

Advances in the mechanisms of *Hibiscus sabdariffa* L. on hypertension

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Abstract. As a traditional edible beverage and medicinal application for high-pressure blood treatment with no side effects, *Hibiscus sabdariffa* L. (HS) has high prospect to be nutraceuticals as diet additive for anti-hypertension. The anti-hypertension effect of HS has also been scientific validated recently especially in diabetic patient and post-partum mothers. In this paper, we reviewed up-to-date mechanisms found for the anti-hypertensive effect of HS extracts. Those included the inhibition of angiotensin-converting enzymes (ACE) activity, diuretic effect, endothelium-derived nitric oxide-cGMP-relaxant pathway and inhibition of calcium (Ca²⁺)-influx into vascular smooth muscle cells, cholinergic and/or histaminergic mechanisms, reduction in the diffusion distance between capillaries and myocytes as well as new vessel formation. Phenolic acids, anthocyanins (e.g. cyanidin-3-sambubioside and delphinidin-3-sambubioside) and anthocyanin metabolites probably contribute mostly to the hypotensive activity.

1. Introduction

The *Hibiscus* genus consists of more than 300 species of annual or perennial herbs, shrubs or trees. Among which, two species, i.e. *H. cannabinus* var. HC-2 ($2n=2x=36$) and *H. sabdariffa* var. HS-24 ($2n=4x=72$), are considered to be the most economically important [1].

In Mexico, HS is widely used in beverages for culinary and medicinal usage. Its decoctions and infusions of calyces, and occasionally leaves, are used extensively in the treatment of hypertension with no reported adverse events or side effects.

The individuals suffering from hypertension is hitherto estimated to be 1 billion all over the world. As one of the top ten factors contributing to the global burden of disease, hypertension cause 13% of total death worldwide per year up to 7.1 million [2]. Anthocyanins and phenolic acids, in the aqueous infusion of the HS calyces, may be the bioactive compounds responsible for reducing the blood pressure. As a traditional edible beverage and dietotherapy for high-pressure blood treatment with no side effects, product of HS has high prospect to be nutraceuticals as diet additive for anti-hypertension [3].

In this paper, we reviewed general activities of HS products and up-to-date mechanisms found for the anti-hypertensive effect of HS extracts.

2. Bioactive constituents of HS

Four constitution categories namely organic acids, anthocyanins, polysaccharides and flavonoids contribute to pharmacological effect of HS.

Organic acids are found in high relative quantities in HS extracts, including citric acid, hydroxycitric acid, hibiscus acid, malic and tartaric acids as major compounds, and oxalic and ascorbic acid as minor compounds. Among those, hibiscus acid has been reported to be a potential bioactive constituent contributes to lowering blood pressure [4]. Anthocyanins belong to the widespread class of phenolic compounds collectively named flavonoids. They are abundant in HS flower and responsible for its red color [5]. Anthocyanins, particularly delphinidin-3-sambubioside and cyanidin-3-sambubioside, are regarded as important nutraceuticals since they play a potential role in the prevention of various diseases associated with oxidative stress [6-9]. Early reports have proved that polyphenol-rich foods could protect and improve endothelial function with vascular relaxation through the NO-cGMP pathway and ACE inhibition, resulting in a decrease of blood pressure [10-11]. Edwards et al. [3] found that anthocyanin metabolites reduced superoxide production and improved NO bioavailability by modulating vascular reactivity via inducing HO-1 and regulating NOX activity. A series of studies [12] [13-15] demonstrated that quercetin glycosides could be a potential ligand for remedy of hypertension and congestive heart failure. Quercetin could reduce blood pressure in spontaneously hypertensive rats [16] via inhibition of ACE activity and improvement of endothelial function [17]. The inhibition of ACE by tannins was also well established [4, 18-20].

HS extracts have a low degree of toxicity with a LD50 ranging from 2,000 to over 5,000 mg/kg/day. After taking HS extract, there is no evidence of hepatic or renal toxicity except for possible adverse hepatic effects at high doses [21].

