



The Effect of Roselle (Hibiscus) Juice on Mood Improvement in Healthy Adults

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ABSTRACT

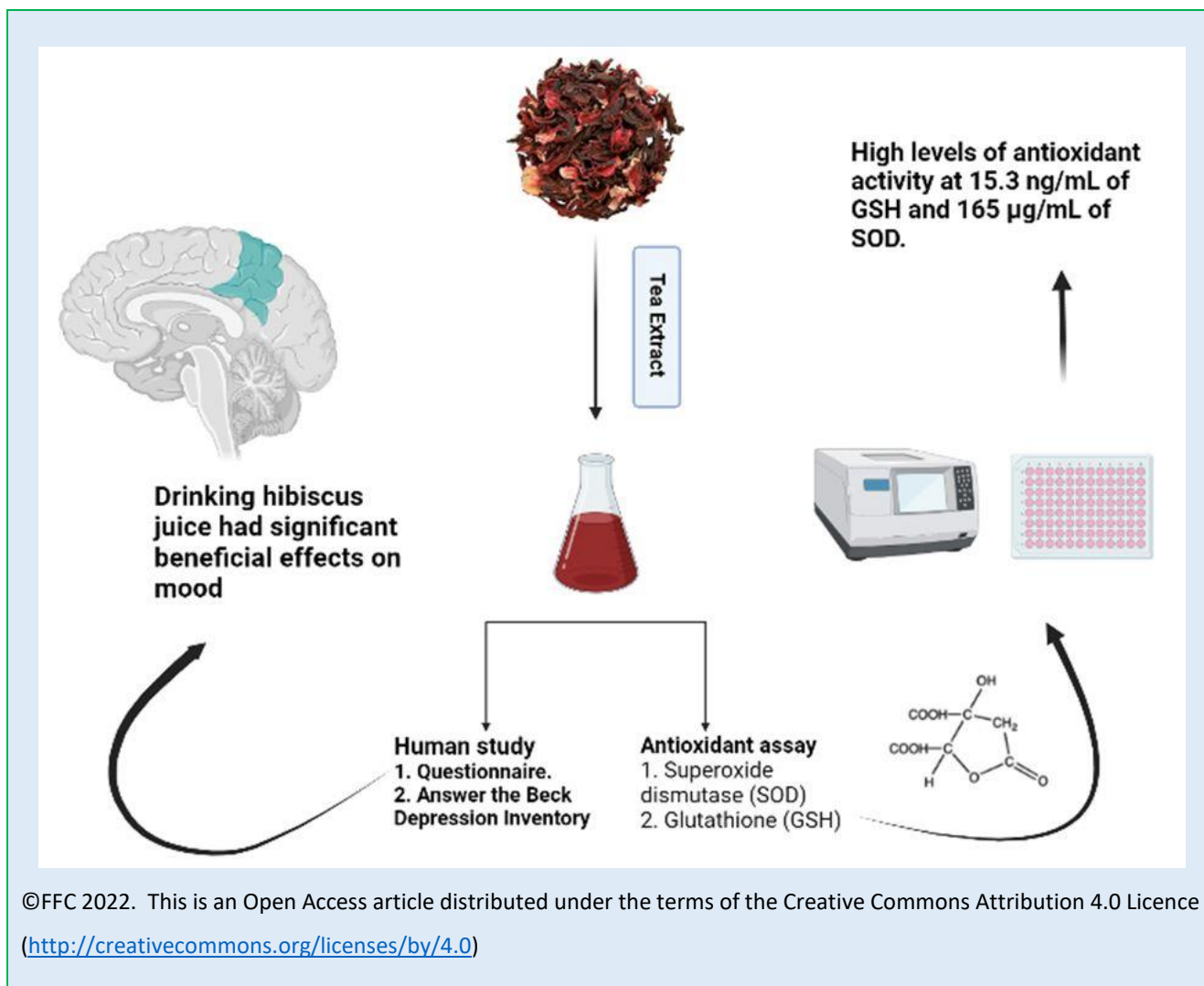
Introduction: The juice of Hibiscus sabdariffa Linn is commonly used as a traditional medicine for hypertension, pyrexia, and mood improvement. This study aimed to examine the effect of hibiscus juice on the mood states of healthy Saudi adults.

