

## REVIEW ARTICLE

### Therapeutic potential of *Hibiscus sabdariffa*: A review of the scientific evidence<sup>☆</sup>



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#### KEYWORDS

*Hibiscus sabdariffa*;  
Oxidative stress;  
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Hypertension;  
Atherosclerosis;  
Lipid profile

#### Abstract

**Background and objective:** Infusion of *Hibiscus sabdariffa* (*H. sabdariffa*) is a very popular drink in many parts of the world. Its phytochemical composition is associated to antioxidant, hypotensive, and antiatherosclerotic effects. However, the molecular mechanisms involved in these processes are not well known. The aim of this review was to report the scientific evidence supporting that regular use of *H. sabdariffa* decreases oxidative stress, atherosclerosis, lipid profile, and blood pressure.

**Materials and methods:** A search of recent publications was made in the following specialized electronic databases: Elsevier Journal, SciELO, FSTA, Science Direct, Springer Link, and NCBI. Results of research conducted in clinical trials in humans and in animal models and cell cultures were recorded. Keywords used included *H. sabdariffa*, oxidative stress, polyphenols, hypertension, atherosclerosis, and lipid profile.

**Results:** Results of the different articles suggested a possible therapeutic effect of *H. sabdariffa* extracts on oxidative stress, lipid profile, hypertension, and atherosclerosis thanks to its composition rich in phenolic compounds. Anthocyanins significantly decrease LDL oxidation, inhibit adipogenesis by regulating adipogenic signaling pathways and transcription factors, and modulate gene expression of certain microRNAs. No adverse events or side effects were reported.

**Conclusions:** Further more homogeneous, placebo-controlled studies in humans are needed to state that *H. sabdariffa* has therapeutic efficacy in humans.

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**PALABRAS CLAVE**

*Hibiscus sabdariffa*;  
Estrés oxidativo;  
Polifenoles;  
Hipertensión;  
Aterosclerosis;  
Perfil lipídico

**Potencial terapéutico del *Hibiscus sabdariffa*: una revisión de las evidencias científicas****Resumen**

**Antecedentes y objetivo:** La infusión de *Hibiscus sabdariffa* (*H. sabdariffa*) es una bebida muy popular en muchos lugares del mundo. Su composición fitoquímica se asocia a efectos antioxidantes, hipotensores y antiateroscleróticos. No obstante, no se conocen con profundidad los mecanismos moleculares implicados en estos procesos. El objetivo de la presente revisión fue describir las evidencias científicas que apoyan que el consumo regular de *H. sabdariffa* reduce el estrés oxidativo, la aterosclerosis, el perfil lipídico y la tensión arterial.

**Material y métodos:** Se realizó una búsqueda de publicaciones recientes en las siguientes bases de datos electrónicas especializadas: Elsevier Journal, SciELO, FSTA, Science Direct, Springer Link y NCBI. Se describieron los resultados de trabajos llevados a cabo en ensayos clínicos en humanos, modelos animales y cultivos celulares. Las palabras clave utilizadas fueron *Hibiscus sabdariffa*, estrés oxidativo, polifenoles, hipertensión, aterosclerosis y perfil lipídico.

**Resultados:** Los resultados de los diferentes artículos evidenciaron un posible efecto terapéutico de los extractos de *H. sabdariffa* sobre el estrés oxidativo, el perfil lipídico, la hipertensión y la aterosclerosis, gracias a su composición rica en compuestos fenólicos. Las antocianinas reducen significativamente la oxidación de la lipoproteína LDL, inhiben la adipogénesis mediante la regulación de las vías de señalización adipogénicas y factores transcripcionales, y modulan la expresión génica de determinados microARN. No se comunicaron acontecimientos adversos ni efectos secundarios.

**Conclusiones:** Son necesarios más estudios en humanos, estudios más homogéneos y controlados con placebo, para poder aseverar que *H. sabdariffa* posee eficacia terapéutica en humanos.

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**Introduction**

Highly reactive free radicals (OFRs) are atoms or atom groups with an unpaired or free electron. To achieve electrochemical stability, OFRs start a chain reaction that may damage biological macromolecules such as lipids, proteins, carbohydrates, and nucleic acids, and imbalance body homeostasis.<sup>1</sup> Most OFRs result from normal cell metabolism.<sup>2</sup> However, OFR production may also increase due to the metabolism of some exogenous substances, exposure to sun rays or ionizing radiation, pesticides, and heavy metals.<sup>3</sup> Other factors affecting OFR production are associated with exposure to the action of some xenobiotics (chloroform, acetaminophen, carbon tetrachloride), tobacco smoke, or inadequate diet, either as excess harmful substances or as deficient antioxidants.<sup>3</sup> All of these factors may cause excess OFRs in cells and increase susceptibility to the occurrence of pathological conditions such as cancer, cell aging, atherosclerosis, high blood pressure (HBP) or hyperlipidemia.<sup>1,4</sup>

HBP is a very important and common cardiovascular risk factor in modern society.<sup>5,6</sup> In fact, HBP is one of the main risk factors for the development of cardiovascular disease together with smoking, dyslipidemia and, especially, high plasma levels of low density lipoprotein (LDL) cholesterol.<sup>7-11</sup> To these should be added other predisposing risk factors, such as obesity and sedentary lifestyles, which exert their action through intermediate, causative or conditional risk factors.<sup>8,9</sup> Cardiovascular diseases are the leading cause of death in Spain, and are responsible for almost 40% of all deaths.<sup>12</sup> The mechanism starting HBP is unknown in 90% of cases, but there is evidence which appears to

suggest that increased OFR production is related to its pathogenesis.<sup>13-15</sup> In fact, it has been noted that individuals with HBP may experience an increase in blood levels of thiobarbituric acid, a marker of lipid peroxidation, and a reduction in antioxidant activities of the enzymes superoxide dismutase, glutathione peroxidase, and catalase.<sup>16,17</sup> Moreover, Ward et al.<sup>18</sup> found a decrease in non-enzymatic antioxidants, such as vitamin E and reduced glutathione, in patients with HBP. The pathogenesis of HBP has also been related to metabolic abnormalities,<sup>19-21</sup> hormonal factors,<sup>22</sup> and genetic changes. Specifically, it has been estimated from epidemiological and familial studies that the genetic component could cause approximately 40% of interindividual variability in HBP values.<sup>23</sup> The central role of lipid metabolism in the homeostasis of hypertension justifies the extensive analysis made of the genetic varieties of genes that encode for proteins of this system. Maximum attention has been paid to polymorphisms related to functional changes in proteins encoding, for example, polymorphisms in apolipoprotein B<sup>24</sup> and A5,<sup>24</sup> CD36 (cluster of differentiation 36),<sup>25</sup> USF1 (upstream transcription factor 1),<sup>26,27</sup> FADS3 (fatty acid desaturase 3),<sup>27</sup> and GCKR genes (glucokinase regulatory protein).<sup>24</sup> Ischemic heart disease and cerebrovascular disease or stroke are manifestations of atherosclerosis.<sup>28</sup> The characteristic lesion is the atheroma plaque, consisting of lipid, fibrous tissue, and immune system cells. One of the earliest events in atherosclerosis is LDL accumulation in the arterial wall. One of the most widely accepted hypotheses to explain the development of atherosclerosis is slow oxidation of LDL trapped in the subendothelial space by the action of OFRs generated by vascular cells, demonstrating a close relationship between

OFRs and LDL.<sup>29</sup> The accumulation of lipoproteins in arterial endothelium triggers the activation of macrophage scavenger receptors, leading to the conversion of macrophages into foam cells. Progressive foam cell accumulation contributes to lesion progression.<sup>30</sup>

Plants and animals have endogenous antioxidant systems to remove excess production of OFRs, such as glutathione, vitamin D and vitamin E, catalase, superoxide dismutase, and several peroxidases.<sup>31</sup> Glutathione peroxidase, superoxide dismutase, or the catalase in peroxisomes are endogenous or primary antioxidant enzymes able to metabolize OFRs generated in cellular redox processes. By contrast, alpha lipoic acid, vitamins C, E and A, and polyphenols are considered non-enzymatic or secondary antioxidants able to directly destroy OFRs.<sup>32</sup>