3. Anti-hypertensive efficacy of HS

Hypertension is a major cardiovascular risk factor with a significant magnitude of morbidity and mortality. Dietary adjustment is one of the most effective ways for regulating high blood pressure. Dietary intakes of polyphenol-rich foods, herbs and beverages could improve vascular health, and subsequently significantly reduce the risk of hypertension and cerebrovascular disease [22]. HS is rich in organic acids, polyphenols, anthocyanins, polysaccharides that are beneficial to the cardiovascular system.

Daily consumption of 500 mg of HS calyx powder could reduce systolic blood pressures and serum triglycerides in metabolic syndrome patients [17]. Another clinical trial showed that the experimental treatment decreased blood pressure from 146.48/97.77 to 129.89/85.96 mmHg, reaching an absolute reduction of 17.14/11.97 mmHg by using HS calyx watery infusions [6]. Notably, this experimental treatment showed a therapeutic effectiveness of 65.12% and a tolerability and safety up to 100%. Mozaffari-Khosravi *et al.* [23] evaluated the antihypertensive effect of HS in diabetic patients through a double blind, randomized trial. After administering the tea contain HS twice per day for 1 month, systolic blood pressure was measured. The mean systolic blood pressure decreased from 134 to 112.7 mmHg after the experimental duration. Nikmah *et al.* [24] reported that HS had significant effect in the treatment of hypertension in postpartum mothers and benefited the healing process more quickly through a combination with antihypertensive medicines.

Considering its wide usage for high-pressure blood treatment as a traditional edible beverage and medicinal application and with no side effects, HS have got a great chance to be nutraceuticals as diet additive for anti-hypertension [25].

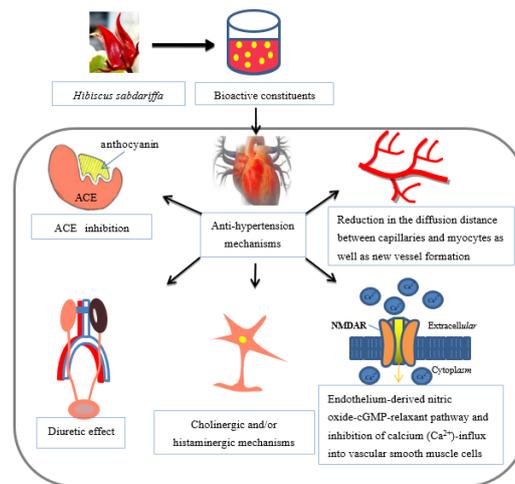


Figure 1. Mechanisms of *Hibiscus sabdariffa* L. in the treatment of hypertension.

4. Antioxidant and anti-inflammatory activities of HS

The most popular explanation for HS's hypertensive effect is that the antioxidant effects of the anthocyanins would inhibit LDL-C oxidation, which impedes atherosclerosis, an important cardiovascular risk factor.

HS contains high-level antioxidant substrates [26] and the antioxidant effects were observed in the extract of HS from calyces, leaves, as well as seeds [15, 27]. There is a significant positive correlation between total phenol content and antioxidant capacity and the highest antioxidant capacity extracts involves high amount of cyanidin 3-sambubioside or delphinidin 3-sambubioside [28-29].

The combination of oxidation, inflammation, and endothelial dysfunction has an interrelated mechanism in the pathogenesis of hypertension [30]. The calyx extract had an anti-inflammatory activity for it was capable to inhibit enzymes cyclooxygenase both COX-1 and COX-2, and down regulate the expression of COX-2 in RAW 264.7 cells treated with lipopolysaccharide [16]. Further, Kao *et al.* [31] found that polyphenols extracted from HS inhibited lipopolysaccharide-induced inflammation by improving antioxidative conditions and regulating cyclooxygenase-2 expression.

