

# Effect of *Hibiscus sabdariffa* Tea Extracts Intake on the Atherosclerosis Biomarkers in Adults

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**Abstract Purpose:** In Saudi Arabia, about 46% of total of deaths caused by Cardiovascular diseases (CVDs). Recently, an association between the bioactive components in *Hibiscus sabdariffa* and atherosclerosis prevention was detected. The present study aimed to examine the influence of anthocyanins from *Hibiscus sabdariffa* tea extracts on the atherosclerosis biomarkers in adults in Saudi Arabia. **Patients and Methods:** 16 female Participants, with mean age was of about 20-35 years were asked to fast for 12 hours after 3 days washout, then blood sample were collected and blood pressure were measured before and after consumption of *Hibiscus sabdariffa* tea extract. All, participants consumed. *H. sabdariffa* tea extract (240 ml tea bag) twice a day for 6 weeks. blood sample and blood pressure were collected at the end of intervention. Biochemical analysis, determination of Antioxidant concentration and determination of oxidized low-density lipoprotein (OxLDL) Levels were carried out. **Results:** A significant decline in blood pressure (systolic and diastolic) and OxLDL for the participants while no changes in levels of LDL, TG, cholesterol and the rate of glucose. A significant increase in HDL was, also, observed. The study found that there were no significant differences in participants antioxidants concentration. **Conclusion:** The study suggests that anthocyanins from *H. Sabdariffa* may have an effective role in preventing of atherosclerosis due to the influence of relevant biomarkers related.

**Keywords:** anthocyanins, cardiovascular diseases, bioactive components, antioxidants

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as the extraction of *H. sabdariffa* is promising as a treatment of hypertension and hyperlipidemia [5].

## 1. Introduction

Globally about 30% of all deaths are caused due to cardiovascular diseases (CVDs) each year, these figures are expected to grow to 23.6 million by 2030 [1]. Moreover, the distribution and proportion of major causes of death are including CVDs in the world. In addition, in Saudi Arabia the relative mortality reaches about 46% of the total deaths, for all ages, and both male and females that are related to Cardiovascular diseases [2]. However, the (OxLDL) had a role in the development of cardiovascular diseases (CVDs), especially, atherosclerosis that can lead to a heart attack or death. Therefore, the increase intake of anthocyanins reduces the risk of CVDs [3]. This research has focused on antioxidants and their atherosclerosis risk factors as biomarkers in targeting the disease, bioactive components in *Hibiscus sabdariffa* especially anthocyanin, (Delphinidin-3-sambubioside and Cyanidin-3-sambubioside) [4] in the preventing of atherosclerosis by anthocyanins, the effect of anthocyanin on lipid profile,

## 2. Case Presentation

There are not enough studies examined the influence of *H. sabdariffa* extract to prevent atherosclerosis. So, the aim of the research to explore the possible influence *H. sabdariffa* tea extract on reducing the risk of atherosclerosis by examining the lipids profile, fasting glucose levels, antioxidant Concentration, amount of OxLDL and blood pressure in human healthy volunteers.

## 3. Research Methodology

### 3.1. Kits and Chemicals

PBS phosphate buffered saline powder PH7.4 (Medicago, Sweden), Antioxidant assay kit96 test (No. 709001), ELISA kit for Oxidized Low-Density

Lipoprotein (OxLDL) 96 well strip plate (No. SEA527Hu), (USCN, China).

### 3.2. Materials

Dried *H. sabdariffa* herbal tea bags were obtained from the local market (Al Wefak Al Saudi for Food Ind, Egypt) each tea bag contains 2g. 4ml tubes for collecting blood samples (Plain, Lithium Heparin) were purchased from (Naseem Al Safsaf, Saudi Arabia)

### 3.3. Equipment's

Digital blood pressure monitor (Riester, Germany), Scales MDW-300L (Pneutrol Spares, UK), Centrifuge/Sigma 2-6E, Microplate washer ELx50 (BioTek, USA), MIR-162 Heated Incubator(SANYO, Japan), Plate Shaker 0S-160 (Human Lab, Korea) , Plate reader ELx808 and ELx50 wavelength range from (405-750 nm) (BioTek, USA), EPSON LQ-680 Pro Printer (EPSON, Japan) , Data analysis and graphing software , Dimension Vista System with Flex reagent cartridge (SIEMENS, Germany) .