Polyphenols are a very numerous and heterogeneous group of molecules which share the characteristic of having several phenol groups in their structures. Many epidemiological studies support the antioxidant properties of polyphenols,<sup>33–36</sup> although their antioxidant capacity depends on their bioavailability and absorption. In turn, this is greatly affected by several factors such as climate, type of soil, type of culture, and sun exposure, amongst others.<sup>37</sup> Most polyphenols are metabolized by colonic microorganisms before being absorbed, and the products resulting from this fermentation are partly responsible for their systemic effects.<sup>38–42</sup> The antioxidant capacity of polyphenols accounts for their vasodilating, antithrombotic, anti-inflammatory, and antiapoptotic actions,<sup>43</sup> and also for their antilipidemic<sup>44,45</sup> and antiatherogenic properties.<sup>46</sup> The antioxidant activity of polyphenols is tenfold higher than that of vitamin A and 100-fold higher than that of vitamin E or carotenoids.<sup>47</sup> More specifically, there are studies suggesting that phenolic compounds may attenuate the oxidation of LDL and high density lipoproteins (HDL).<sup>48–53</sup> In healthy humans, it has been suggested that resveratrol, one of the main phenolic compounds in wine, could prevent the oxidation of LDL and decrease lipid hydroperoxide levels.<sup>54</sup> Recently, Castaner et al.<sup>48</sup> showed that phenolic compounds in olive oil could decrease LDL oxidation and the expression of the CD40L (CD40 ligand) gene, as well as genes related to inflammation processes in humans. The molecular processes associated with the antilipidemic and antiatherogenic properties of polyphenols result from their ability to regulate the expression of different genes associated with the immune system and energy metabolism, and/or from their epigenetic regulation capacity<sup>55</sup> through the induction of changes in the methylation pattern of DNA CpG islands,<sup>56,57</sup> histone acetylation,<sup>58</sup> and modulating the expression of some miRNAs.<sup>59</sup> In this regard, for example, quercetin, the active component of *Hibiscus sabdariffa* (*H. sabdariffa*), has been reported to inhibit the activity of histone acetyltransferase in the promoter region of genes associated with the manifestation of inflammation.<sup>58</sup> Joven et al.<sup>60</sup> used hyperlipidemic mice with LDL receptor deficiency to assess the role of polyphenols in the prevention of liver disease by regulating the expression of hepatic microRNAs miR103/107 and miR122. In their results, these authors emphasized that the oral administration of polyphenols reverted changes induced in non-specific microRNAs miR103/107 after chronic polyphenol intake, and the lack of response of the specific miRNA miR122, speculating about the potential implication

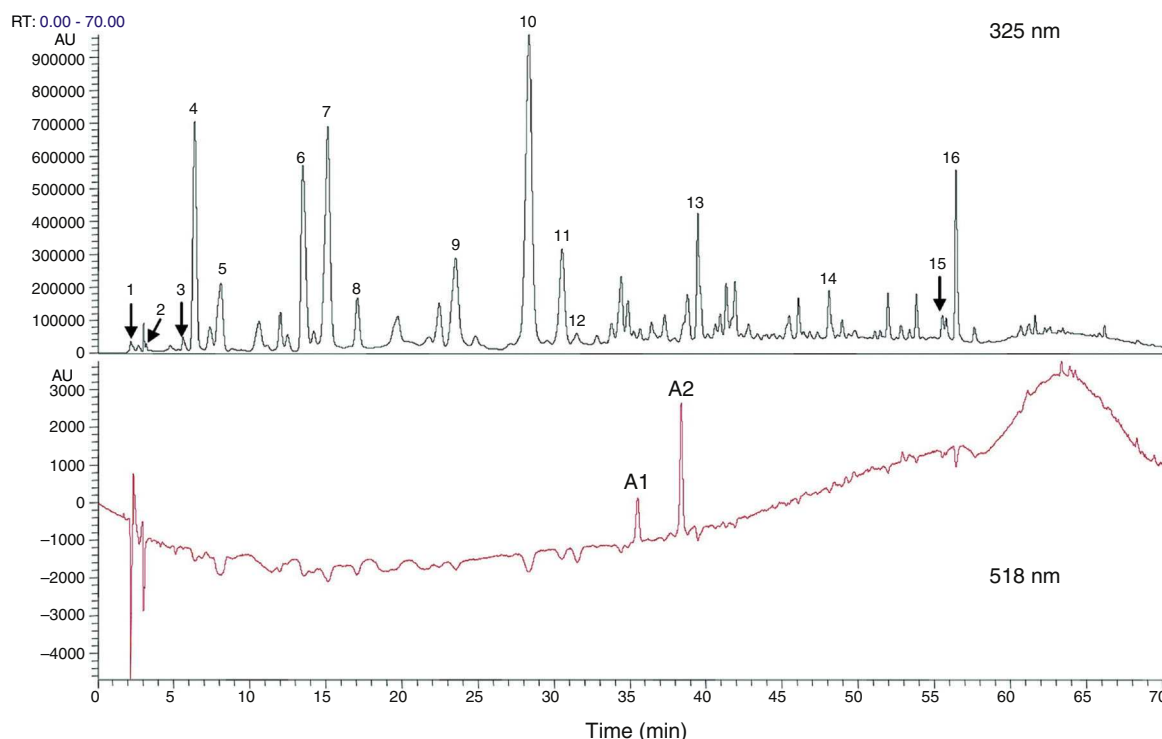
of polyphenols in cell metabolism. The authors reported that modulation of microRNA expression may be a significant and additional intervention mechanism in chronic diseases. Crozier et al.<sup>61</sup> have shown that polyphenolic extract-miRNA specificity may exist, given the potential variety of different structures and compositions of extracts, depending on their botanical origin. Additional studies in humans are however needed to clarify the epigenetic effects of polyphenols.

*H. sabdariffa* has a high polyphenol content.<sup>47</sup> It is also called Jamaica sorrel, roselle, or karkade, and belongs to the malvaceae family native to tropical Africa, although it is cultivated in Mexico, Central America, and south-east Asia. It is an annual herbaceous plant living in dry, subtropical, mountain climates. Its calyces, fleshy and of a vivid red color, have high concentrations of L-ascorbic, arachidic, citric, stearic, and malic acids, in addition to pectins, phytosterols (e.g.  $\beta$ -sitosterol and ergosterol) and polyphenols.<sup>62</sup> Peng et al.,<sup>63</sup> in their research to assess the hypoglycemic and hypolipidemic effect of the polyphenolic extract of *H. sabdariffa*, found at least 18 different phenolic compounds in *H. sabdariffa* (Fig. 1). Commercial preparations of concentrates of calyces, and occasionally leaves, of *H. sabdariffa* are very commonly found as fluid or powder for the preparation of instant drinks or infusions.<sup>62</sup> In addition, its widespread use as a herbal treatment in popular medicine has led to a high acceptance rate by the general population,<sup>64</sup> especially in countries such as the United States,<sup>65</sup> Mexico,<sup>62,66,67</sup> some eastern African countries,<sup>68–70</sup> Iran,<sup>71,72</sup> India,<sup>73</sup> Taiwan,<sup>63</sup> Brazil,<sup>74</sup> and Greece.<sup>75</sup> The ethnobotanical studies available usually describe the origin and parts of the plant used, the properties attributed to it and the form of preparation, without specifying the dosage. No demographic studies supporting the role of the plant in disease prevention have been found.<sup>74,76</sup> On the other hand, scientific studies have shown that the antioxidant effects of polyphenols in *H. sabdariffa* have an antiatherogenic action and decrease hypertension and hyperlipidemia with no reported adverse events or side effects in animals and humans.<sup>65–67,72,77–79</sup>

The purpose of this paper was therefore to collect and unify the evidence showing that regular consumption of *H. sabdariffa* may have a beneficial effect on human health because of the antioxidant, antihypertensive, and lipid-lowering effects of its phenolic components.