5. Mechanisms in the treatment of hypertension by HS

The pathogenesis of hypertension has been extensively studied for decades and factors are involved in body and blood pressure regulations, including the sympathetic nervous system hyperactivity[32], renin-angiotensin-aldosterone system (RAAS) activation, vascular endothelial and smooth muscle cell dysfunction [33]. However, the pathogenic mechanism has not yet been fully illuminated. Among those well-known factors, the top two are water and sodium retention in the vascular compartment and a raise in angiotensin II (Ang II) production and activity by increasing the plasmatic ACE activity [34]. Ang II is able to promote the formation of vascular remodeling of hypertension and accelerates the growth and development of vascular smooth muscle cells by stimulating the proliferation of collagen fibers and the deposition of extracellular matrix by several growth factors or inflammatory mediators [35]. Rather than taking medicine, more and more people tend to reduce the risk of hypertension by taking healthy diet. Herrera *et al.* [7] found that hypertensive patients who drank 10 grams of HS petals daily could control mild to moderate hypertension as effectively as captopril. Similarly, Nikmah *et al.* [24] suggested that the combination of antihypertensive medicines and HS petals could help the healing process more quickly. As we have stated above, HS is rich in phenolic acids and anthocyanins (esp. delphinidin-3-sambubioside and cyanidin-3-sambubioside), which can decrease the blood pressure effectively and are beneficial to cardiovascular.

5.1 Inhibition of ACE activity

RAAS could control the blood pressure through the homeostatic control system [36]. Dysregulation of some components of RAAS, such as ACE in essential hypertension, has been reported previously [18, 37].

ACE is a zinc-containing peptidyl dipeptide hydrolase. The active site of ACE is composed of three parts including a carboxylate binding functionality such as the guanidinium group of Arg, a pocket that accommodates a hydrophobic side chain of C-terminal amino acid residues, and a zinc ion [12].

Flavonoids have been confirmed to inhibit the ACE in certain previous literatures [14, 38-39]. The inhibition of ACE activity by flavonoids and other polyphenols is largely due to the formation of chelate complexes with the zinc atom within the active center of zinc-dependent metalloproteinases [40] and the formation of hydrogen bridges between the inhibitor and amino acids near the active site [39, 41]. Ojeda D *et al.* indicated that delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside characterized from the active aqueous extract of HS was able to inhibit the enzyme activity by competing with the substrate for the active site [12]. The rigid planar structure of delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside and the presence of ortho-dihydroxylation on the aromatic ring probably contributed to the inhibition of ACE [12]. Another clinical trial, conducted on 193 patients with hypertension stages I and II, carried out by Herrera-Arellano *et al.* also confirmed the inhibition of ACE activity through using watery infusions of HS [42]. The trial showed that HS calyxes could significantly reduce plasma ACE activity. Specifically, ACE plasmatic activity was inhibited by HS calyxes range from 44.049 to 30.1 Units (Us; $p = 0.0001$). Moreover, HS calyxes also exerted important antihypertensive effectiveness with a wide margin of tolerability and safety.

In a double-blind controlled randomized clinical study conducted with hypertensive to investigate effects of aqueous extract of HS on the RAAS, Placebo (150 mg/kg/day), lisinopril (10 mg once daily) and aqueous extract of HS (150 mg/kg/day) were given respectively for 4 weeks [43]. The results showed that HS reduced serum ACE and plasma aldosterone in patients with equal efficacy as lisinopril. Apart from ACE inhibition, HS exerts antihypertensive action may also through additional mechanisms including direct blockage of angiotensin II type 1 receptor binding to Ang II and high Mg^{2+} content in HS contributed to the decreased secretion of aldosterone.

5.2 Regulating serum electrolyte by diuretic effect

The diuretic effect of HS has been characterized pharmacologically both in clinical trials [42] and in pre-clinical experiments in rats [10, 44].