### 3.4. Preparation of *H. Sabdariffa* Extract

*H. sabdariffa* tea was prepared by adding (240 ml) of boiling water (100°C) to *H. sabdariffa* herbal tea bags that measure (2g) each, the bag will be infused for 10 min then one cube of sugar (5 g) may add if needed [6].

### 3.5. Human Study Design

The research is seeking to assess the effectiveness of a commercially available *H. sabdariffa* tea extract on reducing the risk of atherosclerosis by carrying out a human intervention study. The study has taken its ethical approval Ref 20-15 to precede the research from ethics committee of king Abdul-Aziz University. Then, twenty adult female volunteers have been conventionally selected 4 withdraw from the study, sixteen female volunteers; based on previous study [7].

The research has been conducted at king Abdul-Aziz University and King Fahad Medical Research Centre. The inclusion criteria; (Female adults, (20-35) years old, BMI of 25-35 kg/m<sup>2</sup>, Ability to consent, healthy Jeddah /Saudi Arabia residents). On the other hand, exclusion Criteria were (no chronic disease, no consumption for dietary supplements as daily routine, no exercise, not Pregnant or lactating).

Volunteers were enrolled and recruited from students and signed the consent form.

There was three-day washout period before starting the intervention. Participants were asked to avoid a list of foods (a low-flavonoid diet) before the studies period of time; that was explained to them and provide them with the list for guidance. Participants were asked to fast for 8 to 12 hours before visiting the researcher at the end of the three-day washout.

Also, volunteers were requested to complete a food dietary record of three days, were collected before the intervention to make sure the results seen are due to the intervention not due a change in dietary pattern. Before intervention blood sample and blood pressure was measured on baseline using one arm and same cuff every time.

Blood pressure reading was taken 3 times with pauses 5 min between each reading before the intervention will start to set the zero line (systolic:120-140 mm hg and diastolic ≤ 90mm hg) in setting position. In addition, height and weight were taken at baseline.

The volunteers were asked to consume *H. sabdariffa* tea one cup (240 ml) (1 tea bag) twice per day for six weeks. The duration of the study was six weeks. By the end of six weeks participants were asked to fast over the night then blood sample and pressure were measured again. Biochemical analysis (lipid profile, glucose level, Antioxidant concentration level and OxLDL levels) were determined.

Compliance of the subjects was measured every week by asking participants to fill a questionnaire and to note any unexpected occurrences encountered during this period to ensure each subject has met the conditions of the study.

## 4. Results and Discussion

### 4.1. Effect of Hibiscus Sabdariffa on Blood Pressure

The current study demonstrated (as showed in Figure 1 and Figure 2) that the *H. sabdariffa* tea significantly lowered the blood pressure (systolic and diastolic) for the volunteers before and after the consumption (115.44 ± 107.56, 73.38 ± 67.19 mm Hg; P = 0.000, 0.005, P < 0.05) respectively.

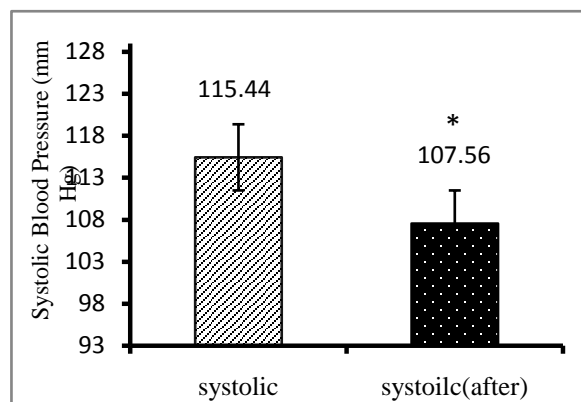


Figure 1. The average of Systolic Blood pressure before and after consumption of *H. sabdariffa* tea, (\*) a statistically significant difference