## Materials and methods

In this review, a search for recent publications was made in the following specialized electronic databases: NCBI, Elsevier Journal Finder, SciELO, ScienceDirect and Springer-Link. Results from studies conducted *in vitro*, in animal models and in humans were collected. Reviews collecting and analyzing the effectiveness of *H. sabdariffa* in given treatments, such as antihypertensive and lipid-lowering therapies, amongst others, and articles referring to the phytochemical, pharmacological, and toxicological aspects of *H. sabdariffa* were also included. The concepts of oxidative stress, antioxidant capacity, hyperlipidemia, high blood pressure, and atherosclerosis were also analyzed to describe in more detail the molecular mechanisms of *H. sabdariffa*. The keywords used included: *H. sabdariffa*, oxidative stress,



**Figure 1** Total phenolic and flavonoid components estimated with high performance liquid chromatography (HPLC) from dry flower extract of *Hibiscus sabdariffa*. Total phenolic and flavonoid components were estimated as  $58.8 \pm 1.34$  mg and  $13.57 \pm 0.65$  mg/g of dry flowers respectively.<sup>63</sup>

1, hibiscus acid; 2, hibiscus acid ester 6-methyl; 3, gallic acid; 4, not identified; 5, 5 hydroxymethylfurfural; 6, protocatechuic acid; 7, 5-caffeoylquinic acid; 8, feruloyl derivative; 9, chlorogenic acid; 10, 4-caffeoylquinic acid; 11, caffeic acid; 12, galloyl ester; 13, feruloylquinic derivative; 14, kaempferol-3-glucoside; 15, quercetin derivative; 16, tilioside; A1, delphinidin-3-sambubioside; A2, cyanidin-3-sambubioside.

polyphenols, high blood pressure, atherosclerosis, and lipid profile.

A total of 104 articles were reviewed in preparing this paper. The articles selected were divided into the following categories: (1) generic articles on the pharmacological, chemical, and ethnobotanical properties of *H. sabdariffa*; (2) articles on the relationship between the consumption of *H. sabdariffa* and oxidative stress, and its antioxidant, antihypertensive, hypolipidemic, and antiatherosclerotic potential.

## Results and discussion

Studies analyzing the therapeutic effects of *H. sabdariffa* used in this review are grouped into three tables. In each table, the part of *H. sabdariffa* used and the procedure to obtain the extract of *H. sabdariffa* are given, as well as a summary of the most relevant results and conclusions. Tables 1 and 2 show the results of studies conducted on different cell lines and animal models respectively. Table 3 shows the results of studies conducted in humans.

The part of the plant used and the extraction method are important because the different parts of *H. sabdariffa*, their color, the harvesting procedure, and the method used to extract its phytochemical components appear to have a great influence on the volatile composition of the extract, which has an influence on dosage.<sup>80</sup> It should be noted, for

instance, that a higher antioxidant capacity has been found in flowers harvested 35 days after maturation as compared to more immature flowers.<sup>81</sup> The extraction of calyx components with ethanol promotes the antioxidant capacity of *H. sabdariffa* as compared to ethanol extraction from leaves or aqueous extraction from both parts.<sup>82</sup> Aqueous extraction is, however, of the greatest interest when testing the effectiveness of the different parts of the plant because it is the traditional preparation procedure and is therefore of greater significance with regard to its implications for public health.<sup>66,67,71</sup>

Specifically, Table 1 shows studies conducted on different cell lines. Various cell lines were selected to conduct the experiments, ranging from rat hepatocytes,<sup>83,84</sup> mouse macrophage cell lines RAW264.7<sup>85</sup> and J774A.1,<sup>86</sup> and preadipocyte cell line 3T3-L1<sup>87</sup> to human leukemia cells HL-60.<sup>88</sup> Most of these studies focused on the evaluation of the antioxidant capacity of *H. sabdariffa* derived from either the effect of its anthocyanin content,<sup>85,86,88</sup> the action of protocatechuic acid,<sup>84,88</sup> or the biological action of other *H. sabdariffa* molecules.<sup>83</sup> Using cell lines of rat hepatocytes pretreated with the cytotoxic compound *t*-BHP, Tseng et al.<sup>83</sup> showed the antioxidant effect (especially cytotoxic and genotoxic) of the different soluble fractions of *H. sabdariffa* through free radical removal and the inhibition of unscheduled DNA synthesis. The antioxidant effect of *H. sabdariffa* was mainly attributed to anthocyanins. The

**Table 1** Cell culture studies showing the different effects of *Hibiscus sabdariffa*.

Authors	Year	Study design	Part used	Extraction method	Type of action analyzed	Animal or cell line used	Dose and treatment time	Main results
Chang et al. <sup>85</sup>	2006	Cell culture	Dry flowers	Methanol extraction	Antioxidant Antiapoptotic capacity	Mouse macrophage cell line RAW264.7	1, 1.2, or 2 mg/mL	Anthocyanins may be used as chemopreventive agents Doses may be reached in a typical diet with no supplementation <i>In vitro</i> , anthocyanins in flower extracts may prevent LDL oxidation and macrophage death. Recent evidence suggests the role of LDL oxidation in the pathogenesis of atherosclerosis, but the <i>in vivo</i> effect of the diet should be assessed
Kao et al. <sup>86</sup>	2009	Cell culture Parameters tested <i>in vitro</i>	Dry flowers	Methanol extraction	Antiatherosclerotic Inhibition of LDL oxidation	Mouse macrophage cell line J774A.1	0.05–2 mg/mL of anthocyanin-rich HS extract	↓ LDL oxidation mediated by the formation of foam cells (a variety of macrophages) and expression of the CD36 gene and its PPAR-gamma transcription factor ↓ protein PPAR-gamma levels in nucleus HS decreases expression of the predominant receptor gene for oxidized LDL, CD36, in both mRNA and at protein level HS inhibits oxidized LDL absorption by macrophages
Kim et al. <sup>87</sup>	2007	Cell culture	Dry flowers	Aqueous extraction	Inhibition of adipocyte differentiation	Preadipocytes 3T3-L1	0 250 1000 2000 5000 µg/mL for 5 days	HS extract showed inhibition of lipid accumulation in cytoplasm, particularly at doses of 2 mg/mL HS inhibited change in adipogenic morphology through reduction of intracellular lipid droplets during adipogenesis HS may block the MAPK pathway and inhibit transcription factors through modulation of the MAPK-mediated signaling pathway during adipocyte differentiation The mechanisms by which the HS extract regulates adipogenesis include inhibition of expression of adipogenic transcription factors C/EBP $\alpha$ and PPAR-gamma through the PI3-K and MPAK pathways

Table 1 (Continued)

Authors	Year	Study design	Part used	Extraction method	Type of action analyzed	Animal or cell line used	Dose and treatment time	Main results
Tseng et al. <sup>88</sup>	2000	Cell culture	Dry flowers	Not specified	Antitumoral and antioxidant Induction of apoptosis by PCA through reduction of phosphorylation of retinoblastoma and expression of Bcl-2 (protooncogene)	Human leukemia cells (HL-60)	0.2–2 mM of PCA 24–48 h	PCA has a dose-dependent inhibitory effect of HL-60 growth At doses above 0.2 mM, PCA has a cytotoxic effect associated with the induction of apoptosis in human leukemia cells HL-60 After 6 h of treatment, the RB hyperphosphorylation level decreases, while the hypophosphorylation level increases. PCA prevents HL-60 cells from entering phase S, where RB is hyperphosphorylated transiently After 1.5 h of treatment, Bcl-2 protooncogene expression ↓ is seen associated with apoptosis PCA exhibits an antiproliferative effect of HL-60 cells through apoptosis, associated with RB phosphorylation and degradation and suppression of protein Bcl-2 Protection against cytotoxicity and genotoxicity of hepatocytes treated with t-BHP HS has free radical uptake function
Tseng et al. <sup>84</sup>	1996	Cell culture	Dry flowers	Ethanol extraction	Antioxidant	Rat hepatocytes	0.05 mg/mL of PCA 0.10 mg/mL of PCA 30 min treatment with t-BHP (1.5 mM)	Protection against cytotoxicity and genotoxicity of hepatocytes treated with t-BHP HS has free radical uptake function

Table 1 (Continued)

Authors	Year	Study design	Part used	Extraction method	Type of action analyzed	Animal or cell line used	Dose and treatment time	Main results
Tseng et al. <sup>83</sup>	1997	Cell culture	Dry flowers	Ethanol extraction	Antioxidant	Rat hepatocytes	1.5 mM of t-BHP to induce cell damage. Doses of 0.1 mg/mL, 0.2 mg/mL, 0.5 mg/mL, 1 mg/mL of HS-C, HS-SE, and HS-R	The HS fraction soluble in chloroform, rich in steroid and flavonoid glycosides, shows a great inhibition of XO activity The HS fraction soluble in ethyl acetate, rich in phenolic components, more effectively scavenges DPPH radicals All fractions show inhibition of unscheduled DNA synthesis at a concentration of 0.20 mg/mL ↓ (substantial) in LDH leak and MDA formation induced by t-BHP by the HS-C and HS-E fractions at 0.10 and 0.20 mg/mL HS-R (0.2 mg/mL) only appears to inhibit genotoxicity induced by t-BHP, but does not inhibit peroxidation or hepatotoxicity

CD36: protein encoding for the CD36 gene; DPPH: radical 1,1-diphenyl-2-picrylhydrazyl; HS: *Hibiscus sabdariffa*; HS-C: HS fraction soluble in chloroform; HS-E: HS fraction soluble in ethyl acetate; HS-R: residual HS fraction; LDH: lactate dehydrogenase; LDL: low density lipoprotein; MAPK: mitogen-activated protein kinase; MDA: malondialdehyde; PCA: protocatechuic acid; RB: retinoblastoma; t-BHP: tert-butyl hydroperoxide; XO: xanthine oxidase activity.