A randomized, double-blind, lisinopril-controlled clinical trial on 193 patients with hypertension stages I and II was conducted after consumption of watery infusions to determine its effect in the treatment of hypertension [42]. The authors found that the serum chlorine level increased from 91.71 to 95.13 mmol/L ($p = 0.0001$), the sodium level decreased from 139.09 to 137.35 mmol/L ($p = 0.07$), while the potassium level was stable under the experimental treatment from HS calyx watery infusions. All these information demonstrated a diuretic effect (probably as an aldosterone antagonist) of HS extracts.

Paradoxically, diuresis mattered less in the antihypertensive action of HS in the established stages of 2K-1C hypertension [10].

5.3 Vasoactivity through NO-relaxant pathway and Ca^{2+} influx

The normal tension of blood vessels cannot be maintained in patients suffered from hypertension ground that endothelial dysfunction will restrain the ability of blood vessels to produce and release endothelium-derived systolic and diastolic factors [45]. NO is essential for endothelial cells to perform their normal physiological functions and decrease in biological activity of NO can result in functional impairment of vascular endothelium [46-49]. Ajay *et al.* [19] examined the effects of a crude methanolic extract of the calyces of HS on vascular reactivity in isolated aortas from spontaneously hypertensive rats and found that HS extracts had a vasodilator effect in the isolated

aortic rings of hypertensive rats. These effects were probably mediated through the endothelium-derived NO-cGMP-relaxant pathway and inhibition of calcium (Ca^{2+})-influx into vascular smooth muscle cells. HS induced relaxation of vascular smooth muscle occurred via endothelium-dependent and -independent mechanisms [50]. The endothelium-dependent vasodilator effect was associated with activation of endothelium-derived NO-relaxant pathway and to the contrast the endothelium-independent effect was related to inhibition of Ca^{2+} influx by the action of quercetin and eugenol present in the HS [51].

5.4 Cholinergic and/or histaminergic mechanisms

Cholinergic and histaminergic mechanisms are closely related to cardiovascular activity and the pathogenesis of hypertension [11, 52-53]. Adegunloye *et al.* [54] investigated the antihypertensive effect of aqueous extracts from HS calyx in anaesthetized rats, from which a dose-dependent decrease in mean arterial pressure in rats was observed. Another antihypertensive mechanism of HS below the results inferred a direct vasorelaxant effect but it was not mediated through inhibition of the sympathetic nervous system. The direct vasorelaxant effect perhaps mediated through cholinergic or histaminergic mechanisms that were produced by membrane stabilization and stimulation of vascular $\text{Na}^+ - \text{K}^+$ ATPase activity and inhibition of Ca^{2+} release from intracellular stores.

5.5 Reformation of capillaries, myocytes and vessel

The effect of the water extract from the dried calyx of HS on left ventricular myocardial capillary length and surface area in spontaneously hypertensive rats had been investigated by Inuwa and Ali [13]. Spontaneously hypertensive rats were given to three different doses of the water extract of HS in lieu of drinking water for 10 consecutive weeks. The results showed that HS ingestion significantly reduced systolic blood pressure, diastolic pressure and left ventricles mass in a dose-dependent fashion but did not affect the heart rate. HS significantly increased surface area and length density of myocardial capillaries by 59%, 65% and 86%, and length density by 57%, 77% and 57%, respectively. Myocyte nuclear volume was significantly decreased in HS-treated rats. These changes suggested that the observed beneficial effect of HS on high blood pressure in spontaneously hypertensive rats could be mediated through a reduction in the diffusion distance between capillaries and myocytes, as well as new vessel formation.

6. Conclusion

Previous phytochemical studies on HS have reported the presence of a bunch of potential bioactive constituents including phenolics, organic acids, sterols, terpenoids, polysaccharides and some minerals. Notably, the phenolics in HS mainly consists of anthocyanins, *e.g.* delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside.