Methods: A total of 32 healthy adults were asked to complete a questionnaire and were subsequently provided with 250 mL of prepared hibiscus juice to drink daily for 1 week. The participants were instructed to answer the Beck Depression Inventory before and after 1 week of drinking the hibiscus juice daily.

Results: The results indicated a significantly positive change in the participants' daily mood after 1 week of drinking 250 mL of hibiscus juice daily. Drinking hibiscus juice enhanced mood status by increasing relaxation and decreasing fear of the future and heart palpitations in most participants. Furthermore, it increased the consumption of caffeinated drinks. However, drinking hibiscus juice had no effect on the number of meals consumed.

Conclusion: Drinking hibiscus juice had significant beneficial effects on mood. These results may be useful for future public health recommendations, especially among young adults and children. Further studies with larger sample sizes and longer experimental durations are needed to confirm and generalise these findings.

Keywords: Mood Improvement, Cognitive, Hibiscus, Saudi Arabia, Adults



INTRODUCTION

In Arabic, *Hibiscus sabdariffa* (*H. sabdariffa*) Linn is known as karkadé. Hibiscus drinks are used worldwide because of their curative properties and is considered a functional food. Functional foods are defined by the Functional Food Centre (FFC) as a “natural or processed foods that contain biologically active compounds, which, in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, to promote optimal health and reduce the risk of chronic/viral disease and manage their symptoms [1]. Hibiscus contains carbohydrates, proteins, fatty acids, flavonoids, minerals, and vitamins and is also rich in antioxidants, such as anthocyanin, quercetin, L-ascorbic acid, and proto-catechuic acid [2], and contains

anisaldehyde, arachidic acid, β-carotene, β-sitosterol, delphinidin, gossypetin, and hibiscetin [3, 4].

Depression, anxiety, and fatigue, prevalent in the Saudi population, can be averted by consuming healthy foods and maintaining a healthy lifestyle [5]. Conscious thoughts and feelings, planning, opinions, and decisions are examples of cognitive processes. However, cognitive processes often involve activities such as perceiving and recognising objects, attending to sounds, learning new responses, and memorising things [6], and many individuals must learn the skills to engage in such processes. Hibiscus drinks are considered mood enhancers and are used to alleviate mental fatigue [7], though it cannot be considered an alternative to mental health therapy. It is commonly used in traditional

medicine to treat hypertension, hyperlipidaemia, liver diseases, and pyrexia. It has also been reported to play a role in the prevention of cancer, diabetes, and heart disease [8, 9]. Cold-pressed hibiscus juice has been shown to decrease blood pressure in patients with hypertension [7]. Daily consumption of hibiscus decreases systolic and diastolic blood pressure in adults and reduces the total cholesterol and low-density lipoprotein cholesterol levels [10]. Anthocyanins, a group of antioxidants, inhibit low-density lipoprotein oxidation, which is a risk factor for cardiovascular diseases. Anthocyanins, polyphenols, and hibiscus acid are also considered to be phytochemicals, as they have antihypertensive and hypocholesterolaemia effects [10]. Hibiscus extract is reported to have antihypertensive, antioxidant, anticlastogenic, antistress, and antispasmodic effects [11-16]. Evidence for the use of hibiscus as an adjuvant to antidepressants has been previously reported in animal studies [17, 18], but not in human studies.

For centuries, herbal remedies have been part of the culture and society of the Saudi population. Previous studies have indicated the acute benefits of phenols in healthy young adults [19, 20]; nonetheless, there is a lack of research on the effects of hibiscus juice in this population. Therefore, this study aimed to investigate the short-term effects of hibiscus juice on the cognition and mood of healthy adults in Jeddah, Saudi Arabia.