**Table 2** Studies in animal models showing the different effects of administration of *Hibiscus sabdariffa*.

Authors	Year	Study design	Part used	Extraction method	Type of action analyzed	Animal or cell line used	Dose and treatment time	Main results
Alarcon-Aguilar et al. <sup>95</sup>	2007	Case-control	Calyces	Aqueous extraction	Antiobesity	Control group and group injected MSG n = 16 male obese mice Subgroup I, n = 8 Subgroup II, n = 8 Eight healthy mice Subgroup III, n = 4 Subgroup IV, n = 4	Subgroup I: 120 mg/kg/day of HS; 60 days Dose was divided in 2 (60 mg/kg). The first half was dissolved in saline and given with a cannula. The second half was dissolved in water and administered <i>ad libitum</i> Subgroup II: 4 mL/kg (ISS); 60 days Subgroup III: 120 mg/kg/day of HS; 60 days Dose was divided in 2 (same procedure as for subgroup I) Subgroup IV: 4 mL/kg (ISS); 60 days	<p>↓ significant in weight of obese mice</p> <p>Aspartate transferase showed no changes</p> <p>↓ blood glucose in the obese group treated with HS. Not in healthy mice</p> <p>Healthy animals showed no significant changes in CHOL and TGC either</p> <p>The antiobesity effect reported in the Mexican population was confirmed</p> <p>Concluded that calyces contain agents which may possibly be used for the prevention and treatment of obesity and hyperglycemia</p> <p>The authors concluded that the mechanisms were not clear</p> <p>This suggests that the specific target of HS in the differentiation process of preadipocytes 3T3-L1 is PPAR-gamma and C/EBP-<math>\alpha</math></p>
Alarcon-Alonso et al. <sup>105</sup>	2012	Case-control	Calyces	Aqueous extraction	Diuretic effect To assess the filtration index in isolated kidney when using HS-extract, furosemide, and amiloride	Male rats	<p>Evaluation of diuresis and electrolyte excretion in urine:</p> <p>Seven groups, n = 6.</p> <p>7.5 mL/100 g of saline were previously given</p> <p>Negative control group = 1.5 mL of distilled water</p> <p>Positive control group = 13 mg/kg of furosemide</p> <p>HS I group = 500 mg/kg</p> <p>HS II group = 1000 mg/kg</p> <p>HS III group = 1500 mg/kg</p> <p>HS IV group = 2000 mg/kg</p> <p>HS V group = 2500 mg/kg</p> <p>Total urine excreted in 5 h was collected. For the tests, the total volume/hour was divided</p> <p>Na, K, and Cl contents were measured</p> <p>In addition, kidney was infused furosemide and amiloride, with or without HS</p>	<p>The diuretic and natriuretic effect shows dose-dependent behavior</p> <p>Pharmacological constants of the natriuretic effect were ED50 = 86 mg/kg and <math>E_{max}</math> = 0.9 mEq/100 g/5 h</p> <p>Urinary excretion with furosemide was 4.8 mL/h. Values with the 1500, 2000, and 2500 doses were 3.0, 4.3, and 4.4 mL/h of urine respectively</p> <p>Evidence of dose dependence</p> <p>Na excretion increased ↑ as ↑ HS doses increased. This data confirms the evident natriuretic effect</p> <p>K excretion did not differ with the different doses as compared to control doses</p> <p>The 1500, 2000, and 2500 ↑ doses increased Cl excretion levels</p> <p>As regards the <i>in situ</i> model using kidneys of study animals, renal filtration increased by 48% with HS extract and an additive effect occurred when infused with furosemide</p> <p>Kidney infusion with amiloride or amiloride with HS showed no different results between them, but differences were seen to the control group, where a 3.9-↑fold greater filtration ratio was seen</p> <p>With furosemide alone and ↑HS there were 2.4-fold and 3.4-fold increases as compared to baseline values respectively</p> <p>According to the authors, the dose to be taken is 300 mg of extract</p> <p>The HS extract show an interesting type of diuretic activity, maintain K concentration in all cases, which corresponds to a healthy Na/K relationship</p> <p>The compound present in HS, as quercetin, had an effect on vascular endothelium, causing nitric oxide release, increasing renal vasorelaxation through increased renal filtration. The diuretic effect may be mediated by nitric oxide release</p>



Table 2 (Continued)

Authors	Year	Study design	Part used	Extraction method	Type of action analyzed	Animal or cell line used	Dose and treatment time	Main results
Ali et al. <sup>89</sup>	2003	Case-control	Calyces/flowers	Aqueous extract	Antioxidant	Thirty-six rats, n = 6	Aqueous extract of HS flowers for 2, 3, or 4 consecutive weeks Oral anthocyanins from HS calyces at doses of 50, 100, and 200 mg/kg for 5 consecutive days 700 mg/kg of acetaminophen were finally administered to induce hepatotoxicity	After the fourth week of administration of aqueous extract, significant improvement was seen in some liver function tests, but histology of acetaminophen-treated rats was not altered After administration of HS anthocyanins: at doses of 200 mg/kg, histology and biochemical indices of liver damage were restored to normal. Capacity to prevent acetaminophen-induced hepatotoxicity was therefore shown Lower doses were ineffective Safety and efficacy studies are still needed to recommend the use of HS as natural treatment against hepatotoxicity caused by acetaminophen, and also probably by other hepatotoxic substances
Carvajal-Zarrabal et al. <sup>94</sup>	2005	Case-control	Calyces	Alcohol extraction	Antiobesity and lipid lowering	Male rats. Hypercholesterolemia was induced through diet	Five groups of rats: Group I, baseline diet Group II, experimental diet with 5 g HS/100 g of diet Group III, experimental diet with 10 g HS/100 g of diet Group IV, experimental diet with 15 g HS/100 g of diet Four weeks	Weight ↑ increase was significantly lower with the SD10 and SD15 doses. SD15 was more effective TGC and LDL ↓ increased in all groups Total lipid levels were lower for SD10 and SD15 Cholesterol levels were lower as compared to the control group, but statistical significance was only seen for SD5 No dose showed a significant result for HDL levels. The hypothesis is that racemization of hibiscus acid, mediated by enzymes in bowel flora, could explain ↓ the significant decrease in triacylglycerol in all experimental groups. This is important bearing in mind that the VLDL, a LDL precursor, mainly consists of triacylglycerols. It is therefore ↓ suggested that decreased LDL levels are due to the inhibition of triacylglycerol synthesis Under the study conditions, 5% HS extract provided the best result in terms of lipid reduction in serum
Chen et al. <sup>79</sup>	2003	Case-control	Not specified	Aqueous extraction	Lipid lowering and antiatherosclerotic	n = 30 albino rabbits induced atherosclerosis	They were divided into 5 groups, n = 5 Control HCD 1% HS HCD + 0.5% HS HCD + 1% HS Ten weeks	↓ Decreased TGC, CHOL, and LDL levels in the group fed HCD HS TGC returned to near normal values with both HS doses The effect on CHOL and LDL was similar for both doses, suggesting that 0.5% is the dose achieving the greatest pharmacological effect ↓ significant decrease in severe aortic atherosclerosis Histologically ↓, HS decreased foam cell formation and inhibited smooth muscle cell migration and blood vessel calcification in rabbits HS has virtually the same lipid-lowering potential as probucol (a lipid-lowering agent) The results suggest that HS inhibits LDL oxidation in arterial wall and therefore exerts an antiatherosclerotic effect t- ↓ BHP decreased levels of GSH peroxidase, a stress marker PCA inhibits the phenomenon The inhibitory effect may partially be related to blockade of the transduction signal of induction of oxidative stress The authors concluded that additional studies are needed on the mechanism of action of PCA on GSH
Liu et al. <sup>91</sup>	2002	Case-control	Not specified	Not specified	Antioxidant and anti-inflammatory	Five groups of rats, n = 6	Doses, 50 and 100 mg/kg of PCA. Five days. t-BHP (0.1 mmol/kg) injected on day 5	

