	16.000	.965
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Table B.1c – Tests of Within-Subjects Effects

Measure: MEASURE_1

Source	Type III Sum of Squares	Df	Mean Square	F
time Sphericity Assumed	141.821	1	141.821	6.457
Greenhouse-Geisser	141.821	1.000	141.821	6.457
Huynh-Feldt	141.821	1.000	141.821	6.457
Lower-bound	141.821	1.000	141.821	6.457
time * Treatment Sphericity Assumed	.044	1	.044	.002
Greenhouse-Geisser	.044	1.000	.044	.002
Huynh-Feldt	.044	1.000	.044	.002
Lower-bound	.044	1.000	.044	.002
Error(time) Sphericity Assumed	351.429	16	21.964	
Greenhouse-Geisser	351.429	16.000	21.964	
Huynh-Feldt	351.429	16.000	21.964	
Lower-bound	351.429	16.000	21.964	

Table B.1d: Test of Between- Subjects Effect

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	500268.081	1	500268.081	5114.010	.000	.997
Treatment	10.303	1	10.303	.105	.750	.007
Error	1565.169	16	97.823			

Table B.2: Descriptive Statistics of DBP

Treatment	Mean	Std. Deviation	N
Pre_DBP Hibiscus	73.6364	6.96093	11
Camellia_sinensis	69.8571	8.74507	7
Total	72.1667	7.68689	18
Post_DBP Hibiscus	71.0000	5.44059	11
Camellia_sinensis	64.5714	9.91392	7
Total	68.5000	7.90569	18

Table B.2a: ANOVA for DBP

Effect	Value	F	Hypothesis df	Error df	Sig.
time Pillai's Trace	.486	15.126b	1.000	16.000	.001
Wilks' Lambda	.514	15.126b	1.000	16.000	.001
Hotelling's Trace	.945	15.126b	1.000	16.000	.001
Roy's Largest Root	.945	15.126b	1.000	16.000	.001
time * Treatment Pillai's Trace	.096	1.692b	1.000	16.000	.212
Wilks' Lambda	.904	1.692b	1.000	16.000	.212
Hotelling's Trace	.106	1.692b	1.000	16.000	.212
Roy's Largest Root	.106	1.692b	1.000	16.000	.212

Table B.2b: Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	166570.759	1	166570.759	1579.684	.000	.990
Treatment	222.870	1	222.870	2.114	.165	.117
Error	1687.130	16	105.446			

Table B.3a- Descriptive Statistics for HR

Treatment	Mean	Std. Deviation	N
Pre_HR Hibiscus	64.2727	10.84519	11
Camellia_sinensis	59.1429	9.90671	7
Total	62.2778	10.50941	18
Post_HR Hibiscus	58.0000	9.02219	11
Camellia_sinensis	57.7143	11.77164	7
Total	57.8889	9.83923	18

Table B.3b -ANOVA test for HR

Effect	Partial Eta Squared	Noncent. Parameter	Observed Powerc
time Pillai's Trace	.266	5.800	.619
Wilks' Lambda	.266	5.800	.619
Hotelling's Trace	.266	5.800	.619
Roy's Largest Root	.266	5.800	.619
time * Treatment Pillai's Trace	.125	2.295	.297
Wilks' Lambda	.125	2.295	.297
Hotelling's Trace	.125	2.295	.297
Roy's Largest Root	.125	2.295	.297

Table B.3c -Tests of Between Subjects Effect

Effect	Partial Eta Squared	Noncent. Parameter	Observed Power ^c
time Pillai's Trace	.266	5.800	.619
Wilks' Lambda	.266	5.800	.619
Hotelling's Trace	.266	5.800	.619
Roy's Largest Root	.266	5.800	.619
time * Treatment Pillai's Trace	.125	2.295	.297
Wilks' Lambda	.125	2.295	.297
Hotelling's Trace	.125	2.295	.297
Roy's Largest Root	.125	2.295	.297

Table B.3d -Tests of Within-Subjects Effect

Source	Type III Sum of Squares	df	Mean Square	F
time Sphericity Assumed	126.858	1	126.858	5.800
Greenhouse-Geisser	126.858	1.000	126.858	5.800
Huynh-Feldt	126.858	1.000	126.858	5.800
Lower-bound	126.858	1.000	126.858	5.800
time * Treatment Sphericity Assumed	50.191	1	50.191	2.295
Greenhouse-Geisser	50.191	1.000	50.191	2.295

Huynh-Feldt	50.191	1.000	50.191	2.295
Lower-bound	50.191	1.000	50.191	2.295
Error(time) Sphericity Assumed	349.948	16	21.872	
Greenhouse-Geisser	349.948	16.000	21.872	
Huynh-Feldt	349.948	16.000	21.872	
Lower-bound	349.948	16.000	21.872	

Table B.4a -Descriptive Statistics for Carotid-femoral Pulse wave velocity (cfPWV)

Treatment	Mean	Std. Deviation	N
Pre_cfPWV Hibiscus	6.1700	.91171	10
Camellia_sinensis	5.4500	.47223	6
Total	5.9000	.83825	16
Post_cfPWV Hibiscus	5.7900	.69833	10
Camellia_sinensis	5.4833	.56362	6
Total	5.6750	.64962	16

Table B.4a – ANOVA Test for cfPWV

Two by two -repeated measures ANOVA
 for testing the time effect and time by treatment group
 effect for the carotid-femoral pulse wave velocity

Effect		Value	F	Hypothesis df	Error df	Sig.
time	Pillai's Trace	.044	.647b	1.000	14.000	.435
	Wilks' Lambda	.956	.647b	1.000	14.000	.435
	Hotelling's Trace	.046	.647b	1.000	14.000	.435
	Roy's Largest Root	.046	.647b	1.000	14.000	.435
time * Treatment	Pillai's Trace	.062	.920b	1.000	14.000	.354
	Wilks' Lambda	.938	.920b	1.000	14.000	.354
	Hotelling's Trace	.066	.920b	1.000	14.000	.354
	Roy's Largest Root	.066	.920b	1.000	14.000	.354

Table B.4b – Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F
time	Sphericity Assumed	.225	1	.225	.647
	Greenhouse-Geisser	.225	1.000	.225	.647
	Huynh-Feldt	.225	1.000	.225	.647

Lower-bound	.225	1.000	.225	.647
time * Treatment Sphericity Assumed	.320	1	.320	.920
Greenhouse-Geisser	.320	1.000	.320	.920
Huynh-Feldt	.320	1.000	.320	.920
Lower-bound	.320	1.000	.320	.920
Error(time) Sphericity Assumed	4.875	14	.348	
Greenhouse-Geisser	4.875	14.000	.348	
Huynh-Feldt	4.875	14.000	.348	
Lower-bound	4.875	14.000	.348	

Table B.5c – Tests of Within-Subjects Contrast

Source time	Type III Sum of Squares	df	Mean Square	F	Sig.
time Linear	.225	1	.225	.647	.435
time * Treatment Linear	.320	1	.320	.920	.354
Error(time) Linear	4.875	14	.348		

Table B.5d – Tests of Between-Subjects Effects

Table showing the time effect and time by treatment group effect between the subjects

Source time	Type III Sum of Squares	df	Mean Square	F	Sig.
time Linear	.225	1	.225	.647	.435
time * Treatment Linear	.320	1	.320	.920	.354
Error(time) Linear	4.875	14	.348		