MATERIAL AND METHODS

Participants: A total of 32 healthy men (n = 16) and women (n = 16) aged 20–40 years from King Abdul-Aziz University, Jeddah, Saudi Arabia, volunteered to participate in this study between March and May 2019. None of the participants reported having severe medical conditions (e.g., diabetes, heart disease, hypertension, epilepsy). Smokers, pregnant women, those who were

desiring to become pregnant, and lactating women were excluded. The participants provided informed consent, and were compensated for study participation. Ethical approval for the study was provided by the Unit of Biomedical Ethics Research Committee at King Abdul-Aziz University (reference no. 631-19), and the study was conducted in accordance with the Declaration of Helsinki.

Study design and treatment: This was a pilot study with two phases, and the study duration was 10 days. In the first phase (lasting 3 days), the participants' baseline measurements, demographic information, and daily habits were collected. In the next phase, the participants were asked to drink hibiscus juice daily for 7 days. Data collection was conducted in a quiet room at King Fahd Medical Research Centre at King Abdul-Aziz University between 1:00 pm and 3:00 pm. The participants were asked to consume the same breakfast, lunch, and dinner on weekdays and weekends. Prior to participation, each participant signed an informed consent form and completed a screening questionnaire. All participants reported that they were in good health and were not taking any prescription/over-the-counter medicines, illicit/social drugs or, herbal/dietary supplements.

Sample preparation of hibiscus drink: Hibiscus drink samples were prepared according to the method described by Abubakar et al. [21] with slight modifications. The active treatment dose consisted of 250 mL of sun-dried hibiscus flowers obtained from a local market (Abazeer) in Jeddah. A stock solution of hibiscus was prepared by adding 90 g of dried hibiscus to 3000 mL of boiling water. The solution was then allowed to simmer for 10 min until it became dark red in appearance. Subsequently, the juice was cooled and stored in individual containers before being chilled in a refrigerator at 4 °C [21]. All drinks were prepared from a

large stock. Each day for 1 week, participants orally consumed 250 mL of hibiscus juice as a single dose per day.

Questionnaire design Each participant was asked to complete a three-section questionnaire. The first section of the questionnaire included demographic information such as age, sex (male or female), family income per month (<Saudi riyal [SR] 4000; SR 4001–8000; >SR 8001), and marital status (single, married), and was completed once at baseline. In the second section of the questionnaire, participants were asked about their daily habits to determine the effect of hibiscus juice on factors such as daily meal frequency (one meal, two meals, and three meals). Data on caffeine consumption (tea/coffee) was also collected (never, one cup, 2–3 cups, 3–4 cups, >4 cups). The final section of the questionnaire focused on psychological characteristics and participants were asked to complete the 21-item Beck Depression Inventory (BDI) to determine stress levels [22]. These sections were completed before and after drinking hibiscus juice daily for 10 days. The English version of the questionnaire was translated into Arabic so that language would not be a barrier for the participants.

Cognitive and mood measures: All cognitive and mood measures were assessed using the BDI [22], a self-report questionnaire consisting of 21 questions related to feelings and thoughts of depression. The participants were asked to select one statement, from a group of statements, that best described how they felt. Progress through the tasks was ensured by providing brief instructions before the start of each task. The questionnaire took approximately 15 minutes to complete. The response to each question was scored using a 4-point scale (indicating degree of severity), with 0 indicating no symptoms and 3 indicating severe symptoms. The scores of the 21 questions were then

summed. A higher total score indicated higher symptom severity (never, 0–13; low, 14–19; medium, 20–28; severe, 29–63) [23].

Biochemical assay for antioxidants of hibiscus juice: The enzyme-linked immunosorbent assay kits for plant superoxide dismutase (SOD) and glutathione (GSH) were obtained from MyBioSource, San Diego, USA. The SOD and GSH concentrations in the hibiscus juice were determined by a 96-well microplate-based assay using specific quantitative colorimetric detection kits (Sigma-Aldrich, USA).

Superoxide dismutase assay: Hibiscus juice samples were assayed using the SOD assay as described by Fridovich [24]. Briefly, using a 96-well plate, 50 μ L of hibiscus juice was added to 100 μ L of HRP-conjugated reagent. The plate was incubated for 60 min at 37 °C. The plate was then washed four times with washing solution. Chromogen Solution A (50 μ L) was added to each well, followed by Chromogen Solution B (50 μ L). The contents of the wells were mixed gently and then incubated for 15 min at 37 °C, during which time the plate was protected from light. Next, 50 μ L of stop solution was added to the plate and the optical density of the contents was recorded (Sigma Aldrich, USA) at 450 nm within 15 min.