Table 2 (Continued)

Authors	Year	Study design	Part used	Extraction method	Type of action analyzed	Animal or cell line used	Dose and treatment time	Main results
Farombi and Ige	2007	Case-control	Flowers	Alcohol extraction	Lipid lowering and antioxidant	Thirty male albino mice Alloxan-induced diabetes	100 mg/kg and 200 mg/kg of ethanol extract of HS calyces versus lovastatin (10 mg/kg) Four weeks	At doses of 200 mg/kg, potent lipid-lowering activity and antioxidant properties are seen in the alloxan-induced diabetic model At doses of 200 mg/g, ↓ decrease in LDL and CHOL Potential therapy to decrease and prevent development of atherosclerosis and diabetes-related cardiovascular disease Antioxidant and lipid-lowering activities attributed to polyphenols and dihydrobenzoic acids
Hirunpanich et al. <sup>97</sup>	2006	Case-control	Calyces	Aqueous extraction	Antioxidant and hypocholesterolemic	Forty-two male mice. Hypercholesterolemia was induced in the case group	Intragastric doses of 250, 500, and 1000 mg/kg of HS in distilled water. Six weeks. Cholesterol-rich diet was continued	No changes in serum HDL levels ↓ Decreased serum cholesterol, TGC, and LDL levels after 4-6 weeks. Decrease with the ↓ 250 dose was not relevant Prior studies show that doses higher than 1000 mg/kg (2000) are not more effective, which suggests saturation of the lipid-lowering effect. In fact, diarrhea and weight loss occur Its pharmacological saturation dose ranges from 250 to 1000 mg/kg <i>In vitro</i> , 0.1 mg/mL is the lowest value with protective effect against LDL oxidation Mechanisms of action not elucidated yet (future studies required)
Lee et al. <sup>92</sup>	2009	Case-control	Flowers	Alcohol extraction	Attenuation of nephropathy in type 1 diabetes	Male rats induced diabetic nephropathy with STZ	Control: injection of 0.05 M of citrate STZ group and standard diet Diabetic rats with 100 mg/kg/day HP Diabetic rats with 200 mg/kg/day HP Eight weeks	HS polyphenols significantly decreased the kidney mass increase induced by STZ Hydropic change (impaired osmotic diuresis caused by hyperglycemia) in renal proximal tubular change improved ↓ Decreased serum triglycerides, total CHOL, and LDL ↑ Significant increase in catalase and GSH activity and decreased ↓ lipid peroxidation Possibly improves cardiovascular damage in diabetic nephropathy It is suggested that HP reverses diabetic nephropathy induced by high glucose in early stages Glutathione only increased with the dose of 200 mg/kg/day ↑. Catalase increased with ↑ both doses
Liu et al. <sup>90</sup>	2010	Case-control	Flowers	Aqueous extraction	Antioxidant	Six groups of mice. n = 10 Each group with a different protocol RT	Doses of 200, 400, or 600 mg/kg of HS. Two weeks, once daily Then, 1000 mg/kg of: APP (drug inducing oxidative stress, causes acute liver damage)	Protects liver cells from acute damage caused by APP Its mechanism of ↓ action decreases oxidative stress and cell death Anthocyanins and protocatechouic acid may be of potential value for improving and preventing liver damage induced by chemical products

Table 2 (Continued)

Authors	Year	Study design	Part used	Extraction method	Type of action analyzed	Animal or cell line used	Dose and treatment time	Main results
Ochani et al. <sup>62</sup>	2009	Case-control	Calyces and leaves	Ethanol/water	Antioxidant and antihyperlipidemic	Mouse liver to study antioxidant activity Albino rats to study antihyperlipidemic capacity Five group, n = 6	Rat group: Group I: control Group II: hyperlipidemic diet Group III: lovastatin, 10 mg/kg/day Group IV: 500 mg/kg/day of ethanol extract of HS calyces Group V: 500 mg/kg/day of ethanol extract of leaves Thirty days After 6 weeks, rats induced hypertension received 250 mg/kg/day of HS, n = 5 A second hypertensive group with no treatment, n = 5 Control group, n = 5 Eight weeks	↑ Increased inhibitory activity of lipid peroxidation of ethanol extract of calyces, followed by ethanol extract of leaves and finally, aqueous extract of leaves ↓ Decreased cholesterol, LDL, VLDL, TGC levels and increased ↑ serum HDL in rats treated with 500 mg/kg/day of alcohol extract of HS as compared to hyperlipidemia induced control rats The group treated with HS alone also showed weight ↓ decrease
Odigie et al. <sup>70</sup>	2003	Case-control	Petals	Aqueous extraction	Antihypertensive and capacity to reverse cardiac hypertrophy	2K-1C hypertensive rats (renovascular hypertension) Renovascular hypertension was induced clamping the left renal artery with a silver clip for 6 weeks	250 mg/kg/day of HS, n = 5 A second hypertensive group with no treatment, n = 5 Control group, n = 5 Eight weeks	In hypertensive rats (BP > 140 mmHg) with HS there ↓ was a decrease to 139.6 ± 1.6 mmHg as compared to the untreated hypertensive group, 174 ± 2.4 mmHg No significant differences were seen from the control group, 139.6 ± 1.6 mmHg versus 32 ± 3.4 mmHg Heart rate ↓ decreased in animals treated with HS as compared to the other 2 groups Heart weight was lower in the group treated with HS, in which it was similar to the control group; cardiac hypertrophy was therefore attenuated in this group Serum creatinine and plasma electrolytes, Cl, Mg, Na, K, showed no differences from the control The study suggests that HS exhibits an antihypertensive and cardiac protection effect <i>in vivo</i> and supports the popular belief that HS may be useful as an antihypertensive agent The antihypertensive mechanism is speculative, and is postulated to result from the effect of anthocyanins
Peng et al. <sup>63</sup>	2011	Case-control	Calyces	Alcohol extraction	Hypoglycemic and hypoinsulinemic Antioxidant	Rats con type 2 diabetes	100 mg/day 200 mg/day Seven weeks	↓ Decreased serum triglycerides and CHOL, and LDL/HDL risk ratio. ↓ Decreased hyperglycemia and hyperinsulinemia, mainly at doses of 200 mg/kg The authors concluded that HS shows its properties as anti-insulin-resistant and its hypoglycemic, lipid-lowering, and antioxidant effect, inhibiting expression of CTFG and RAG, which may be 2 biomarkers of type 2 diabetes associated with vascular disease HS has potential as an adjuvant in diabetic treatment

Table 2 (Continued)

Authors	Year	Study design	Part used	Extraction method	Type of action analyzed	Animal or cell line used	Dose and treatment time	Main results
Ajiboye et al. <sup>96</sup>	2011	Case-control	Calyces	Alcohol/aqueous extraction	Antioxidant and drug detoxification	Thirty male albino mice weighing 175 ± 6.6 g	Control group Second group: interperitoneal carbon tetrachloride 0.5 mL/kg on the last day of treatment Third group: 200 mg/kg of HS anthocyanins Fourth, fifth, and sixth groups: 200 mg/kg of butylated hydroxyanisole, α-tocopherol, and HS anthocyanins. This was changed by carbon tetrachloride 0.5 mL/kg on the last day of treatment Fourteen days	↑ Increased scavenging effect as compared to DPPH, 92% at a concentration of 2 mg/mL More effective than the synthetic oxidant used in the study 69% and 90% increase in the scavenging effect on superoxide and peroxide ions respectively at a concentration of 1 mg/mL ↓ Significant decrease in oxidative potential of $K_3Fe(CN)_6$ Potential use in cancer prevention Inducer of drug detoxification mechanisms
Wang et al. <sup>93</sup>	2011	Case-control	Flowers	Aqueous extraction	Diabetic nephropathy improvement through improved oxidative status and regulation of Akt/Bad/14-3-3 signaling	Twenty-five male rats induced diabetic nephropathy with STZ	Control: injection of 0.05 M of citrate STZ group and standard diet Diabetic rats with 100 mg/kg/day HP Diabetic rats with 400 mg/kg/day HP Eight weeks	Hydropic change (impaired osmotic diuresis caused by hyperglycemia) in renal proximal tubular change improved ↑ Significant increase in catalase and GSH activity and ↓ decreased lipid peroxidation ↓ Decreased serum TGC, CHOL, and LDL levels HDL only increased with the dose of 400 mg/↑kg/day, which means that HS may have a promising effect in slowing metabolic syndrome in diabetes At both doses, expression of Akt/Bad/14-3-3γ recovered after the decrease ↓ seen as compared to normal rats upon STZ injection It appears reasonable to assume that HS may induce recovery of Akt levels and the resultant signaling cascade through oxidative stress improvement in diabetic rats HS has been shown to have a potential to attenuate the effects of diabetic nephropathy by antioxidant and antiapoptotic mechanisms. The relationship of HS with the Akt pathway in diabetes and the mechanism of action have not been elucidated yet ↓ Significant decrease in plasma total cholesterol with 1.5 mg/kg of both varieties ↓ LDL decrease with both doses of both varieties No significant changes in HDL and TGC levels
Olatunji et al. <sup>68</sup>	2005	Case-control	Petals	Aqueous extraction	Cholesterol-lowering Cardioprotective effect	Thirty albino rats n = 6	1 mg/kg/day or 1.5 mg/kg/day of the red or green variety of <sup>1</sup> HS Twenty-eight days	↓ Significant decrease in plasma total cholesterol with 1.5 mg/kg of both varieties ↓ LDL decrease with both doses of both varieties No significant changes in HDL and TGC levels