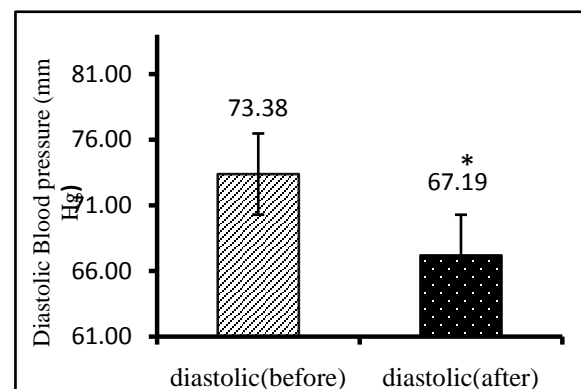


Figure 2. The average of Diastolic Blood pressure before and after consumption of *H. sabdariffa* tea, (\*) there is a statistically significant difference

These results were related to the study done by McKay *et al.*, (2010) that examined the effects of *H. sabdariffa* tea intake on hypertensive human, the results revealed that the daily consumption of *H. sabdariffa* tea extract, lowered systolic BP. Diastolic BP was also reduced [8]. So, the daily consumption of *H. sabdariffa* tea may be has a great effect in preventing atherosclerosis by its effect in lowering high blood pressure.

Anthocyanins had many biological activities including antioxidant, anti-inflammatory activities [9] and antihypertensive properties [8]. Since Hypertension is a main factor in coronary heart disease and atherosclerotic vascular disease of the coronary [10], it was aimed to reduce blood pressure by consuming *H. sabdariffa* tea as prevention step of atherosclerosis.

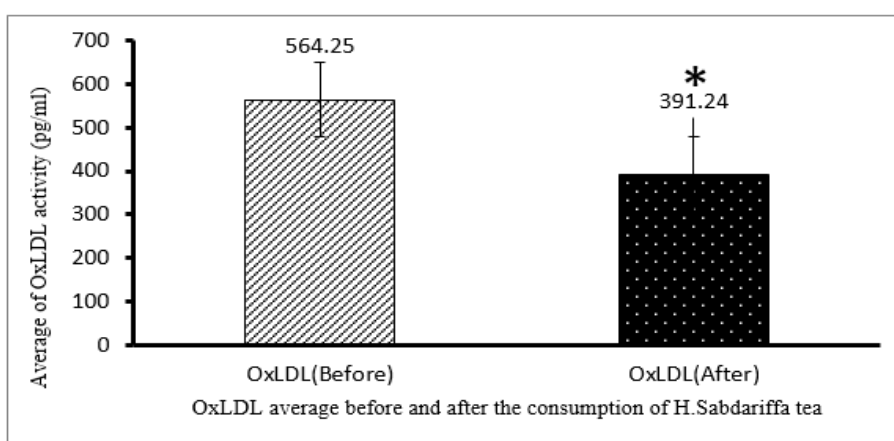
#### 4.2. Effect of *Hibiscus sabdariffa* on OxLDL

The early step in development of atherosclerosis and complications includes enhance of low-density lipoprotein oxidation that was promoted by the involvement of endothelial dysfunction [11]. Consequently, it has been focused in the current study to decrease the OxLDL levels of the participants, and the results shows that there was a significant difference ( $P=0.013$ ,  $P< 0.05$ ); among the participants OxLDL levels before and after the consumption of *H. sabdariffa* tea. This is in agreement with the study conducted by Long Yi *et al.*, (2010), that suggested that anthocyanins may inhibit OxLDL-induced of cell possibility at various different extend, and since compared to OxLDL-treated group has proved that most of examined anthocyanins showed a significant effect ( $P < 0.05$ ) [12]. Moreover, Yun-Ching Chang *et al.*, (2006) study which amid to evaluate the anti-oxidant activity of *H. sabdariffa* by measuring their influences on LDL oxidation in RAW264.7 cells. Resulting that Hibiscus anthocyanins can be used as an inhibitor for LDL

oxidation, reduce oxidative damage in endothelial cells [13].