Determination of hepatic reduced glutathione: Glutathione peroxidase (GPx) activity was measured in the hibiscus juice using an enzyme-linked immunosorbent assay kit as per the manufacturer's instructions (MyBioSource, San Diego, USA) in accordance with the method of Sedlak and Lindsay [25]. Briefly, 50 μ L of standard dilutions and hibiscus juice were added to the 96-well plate with a blank. Then, 50 μ L of detection reagent A was added to each well immediately. The plate was gently shaken using a microplate shaker, covered with a plate sealer and

incubated for 1 h at 37 °C. The plate was then washed three times with 350 µL of washing solution. After the last wash, 100 µL of Detection Reagent B working solution was added to each well and the plate was incubated again for 30 min at 37 °C while covered with a plate sealer. The plate was then washed five times as previously described. Next, 90 µL of substrate solution was added to each well and the plate was covered with a new plate sealer and incubated for a further 15–25 min at 37 °C. Finally, 50 µL of stop solution was added to the plate, and the microplate reader (Sigma Aldrich, USA) was run and immediately measured at 450 nm.

Statistical analysis: Statistical analyses were performed using SPSS version 28 (IBM Corp., Armonk, NY, USA). Categorical data were compared using the non-parametric chi-square test. The Kruskal–Wallis and Mann–Whitney U tests were used to compare the

questionnaire scores between groups. Any difference that resulted in a p-value of less than 0.05 (95% confidence interval) was considered statistically significant.

RESULTS

Demographic data: The demographic characteristics of participants, stratified by sex, are summarised in Table 1. Thirty-two patients were eligible to participate in the current study, and the proportions of men and women were equal. More than half of the participants were aged ≥26 years. Approximately 26.5% and 73.5% of male and female participants, respectively, were unmarried. The majority of participants (53%) had an income of SR 8001 or more, followed by participants with an income of SR 4000 or less. However, there were no significant differences in other demographic characteristics between the men and women.

Table 1. Demographic characteristics of the study participants by sex (n = 32)

Characteristics	Men (n = 16)		Women (n = 16)		p value
	n	%	n	%	
Age, years					
<18	1	6.25	0	0.00	0.34
19–25	7	43.75	7	43.75	
≥26	8	50.00	9	56.25	
Social status					
Single	10	62.50	6	37.50	0.07
Married	6	37.50	10	62.50	
Monthly income, Saudi riyal					
4000 or less	7	43.75	7	43.75	0.23
4001–8000	0	0.00	1	6.25	
8001 or more	9	56.25	8	50.00	

The data are presented as frequency and percentage.

Chi square test was used to examine statistical difference between the groups.

Saudi riyal (currency 3.75 RS = 1\$).

Daily habits: Consumption of caffeinated drinks significantly changed before and after drinking hibiscus juice ($p < 0.001$), with 19 participants who did not consume caffeine at baseline compared to 23

participants who consumed 3–4 cups of caffeinated drinks after drinking hibiscus juice. The number of meals consumed remained approximately the same before and after drinking hibiscus juice (Table 2).

Table 2. Amount and frequency of meal/caffeine consumption among participants before and after intervention (n = 32)

	Baseline	After intervention	p value
Number of meals			
One meal	3	6	0.54
Two meals	17	16	
Three meals	12	10	
Number of caffeine drinks			
None	19	9	<0.0001
One cup	8	0	
2–3 cups	1	0	
3–4 cups	4	23	

The data are presented as frequency.

Chi square test was used to examine statistical difference between the groups.