In the last decade, the relationship between oxidative stress and hypertensive renal injury has attracted more and more attentions. Hypertension would stimulate myocardium to produce oxidative stress, resulting in increased reactive oxygen species, thus leading to a decrease in myocardial antioxidant capacity. Myocardial oxidative stress could activate proteins NF- κ B family and enhance the expression. Proinflammatory cytokines can increase the accumulation of neutrophils in the capillary, thus increase vascular resistance, resulting in vascular injury. They also damage the structure and function of vascular endothelial cells and make these endothelial cells produce vasodilator substances such as NO reduction, leading to blood vessel contraction and an elevated blood pressure.

HS exert important antihypertensive effectiveness with a wide margin of tolerability and safety. Flavonoids and other polyphenols in HS exert ACE inhibitory activity probably through the formation of chelate complexes with the zinc atom within the active center of zinc-dependent metalloproteinases and the formation of hydrogen bridges between the inhibitor and amino acids near the active site. Delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside characterized from the active aqueous extract of HS could inhibit the enzyme activity by competing with the substrate for the active site. Although diuretic effect of HS has been proved both in clinical trials and in pre-clinical experiments in

rats, it serves as an important anti-hypertension role is still controversy. The effects of a crude methanolic extract from HS calyces on vascular reactivity in isolated aortas from spontaneously hypertensive rats have been studied and the results show that HS extracts has a vasodilator effect in the isolated aortic rings of hypertensive rats. These effects may be mediated through the endothelium-derived NO-cGMP-relaxant pathway and inhibition of calcium (Ca^{2+})-influx into vascular smooth muscle cells. While Adegunloye and Omoniyi et al. suggests that the mechanism of the antihypertensive action of HS may be responsible to direct vasorelaxant effect. This effect may be mediated through cholinergic or histaminergic mechanisms which are produced by membrane stabilization and stimulation of vascular $\text{Na}^+ - \text{K}^+$ ATPase activity and inhibition of Ca^{2+} release from intracellular stores, but is not mediated through inhibition of the sympathetic nervous system. In addition, Inuwa *et al.* [13] found that a reduction in the diffusion distance between capillaries and myocytes, as well as new vessel formation after taking the water extract from the dried calyx of HS in spontaneously hypertensive rats, raising another mechanism for anti-hypertension effect of HS. All these mechanisms of Hibiscus sabdariffa L. in the treatment of hypertension concerned above are illustrated in figure 1.

Comprehensive body of evidence proves up to the hilt that extracts of HS is a promising treatment for hypertension. However more high quality animal and human studies inspired by actual clinical practices are in need for further insight of underlying therapeutic mechanisms of HS extracts.

7. Future directions and scope in anti-hypertension application and in food industry

As a traditional beverage, infusion of calyces or leaves of HS shows its efficacy in anti-hypertension with rare degree of toxicity. HS are promising as a treatment of hypertension, however more researches should be focused on following topics for its better use in anti-hypertension application and in food industry.

a. Most reported mechanisms of anti-hypertension of HS confirmed the inhibition of ACE, while scarce research illustrated clearly other mechanisms that also significantly related. The researches focusing on the interaction between different mechanisms is yet blank.

b. More high quality animal and human studies informed by actual therapeutic practices are needed to provide recommendations for use that have the potential for widespread public health benefit.

c. Despite of anthocyanins, the chemicals that also play role in the anti-hypertension, should be defined by in vivo or vitro experiment with pure chemicals. And the interaction effect between these bioactive constituents should be explored.

d. Clinical data for the comparison of anti-hypertension effect between HS and common hypotensor is in need.

Notes: Mechanisms of Hibiscus sabdariffa L. in the treatment of hypertension include the inhibition of ACE activity, diuretic effect, endothelium-derived nitric oxide-cGMP-relaxant pathway and inhibition of calcium (Ca^{2+})-influx into vascular smooth muscle cells, cholinergic and/or histaminergic mechanisms, reduction in the diffusion distance between capillaries and myocytes as well as new vessel formation.

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