APP: acetaminophen; COL: high-fat diet; CTGF: connective tissue growth factor; DPPH: α-diphenyl-β-picrylhydrazyl; GSH: glutathione; HCD: high cholesterol diet; HDL: high density lipoprotein; HS: *Hibiscus sabdariffa*; ISS: saline solution; LDL: low density lipoprotein; MSG: monosodium glutamate; PCA: protocatechuic acid; BP: blood pressure; RAGE: receptor for advanced glycation end-products; SD: supplemental diet; STZ: streptozotocin injection; t-BHP: tert-butyl hydroperoxide; TGC: triglycerides; VLDL: very low density lipoprotein.

**Table 3** Studies in humans showing the different effects of intake of *Hibiscus sabdariffa*.

Authors	Year	Study design	Part used	Extraction method	Type of action analyzed	Animal or cell line used	Dose and treatment time	Main results
Frank et al. <sup>98</sup>	2012	Case-control	Indeterminate	Aqueous extraction	Antioxidant	Eight healthy humans	Experimental drink: 10 g of HS extract in 200 mL of water. Taken once Reference drink: 200 mL of water. Taken once A blood sample was taken from each group at different time intervals (every half an hour for 3 h, and hourly thereafter until hour 10) The process was repeated 2 weeks later	Significant differences in antioxidant potential in plasma and urine Significant differences in malondialdehyde, an oxidative stress biomarker High biotransformation of HS polyphenols ingested, most likely due to colonic microbiota
Gurrola-Diaz et al. <sup>64</sup>	2010	Case-control	Calyces	30% ethanol extraction	Preventive treatment of lipid profile	n = 222 volunteers of both sexes. Age, 30–71 years T1: diet T2: HSPE T3 HSPE + diet in subjects with and without MeSy	100 mg HSPE daily in capsules for one month Biochemical evaluation was performed on day 0 and 31	Subjects with MeSy treated with HSPE, ↓ decreased glucose and total ↑ CHOL, increased HDL, and improved TGC/HDL-C ratio, an insulin resistance marker Group T3 subjects with MeSy and group T2 subjects with no MeSy showed TGC decrease <sup>3</sup> HSPE may be used by patients with metabolic syndrome
Herrera-Arellano et al. <sup>66</sup>	2004	HS and captopril	Dry calyx extract	Aqueous extraction	Antihypertensive	Study in 75 subjects with mild and moderate hypertension to test the antihypertensive effect and tolerability of HS versus captopril	9.6 mg of anthocyanins (10 g HS) as a daily infusion before breakfast 25 mg of captopril/2 sugar-coated tablets daily Four weeks	The antihypertensive effectiveness ratio was 0.7895 for HS and 0.8438 for captopril 100% tolerability for both treatments HS significantly increased urinary sodium excretion. No significant changes in other urinary electrolytes Short-/long-term administration of HS extract is safe

Table 3 (Continued)

Authors	Year	Study design	Part used	Extraction method	Type of action analyzed	Animal or cell line used	Dose and treatment time	Main results
Herrera-Arellano et al. <sup>67</sup>	2007	HS and lisinopril	Dry calyx extract	Aqueous extraction	Antihypertensive Tolerability, efficacy, and safety analysis	Randomized, double-blind trial 193 patients with hypertension I or II	Experimental group, n = 100, 250 mg total anthocyanins per dose Control group, 10 mg of lisinopril Total duration, four weeks Tolerability (lack of side effects), efficacy (BP reduction $\geq$ 10 mmHg), and safety (no pathological changes in biochemical tests of renal and hepatic parameters) were analyzed	Efficacy and tolerability of HS were lower as compared to lisinopril HS has an antihypertensive effect with a high degree of safety and tolerability, and also inhibits the action of angiotensin-converting enzyme in plasma
Mozaffari-Khosravi et al. <sup>72</sup>	2009	HS-BT	Indeterminate	Infusion (commercial bags)	Antihypertensive	Sixty diabetic patients with mild hypertension	Infusion of HS or BT. 2 g twice daily for one month BP was measured on days 0, 15, and 30	HS has a positive effect in people with type 2 diabetes and mild hypertension
Kuriyan et al. <sup>73</sup>	2010	Case-control Double-blind, placebo-controlled	Leaves	Water/alcohol extraction: 50% water- 50% ethyl alcohol	Lipid-lowering effect	n = 57 subjects with LDL levels ranging from 130–190 mg/dL with no history of coronary artery disease Thirty-one males, 26 females aged 35–60 years	The experimental group received 1 g of extract (two 500 mg capsules) for 90 days The placebo group received a similar amount of maltodextrine Physical activity and standard diet were also prescribed	No significant differences in body weight, LDL, TGC The effects seen result from exercise and diet compliance. The dose of 1 g of leaf extract did not appear to have a reducing effect on blood lipids
McKay et al. <sup>65</sup>	2010	Case-control	Dry calyx extract	Aqueous extraction	Antihypertensive	Sixty-five prehypertensive and mildly hypertensive subjects	HS infusion (one bag of 1.25 g of HS), 240 mL/3 times daily for 6 weeks Blood pressure was monitored at weekly intervals throughout the study	Participants with higher SBP showed an excellent response to HS treatment HS in diet is recommended to prehypertensive and mildly hypertensive subjects

Table 3 (Continued)

Authors	Year	Study design	Part used	Extraction method	Type of action analyzed	Animal or cell line used	Dose and treatment time	Main results
Mohagheghi et al. <sup>71</sup>	2011	HS-BT	Dry calyces	Aqueous extraction	Short-term serum lipid reduction in hypertensive patients	n = 84 hypertensive patients treated in the prior 3 months	Random administration of BT or HS from commercial bags (500 mg of HS or BT) Fifteen days twice daily (20 min after preparation) Blood glucose levels and lipid profile were tested on days 0 and 30	There were no significant differences between pre- and post-treatment in the 2 groups HS is a safe medicinal plant HS consumption caused no harmful changes in serum cholesterol, TGC, and creatinine or in Na and K levels within 15 days of discontinuation of medication

CHOL: cholesterol; HDL: high density lipoprotein; HS: *Hibiscus sabdariffa*; HSPE: powder extract; LDL: low density lipoprotein; MeSy: metabolic syndrome; BP: blood pressure; DBP: diastolic blood pressure; SBP: systolic blood pressure; TGC: triglycerides; BT: black tea; T1, T2, T3: treatment.