BDI scores before and after drinking hibiscus: After drinking hibiscus juice, significant improvements in mood status were observed ($p = 0.043$), as reflected by a significant reduction in mean BDI scores (13.6 ± 1.6 before drinking hibiscus juice vs. 9 ± 1.5 after drinking hibiscus juice; Figure 1). Regarding specific items of the BDI, the difference in the responses to three out of 21 questions were statistically significant before and after drinking hibiscus juice (Table 3). These included questions related to relaxation (26 participants were able

to relax after drinking hibiscus juice compared with 12 participants at baseline [$p < 0.001$]); feelings of fear and impending doom (24 participants felt less afraid of bad things happening in the future compared to 14 participants at baseline [$p = 0.04$]); and heart palpitations (26 participants responded that their heart palpitations reduced after drinking hibiscus juice in comparison to 17 participants who never experienced palpitations at baseline [$p = 0.01$]).

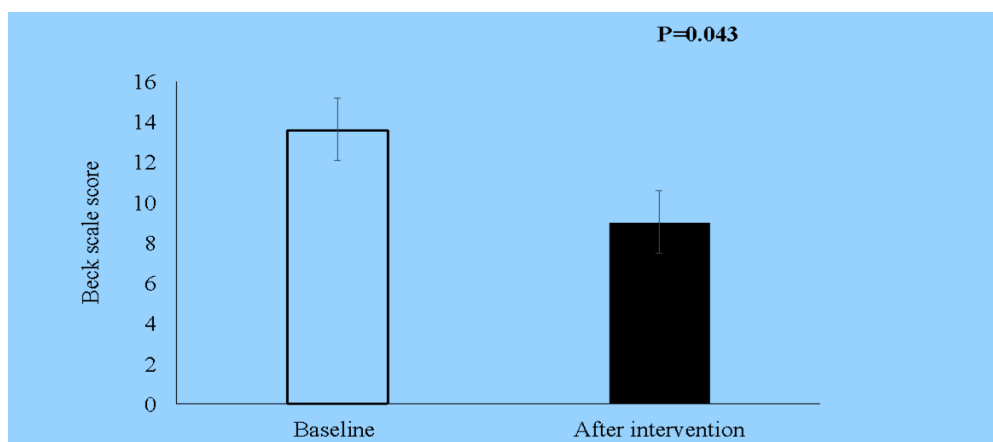


Figure 1. Mean Beck Depression Inventory scores at baseline and after intervention

Table 3. Comparison of baseline and post-intervention responses to the Beck Depression Inventory (n = 32)

Questions	Baseline	After intervention	p value
I feel tingling in my body			0.050
Never	13	21	
Low	15	6	
Medium	4	5	
Feeling hot			0.08
Never	19	23	
Low	8	3	
Medium	3	6	
Severe	2	0	
Wobbliness in legs			0.57
Never	30	28	
Low	1	1	
Medium	1	3	
Unable to relax			0.001
Never	12	26	
Low	16	3	
Medium	3	3	
Severe	1	0	
Fear of worst happening			0.037
Never	14	24	
Low	11	4	
Medium	6	2	
Severe	1	2	
Dizzy or lightheaded			0.31
Never	20	24	
Low	8	7	
Medium	4	1	
Heart palpitations			0.013
Never	17	26	
Low	11	2	
Medium	3	1	
Severe	1	3	
Unsteady			0.36
Never	23	25	
Low	4	5	
Medium	3	2	
Severe	2	0	
Terrified or afraid			0.51
Never	21	25	
Low	7	5	
Medium	3	2	
Severe	1	0	
Nervous			0.11
Never	5	13	
Low	11	10	
Medium	9	6	
Severe	7	3	
Feeling of choking			0.43
Never	21	25	
Low	8	6	
Medium	3	1	

Questions	Baseline	After intervention	p value
Hands trembling			0.93
Never	25	26	
Low	5	4	
Medium	2	2	
Shaky/unsteady			0.09
Never	26	27	
Low	3	5	
Medium	3	0	
Fear of losing control			0.44
Never	17	22	
Low	11	6	
Medium	3	2	
Severe	1	2	
Difficulty in breathing			0.21
Never	20	26	
Low	8	3	
Medium	3	3	
Severe	1	0	
Fear of dying			0.68
Never	12	16	
Low	13	12	
Medium	3	2	
Severe	4	2	
Scared			0.12
Never	21	24	
Low	7	8	
Medium	3	0	
Severe	1	0	
Indigestion or abdominal discomfort			0.50
Never	9	16	
Low	10	8	
Medium	9	8	
Severe	4	0	
Faint / lightheaded			0.55
Never	29	28	
Low	1	2	
Medium	2	1	
Severe	0	1	
Face flushed			0.76
Never	11	14	
Low	12	12	
Medium	5	4	
Severe	4	2	
Hot / cold sweats			0.61
Never	14	18	
Low	11	7	
Medium	6	5	
Severe	1	2	

The data are presented as frequency.