#### 4.3. *Hibiscus sabdariffa* and Biochemical Analysis

The current study showed that there was insignificant difference for the LDL, TG, TC and GLU in the samples before and after drinking of *H. sabdariffa* tea (Table 1), and that agreed with a review done by Hopkins *et al.*, (2013) suggested that *Hibiscus Sabdariffa* tea may use as a treatment to cure hypertension and hyperlipidaemia [5]. But about six researches involving 474 subjects from relevant randomized controlled trials demonstrated that daily consuming *H. sabdariffa* tea or extracted does not support the ability of *Hibiscus sabdariffa* to lower serum lipids levels of LDL, TG, TC and HDL. [14], and as in the study coordinated by M Lefevre *et al.*, (2008) who showed that glucose, triglycerides and serum cholesterol levels were not different after six weeks of feeding on a pro-atherogenic diet to increase oxidative stress, with or without an anthocyanin extract (70% anthocyanins), between two groups of C57BI/6 mice [15]. As for the HDL concentration there was a statistically significant difference between the HDL concentration in the samples before and after the consumption of *H. sabdariffa* tea, which has increased, as for the study done by Mozaffari-Khosravi *et al.*, (2009), which reported that the average of high-density lipoprotein-cholesterol (HDL) significantly increased, after the patients with diabetes consumed *H. sabdariffa* tea for 1 month, two times a day [6]. However, according to Aziz Z. *et al.*, (2013), who studied the effect of *H. sabdariffa* tea on lipid profile, the study found that there was an insignificant effect on LDL, TG, TC [14]. So, the effect of *H. sabdariffa* tea on reducing lipid profile and GLU levels can be confirmed by further researches with a larger sample size.



**Figure 3.** The total average of the OxLDL activity levels before and after consumption of *H. sabdariffa* tea, (\*) there is a statistically significant difference

**Table 1. Lipid profile and GLU test of normality.**

	LDL		HDL		TC		TG		GLU	
	Before	After	Before	After	Before	After	Before	After	Before	After
Average (mg/dl)	2.21	2.63	1.36	1.58	3.59	4.11	0.86	0.82	4.93	4.76
T-Test (Sig (2).)	0.08		0.045		0.60		0.11		0.23	

\*1: if  $P>0.05$  the sample is normally distributed, \*2: if  $P<0.05$  the sample is significant.

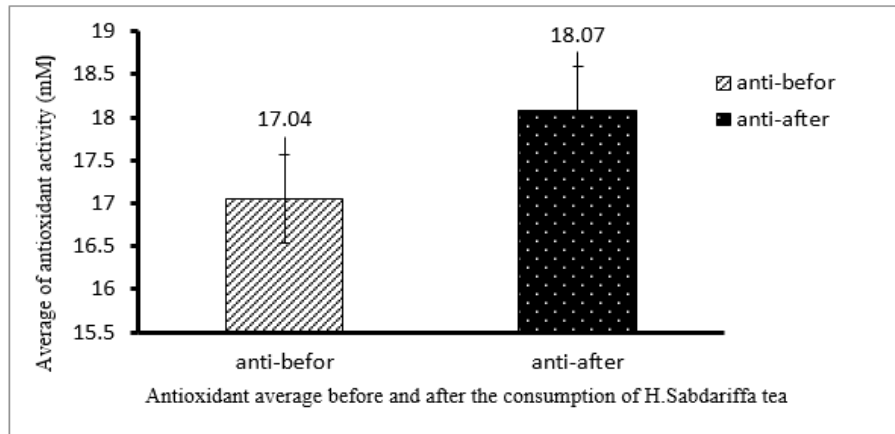


Figure 4. The total average of the antioxidant's activity levels before and after consumption of *H. sabdariffa* tea