antiatherosclerotic capacity of anthocyanins in *H. sabdariffa* through the inhibition of LDL oxidation was analyzed by Kao et al.<sup>86</sup> using mouse macrophages. Their objective was to assess anthocyanin action in foam cell formation and in the expression of the predominant receptor gene for oxidized LDL, CD36, and its transcription factor PPAR-gamma (peroxisome proliferator-activated receptor gamma). The authors showed that *H. sabdariffa* inhibits the absorption of oxidized LDL by macrophages by decreasing the expression of the CD36 receptor gene. Chang et al.<sup>85</sup> similarly reported the *in vitro* role of anthocyanins in *H. sabdariffa* in the inhibition of LDL oxidation and, thus, in the prevention of atherosclerosis. Kim et al.<sup>87</sup> analyzed the capacity of *H. sabdariffa* extract for the inhibition of adipocyte differentiation and its potential benefit in obesity prevention. They suggested that *H. sabdariffa* may inhibit adipogenesis through the inhibition of three different pathways: (1) the inhibition of adipogenic transcription factors C/EBP $\alpha$  (enhancer binding  $\alpha$ protein) and PPAR-gamma (peroxisome proliferator-activated receptor gamma), (2) the inhibition of PI3K (phosphoinositide 3-kinase) pathways, and (3) the inhibition of metabolic pathways associated with MAPK (map kinase). Treatment with *H. sabdariffa* extract during the adipogenic differentiation process showed a significant reduction in protein and mRNA expression of factors C/EBP $\alpha$  and PPAR-gamma. The authors also reported a decrease in the mRNA of leptin, a hormone regulating intake and energy expenditure and which is partially activated by C/EBP $\alpha$  at the transcriptional level. It should also be noted that the *H. sabdariffa* extract inhibited PI3K phosphorylation and expression during adipogenesis. Because of these results, the authors stated that *H. sabdariffa* is able to block the PI3K and MAPK signaling pathways and inhibit transcriptional factors during the early differentiation phases of adipocytes. Based on all of the foregoing, the authors postulated that *H. sabdariffa* extracts are beneficial for obesity prevention and may lead to body fat loss *in vivo*. To sum up, Table 1 clearly states the antioxidant effect of *H. sabdariffa*, mainly attributed to anthocyanins, which leads one to hypothesize about its capacity to take up OFRs and, among other properties, the inhibition of unscheduled DNA synthesis and the epigenetic changes which could be derived from them. These results open the way to continuing research in animal models and humans to validate the postulated hypotheses.

In the animal models in Table 2, healthy animals<sup>68,89-91</sup> or animals previously manipulated to induce diabetes or atherosclerosis,<sup>69,92,93</sup> hypercholesterolemia or hyperlipidemia,<sup>82,94</sup> obesity,<sup>94,95</sup> or high blood pressure<sup>70</sup> to test the effects of *H. sabdariffa* consumption were used. Ajiboye et al.<sup>96</sup> reported the potential of *H. sabdariffa* to take up the radicals di-phenyl-2,4,6-trinitrophenyl iminoazanium and 2,2-diphenyl-1-picrylhydrazyl at a dose of 2 mg/mL of anthocyanin extract from *H. sabdariffa*, and to take up superoxide ion and hydrogen peroxide at a concentration of 1 mg/mL. The antioxidant potential of *H. sabdariffa* was analyzed in blood and liver of albino rats. Liu et al.<sup>90</sup> showed that doses of 200, 400, and 600 mg/kg of aqueous extract of *H. sabdariffa* flowers decreased lipid peroxidation and increased catalase activity and GSH levels in plasma and liver tissue from mice. Ali et al.<sup>89</sup> reported that doses of 200 mg/kg of aqueous extract of calyces restored to normal the biochemical indices of

**Table 4** Studies on HS toxicity and safety.

Authors	Year	Study design	Part used	Extraction method	Type of action analyzed	Animal or cell line used	Dose and treatment time	Main results
Akindahunsi et al. <sup>102</sup>	2003	Case-control	Calyces	Methanol-water (4:1)	Toxicity	Six groups, n = 4, of Wistar albino rats	Group I: 0, physiological saline alone G II: a dose of 250 mg/g G III: 3 doses G IV: 5 doses G V: 10 doses G VI: 15 doses Treatment duration was not specified	AST and ↑ ALT significantly increased in all groups as compared to control group Histopathological studies showed no pathological damage in liver and heart in any groups Long-term administration of dose 15 may cause liver damage It was concluded that although mean daily consumption of 150–180 mg/kg appears safe, extract should be taken with caution because higher doses may induce liver damage
Fakeye <sup>104</sup>	2008	Case-control	Flowers	Water-ethanol (50:50)	Study of the immunomodulatory properties and subacute toxicity profile of 2 fractions of alcohol-water extract of HS calyces	Male albino mice	Three groups, n = 4, administered: 50 mg/kg, 100 mg/kg of fraction soluble in ethyl acetate 100 mg/kg of the residual part Group 4: control Seven days	No toxicity was found with doses used, which showed immunostimulating activities Fractions have an impressive immunostimulating activity The residual water-soluble fraction caused a marked weight ↓ decrease as compared to the control group Both fractions caused a marked basophil ↓ decrease The residual aqueous fraction caused ↓ neutrophil decrease Both fractions were found to be susceptible to becoming drug entities



Table 4 (Continued)

Authors	Year	Study design	Part used	Extraction method	Type of action analyzed	Animal or cell line used	Dose and treatment time	Main results
Fakeye et al. <sup>103</sup>	2009	Case-control	Flowers	Water-ethanol (50:50)	Study of hematological, biochemical, and histopathological toxicity from oral intake of HS	Thirty-five albino rats. Divided into 7 groups, n = 5	Administration – 50:50 water/ethanol – 100% ethanol 300 mg/kg and 2000 mg/kg/day of both extracts were administered. Ninety days A seventh, control group. 2 mL of water/day	Death of animals was preceded by severe weight loss, associated with diarrhea in animals with doses of 2000 mg/kg AST activity improved with the administration of aqueous extract and 50% of ethanol, with a significant ↑ AST increase with higher doses ALT and creatinine levels were significantly affected by both extract types at different doses. A significant increase in creatinine levels was seen ↑ with the aqueous extract at higher doses Cholesterol levels were not significantly affected by extracts No significant histopathological changes were seen ↓ Decreased erythrocyte count. No leukocyte decrease Long-term use of high doses causes ↑ increased liver enzyme levels, and their effects may be confused with chronic hepatitis, with no clear damage seen in hepatocytes

AST: aspartate aminotransferase; ALT: alanine aminotransferase; HS: *Hibiscus sabdariffa*.

liver damage caused by acetaminophen in mice, showing the capacity of *H. sabdariffa* to prevent liver damage. Olatunji et al.<sup>68</sup> showed that chronic intake for 28 days of 1–1.5 mg/kg/day of aqueous extract of *H. sabdariffa* petals significantly decreased plasma LDL levels in experimental rats. Only the 1.5 mg/kg/day doses of aqueous extract of *H. sabdariffa* petals showed a significant decrease in plasma total cholesterol levels, with no significant changes in HDL or total triglyceride levels.<sup>68</sup> No relevant changes were seen either in serum HDL levels after the use of *H. sabdariffa* in experiments conducted in models of hypercholesterolemia, hyperlipidemia, or induced obesity,<sup>63,69,94,97</sup> but significant improvements occurred in total cholesterol, LDL and/or total triglyceride levels. In parallel, Hirunpanich et al.<sup>97</sup> also showed its effectiveness at doses higher than 250 mg/kg/day after 6 weeks of intake of aqueous extract of calyces. These same authors noted that doses higher than 1000 mg/kg were not associated with greater effectiveness, which suggested saturation of the lipid-lowering effect. The authors showed the pharmacological dose of aqueous extract of calyces to range from 250–1000 mg/kg/day. By contrast, Ochani and D’Mello<sup>82</sup> and Wang et al.<sup>93</sup> showed that doses of 500 mg/kg/day of alcoholic extract of leaves and calyces and 400 mg/kg/day of aqueous extract of flowers, respectively, not only decreased total cholesterol, LDL and total triglyceride levels in serum, but also increased HDL levels. In rat models with induced diabetes, alcoholic extracts of *H. sabdariffa* flowers or calyces caused a reduction of serum levels of total cholesterol, LDL and/or total triglycerides.<sup>69,92</sup> So far, there have been no articles relating the use of *H. sabdariffa* to epigenetic changes. Further studies are therefore needed to understand the molecular mechanisms associated with the use of *H. sabdariffa* and disease pathogenesis, particularly in humans. Continuing with the study of the effects of *H. sabdariffa* on the regulation of energy and cell metabolism, Chen et al.<sup>79</sup> worked with an atherogenic rabbit model to assess the antiatherosclerotic and lipid-lowering effect of *H. sabdariffa* extracts. Histologically, they showed that exposure for 10 weeks to doses of a 0.5–1%/diet of *H. sabdariffa* aqueous extract resulted in decreased foam cell formation and the inhibition of blood vessel calcification, as well as serum total cholesterol, LDL, and triglyceride levels. The authors concluded that the antiatherosclerotic activity of *H. sabdariffa* is related to the prevention of LDL oxidation in the arterial wall and that its consumption may be beneficial for decreasing disease incidence. Wang et al.<sup>93</sup> emphasized antioxidative and antiapoptotic mechanisms as a potential explanation for decreases in total cholesterol, LDL, and total triglyceride levels. Lee et al.<sup>92</sup> reported increased catalase and glutathione activities at doses of 200 mg/kg and decreased lipid peroxidation as mechanisms for regulating plasma lipid levels. The results of animal studies show the antihypertensive and antioxidant effects of *H. sabdariffa*. However, the results on the effect of *H. sabdariffa* intake on cholesterol metabolism, and thus on the parameters of the associated diseases, are more variable. In this regard, the heterogeneity of the results in terms of HDL levels after the consumption of *H. sabdariffa* should be noted, because although HDL levels significantly increased with high doses of *H. sabdariffa* extract in a few studies, they did not usually change. A single conclusion

is difficult to reach because of the high diversity in the methods used, the number of subjects used in each group, and the doses used in the different experimental protocols.