Chi square test was used to examine statistical difference between the groups.

Never coded: 0; low coded: 1; medium coded: 2; and severe coded: 3.

The total score was obtained by adding the statement scores (range: 0–63).

Biochemical assay for antioxidants of hibiscus juice: SOD is an antioxidant enzyme responsible for the elimination of superoxide radicals. GPx is a selenocysteine-dependent enzyme and the most important hydrogen peroxide-scavenging enzyme that converts hydrogen peroxide into water [26]. SOD and GPx can directly counterbalance the attack of oxidants and protect cells against DNA damage. As per our results, hibiscus juice showed high levels of antioxidant activity at 15.3 ng/mL of GSH and 165 µg/mL of SOD.

Discussion: The current study aimed to investigate the benefits of hibiscus juice on the mood and cognition of healthy adults. The findings revealed that hibiscus juice had significant beneficial effects on the participants' mood, as reflected by the reduction in BDI scores after the consumption of hibiscus juice. Specifically, the results indicated that consumption of hibiscus juice increased feelings of relaxation and decreased feelings of fear (in relation to the future) and heart palpitations in the study participants. Increased consumption of caffeinated drinks was also observed after drinking hibiscus juice; however, the number of meals consumed remained approximately the same.

Hibiscus juice is rich in vitamin C and is consumed in many regions worldwide for different purposes. Hibiscus juice is recommended for several medical conditions, such as cardiac and neurologic diseases, hypertension, and calcified vessels. Moreover, hibiscus juice has several benefits, including antihypertensive, antibacterial, and muscle relaxant effects [27, 28]. Different nutrients have been shown to affect depression status. A cross-sectional study conducted in Brazil examined the association between dietary antioxidants and depression among postmenopausal women and found that lower intake levels of polyphenols, vitamin B6, vitamin A, and vitamin C were associated with depression status among women

[29]. Another study involving Iranian postmenopausal women confirmed an inverse relationship between total dietary antioxidant capacity and the prevalence of depression [30]. Hibiscus extract is a good source of antioxidants, which are important biomolecules that protect against chemically induced cytotoxicity. Antioxidants are also involved in the elimination of reactive intermediates by conjugation and hydroperoxide reduction and have free radical quenching potential [31]. Hibiscus is considered a good source of phenolic compounds (2.16–18.78 mg gallic acid equivalent /g), with a total flavonoid content over 1.64–2.94 mg rutin equivalents /g extract [32, 33].

To the best of our knowledge, no previous study has examined the relationship between consumption of hibiscus juice and prevalence of depression. Historically, there has been evidence from animal research on the use of hibiscus extract as an adjuvant to treatment with antidepressants. Mice exposed to restraint stress for 2 hours daily for 3 weeks and then treated orally with hibiscus extract (100, 200, or 400 mg/kg/day) showed a reduction in depressive behaviour [34]. Another study used two bioactive components of hibiscus (methanol extract and ethanolic extract of the roots of hibiscus) for the treatment of mice and reported antidepressant activity [35, 36]. The effect of hibiscus extract on different factors related to emotion and depression may explain its positive influence on BDI scores in our study. Animal studies have reported that hibiscus extract increases levels of serotonin, a neurotransmitter in the central nervous system that is closely linked to emotional disorders and depression as well as brain-derived neurotrophic factor, which is essential for the maintenance of neurons in the core brain and associated with emotional functions. In addition, hibiscus was found to have antidepressant effects through the cyclic adenosine monophosphate response element-binding

protein/brain-derived neurotrophic factor signalling pathway in mice after administration of hibiscus extracts [33].