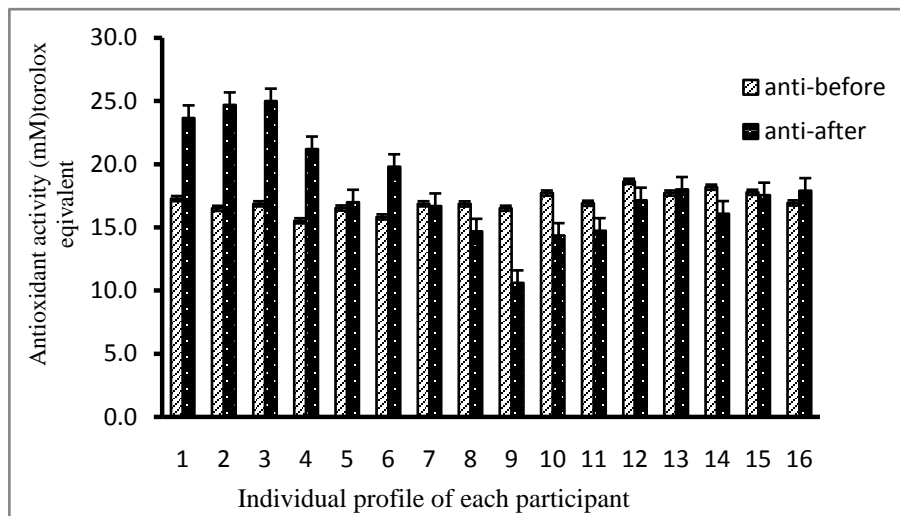


Figure 5. The average of the antioxidant's activity levels before and after consumption of *H. sabdariffa* tea

#### 4.4. Effect of *Hibiscus sabdariffa* on Antioxidant Levels

Several studies have shown that *H. Sabdariffa* extract have a potential antioxidant influence due to strong scavenging effect on reactive oxygen and free radicals [16]. But unfortunately, there were insignificant differences between the antioxidant in the samples before and after the consumption of *H. Sabdariffa* tea extract Figure 4, the statistical analysis conducted by T-test confirmed that the antioxidant levels before and after the consumption of *H. sabdariffa* tea showed  $P=0.346$ ,  $P> 0.05$  therefore, appeared not significant, these results agree with the study done by Mauray *et al.*, (2010), which found that a two week of a nutritional dose of bilberry anthocyanin-rich extract (0.02%) and a standard diet supplementation on E-deficient (apo E<sup>-/-</sup>) mice, as will no significant difference was observed in antioxidant capacity between the bilberry anthocyanin-rich extract and the control groups [17] This can be attributed to the small size of the sample. However, a closer look of Figure 5 shows that antioxidant levels in 43.75% of volunteers increased while in 37.5% decreased. Only 18.75% recorded the same level of antioxidants in the prior- and the post- observation, So, the insignificant can be related to the short duration of the study, as in the study conducted by Mauray *et al.*, (2012), insignificant

difference was achieved between the bilberry extract and control mice during the two-week supplementation in antioxidant capacity (ORAC) [18].

## 5. Conclusion

*H. sabdariffa* is an anthocyanin rich plant, which has a pharmacological purpose especially as an antioxidant. However, the aim of the study was to examine the possible influence of *Hibiscus Sabdariffa* tea extracts on the atherosclerosis biomarkers. The current study has showed that *H. sabdariffa* tea lowered OxLDL, and as for its effect on reducing the risk of atherosclerosis by examining the biomarkers related, it showed a significantly reduction in the participants blood pressure, whereas, but the lipid profile and the antioxidant levels of the participants showed an insignificant reduction, may be due to the sample size.

## 6. Statistical Analysis

Data was analyzed as mean±standard deviation (± S.D.) from individual magnitude. Statistical differences were analyzed with paired sample T-test after examining the normality distribution of the samples before and after the

consumption of the *H. sabdariffa* tea extract. SPSS program version (20) was used, and  $P < 0.05$  was considered significant in all types of statistical analysis.

## 7. Perspectives

In a future study the size of the sample will be increased to be (100) participant and it will contain both male and female as well, a control group will be engaged in the study. In addition, the duration of the study will be for (8 weeks). There will be further evaluation of the effect of delphinidin-3-sambubioside and cyanidin-3-monoglucoside extracted from *Hibiscus sabdariffa* on oxidative stress and the formation of foam cells.

## Consent

Written informed consent was obtained from the patient for publication of this case report.

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## Disclosure

The author reports no conflicts of interest in this work.

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