Table 3 shows the results of studies in humans. Observational studies with<sup>65</sup> and without placebo,<sup>98</sup> epidemiological, interventional, randomized case–control studies with<sup>73</sup> and without placebo,<sup>66,67,72</sup> and randomized studies are shown.<sup>64,71</sup>

The different studies assessed the antioxidant, lipid-lowering, and antihypertensive effects of *H. sabdariffa* in healthy subjects,<sup>98</sup> patients with metabolic syndrome,<sup>64</sup> diabetic patients with mild hypertension,<sup>72</sup> prehypertensive and mildly hypertensive subjects,<sup>65,66,72</sup> hypertensive patients previously given standard antihypertensive medication,<sup>66,67</sup> and subjects with hyperlipidemia.<sup>73</sup> Frank et al.,<sup>98</sup> in a study on the antioxidant capacity of *H. sabdariffa*, administered 10 g of *H. sabdariffa* as an infusion to an experimental group of eight healthy subjects. After intake, blood and urine were collected from the participants for 24 h. The authors showed a significant decrease in malondialdehyde, an oxidative stress biomarker, and a marked increase in the antioxidant potential of human plasma and urine. They also found a significant increase in urinary excretion of hippuric acid, which showed a high biotransformation of the *H. Sabdariffa* polyphenols ingested and pointed to the role of colon microbiota in this transformation. Gurrola-Diaz et al.<sup>64</sup> focused their study on the preventive and improving effects of *H. sabdariffa* on the lipid profile in subjects with or without metabolic syndrome. The most significant data from this study showed that subjects with evidence of metabolic syndrome and who had received experimental treatment with dry extracts of *H. sabdariffa* calyces had significant decreases in blood glucose and total cholesterol levels, increased HDL levels, and an improvement in the total triglycerides/HDL ratio, an insulin resistance marker. The authors showed that anthocyanins in *H. sabdariffa* could regulate adipocyte function, as postulated by Tsuda.<sup>99</sup> Similarly, Mohagheghi et al.<sup>71</sup> subsequently analyzed the short-term efficacy of extract from *H. sabdariffa* calyces on the reduction of serum glucose and lipid levels in hypertensive patients with a history of conventional antihypertensive treatment *versus* intake of the same amount of black tea. Increased levels of total cholesterol and lipoproteins bound to cholesterol (LDL and HDL) were seen with both treatments. Increases in total cholesterol and HDL levels as compared to baseline were significant in both cases. No changes harmful for health were seen in cholesterol, total triglycerides, serum creatinin, Na<sup>+</sup>, or K<sup>+</sup> levels within 15 days of discontinuation of the medication. They, therefore, concluded that *H. sabdariffa* is a safe medicinal plant. Kuriyan et al.<sup>73</sup> could not show the lipid-lowering effect of *H. sabdariffa* leaves in patients on a standard diet and physical exercise. The authors reported decreased body weight and LDL and total triglyceride levels in control and treated patients, and suggested that the results seen were secondary to exercise and diet.

As regards the antihypertensive effect of *H. sabdariffa*, McKay et al.<sup>65</sup> showed that daily intake of three cups of tea of *H. sabdariffa* significantly decreased systolic pressure in prehypertensive and moderately hypertensive subjects. Ajay et al.<sup>100</sup> showed that the antihypertensive effect with vasodilating effects and/or decreased heart rate of

*H. sabdariffa* could be related to its anthocyanin-rich composition. Wahabi et al.,<sup>78</sup> in a meta-analysis of the quality of studies conducted on the effectiveness of *H. sabdariffa* in the treatment of hypertension in prehypertensive or mildly hypertensive subjects, reported that no adequate scientific evidence was available to validate the antihypertensive properties of *H. sabdariffa*. In fact, these authors stated that they were studies of inadequate quality, short duration, and high methodological diversity, which did not allow them to adequately assess the potential adverse effects of continued *H. sabdariffa* intake.

The results given in the different tables suggest that *H. sabdariffa* has antioxidant potential and the capacity to attenuate high blood pressure and the diseases associated with obesity and diabetes such as nephropathy, atherosclerosis, and cardiovascular diseases. The diversity of the effects of *H. sabdariffa* seen in humans may be attributed to the different polyphenol concentrations in *H. sabdariffa* and other concomitant nutrients, to the solvents used for extraction, to the time and form of administration, as well as to the small number of studies available, methodological differences (e.g. the use of black tea as control instead of placebo), different population sizes, and inter-individual heterogeneity.

Finally, some authors suggest that *H. sabdariffa* extracts have a low grade of acute toxicity with a mean lethal dose (LD<sub>50</sub>) ranging from 2000 to more than 5000 mg/kg/day.<sup>77,101</sup> However, a possible adverse effect has been seen in the liver at high doses<sup>77</sup> (Table 4). Akindahunsi and Olaley<sup>102</sup> reported the occurrence of liver toxicity with chronic intake of doses higher than 3000 mg/kg of extract of *H. sabdariffa* calyces. Fakeye et al.<sup>103</sup> pointed out that long-term consumption of high doses of flower extract may cause toxic reactions which may even be confused with episodes of chronic hepatitis. In fact, doses of 2000 mg/kg/day for 90 days induced severe weight losses and diarrhea in their experimental animals. By contrast, experiments using doses ranging from 50 to 100 mg/kg led to the hypothesis that ethanolic and aqueous fractions of extracts from *H. sabdariffa* flowers could have the potential to become pharmacological entities for stimulating immunity.<sup>104</sup>

## Conclusions

The traditional consumption of *H. sabdariffa* as an infusion has been related to different therapeutic properties. Specifically, the results of this review suggest that *H. sabdariffa* is able to take up free radicals inhibiting, for example, LDL oxidation. In addition, it appears that daily consumption of *H. sabdariffa* extract can significantly improve blood pressure in prehypertensive and mildly hypertensive patients and patients with type 2 diabetes. On the other hand, the use of *H. sabdariffa* can improve lipid profile, reducing serum levels of total cholesterol, LDL, and total triglycerides. Anthocyanins in *H. sabdariffa* have been shown to be able to inhibit LDL oxidation and possibly to decrease the risk of atherosclerosis. Although different studies have attempted to show the antioxidant, antihypertensive, and lipid-lowering effects of regular consumption of *H. sabdariffa* in humans and animal and cell models, the cellular, biological, and epigenetic mechanisms of the specific effects

of *H. sabdariffa* have yet to be elucidated. The results published to date do not allow us to define any dose of *H. sabdariffa* with regard to its therapeutic properties. The lack of homogeneity in the experimental design of the different studies, as well as the low number of subjects and their heterogeneity, could be some of the causes of the lack of consistency in the results. However, the results represent interesting findings which should be thoroughly analyzed, because understanding of the distribution and function of polyphenols from *H. sabdariffa* in patients with different conditions may be helpful for achieving effective treatment. Continued research in the field of alternative non-drug treatments, such as functional foods, is also required. Only a limited number of *H. sabdariffa* extracts have been tested to date, and since the effects of different components are not equivalent, the results cannot be generalized. Future large scale studies with control of doses, active components, bioavailability, and other critical variables will therefore be crucial to provide the scientific evidence required to ascertain the efficacy of any therapeutic approach with *H. sabdariffa* and its doses.

## Conflict of interest

The authors state that they have no conflicts of interest.

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