Hibiscus flowers are a good source of several minerals, including potassium, magnesium, calcium, iron, phosphorus, cobalt, manganese, sodium, and zinc. Potassium is required in the body to preserve osmotic balance, pH of body fluid, muscle regulation, and nerve irritability [37]. Hibiscus can exert a positive effect in different forms (as cold juice or hot tea), on lipid profile, creatinine level, and serum electrolytes [37]. Some aspects of mood, such as feeling scared and fear of dying, were not significantly affected by hibiscus juice; however, this does not necessarily mean the juice does not affect the functions of the brain and thinking processes. Some studies show that extracts of different fruits improve different functions of the brain. For example, grape juice was reported to improve cognitive perception and responsiveness in adults [20]. In addition, it was also reported to improve nerve relaxation and nerve conduction by 40.63% and 15.63%, respectively [20].

Studies have also shown that hibiscus juice can reduce systolic and diastolic blood pressure [38]. Several studies have shown that hibiscus extract can increase satiety among overweight and obese subjects as well as providing other health benefits, including reduction of hypertension and levels of blood lipids [21, 39, 40]. In the present study, the number of meals consumed remained approximately the same before and after drinking hibiscus juice, indicating no reduction in hunger in the study sample; however, the consumption of caffeinated drinks such as coffee and tea significantly increased after drinking hibiscus juice. The reason for this increased consumption was unclear, as no previous studies have shown similar results. In Saudi Arabia, coffee and tea are popular beverages despite the hot weather [41]. In

addition, a previous animal study showed that hibiscus extract significantly affected muscle relaxation and had a calming action in rats [42]. Hence, the relaxation effect of hibiscus juice in our study may have led to an increase in the consumption of caffeinated drinks among the study participants. However, further studies are required to confirm these findings.

The current study has some limitations. As this was a pilot study, the sample size was small and the experimental period was relatively short. Time was a particularly limiting factor. In relation to the effects of functional foods, an intervention period of at least 14 weeks is recommended according to Food for Specific Health Use (FOSHU) standards. Therefore, studies with larger sample sizes and longer experimental durations are needed to confirm and generalise the findings. Additionally, further studies are required to explain the underlying mechanism by which hibiscus extract improves mood, enhances relaxation, and increases the consumption of caffeinated drinks. The measurement of other biomarkers in future studies may help to reveal these, as well as additional, underlying mechanisms.

CONCLUSIONS

Our results showed that drinking hibiscus juice had significant beneficial effects on mood, as reflected by the reduction in BDI scores. Specifically, hibiscus juice increased feelings of relaxation and decreased feelings of fear regarding the future and heart palpitations among the study participants. In addition, drinking hibiscus juice had no effect on the amount or frequency of daily meals consumed, though an increased consumption of caffeinated drinks was observed. These results may help to inform future public health recommendations, especially those targeted towards young adults and children, such as the replacement of soda drinks and

unhealthy juices with healthy options such as hibiscus juice.

List of abbreviations: Superoxide dismutase (SOD); Glutathione (GSH); Glutathione peroxidase (GPx); Beck Depression Inventory (BDI); Saudi riyal (SR)

Competing Interests: The authors declare no conflict of interest.

Authors' Contributions: Conceptualization, N.M.A. (Noha M. Almoraie), I.M.S. and M.A.A.; methodology, N.M.A. (Noha M. Almoraie), I.M.S and H.A.W.; software, I.M.S; validation, N.M.A. (Najlaa M. Aljefree); formal analysis, I.M.S.; investigation, N.M.A. (Noha M. Almoraie); data curation, N.M.A. (Noha M. Almoraie) and I.M.S.; writing—original draft preparation, N.M.A. (Noha M. Almoraie), I.M.S, N.M.A. (Najlaa M. Aljefree) M.A.H. and H.A.W; writing—review and editing, N.M.A. (Najlaa M. Aljefree), M.A.H. and M.A.A.; project administration N.M.A. (Noha M. Almoraie). All authors have read and agreed to the published version of the manuscript.

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Ethics Approval: The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Unit of Biomedical Ethics Research Committee at King Abdulaziz University (reference no. 631-19).

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