

Effects of *Hibiscus sabdariffa* L. tea intake on antioxidant response and biochemical profile in healthy Wistar rats

Efeitos do consumo do chá de *Hibiscus sabdariffa* L. Sobre a resposta antioxidante e perfil bioquímico em ratas Wistar saudáveis

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Abstract

Introduction: *Hibiscus sabdariffa* L. have therapeutic potential characteristics and is rich in phytochemicals with anti-inflammatory, antibacterial and antioxidant effects. **Objective:** the study aims to evaluate the daily consumption of hibiscus tea, in a concentration usually consumed by the population, on biochemical parameters and antioxidant activity in healthy female adult Wistar rats. **Methodology:** animals were divided into Control Group (CG), which received filtered water and commercial diet, and Hibiscus Group (HG), which received 15 ml/day of *Hibiscus sabdariffa* L. flower tea (prepared 1g of flower/100ml of water), commercial diet and filtered water. Hibiscus flower infusion for tea preparation was performed daily (ratio of 1.0g of dried flower to 100 ml of filtered, boiled water), infused for 10 minutes, strained, and offered daily to animals (15 ml/day/animal) for 84 days. After 84 days of experimental protocol, animals were euthanized and evaluated serum biochemical parameters such as urea, creatinine, calcium, magnesium, phosphorus, iron, total proteins, albumin, lipid profile and fasting glycemia. Analysis of antioxidant components was performed on hibiscus tea, and serum antioxidant activity data was also analyzed by 2,2-difenil-1-picrylhydrazyl (DPPH), Oxygen Radical Absorbance Capacity (ORAC) and total antioxidant activity by iron reduction (FRAP) methods. **Results:** hibiscus tea has a high antioxidant potential and reduces fasting glucose ($p < 0.05$), but in the concentration offered, this antioxidant capacity was not observed at serum level, without changes in body mass, water intake, food consumption, lipid profile, liver and kidney biomarkers. **Conclusion:** hibiscus tea daily consumption showed higher antioxidant potential and reduced fasting blood glucose without toxic effects. **Keywords:** *Hibiscus sabdariffa*; antioxidants; functional foods; diabetes.

Resumo

Introdução: *Hibiscus sabdariffa* L. possui características de potencial terapêutico, rico em fitoquímicos com ação anti-inflamatória, antibacteriana e antioxidante. **Objetivo:** avaliar os efeitos do consumo diário de chá de hibisco, na concentração usualmente consumida pela população, sobre parâmetros bioquímicos e atividade antioxidante em ratas Wistar adultas saudáveis. **Metodologia:** os animais foram divididos em Grupo Controle (GC), recebeu água filtrada e dieta comercial, e Grupo Hibisco (GH), que recebeu 15 ml/dia de chá da flor *Hibiscus sabdariffa* L. (preparado 1g da flor/100ml de água), dieta comercial e água filtrada. A infusão da flor de hibisco para preparo do chá foi realizada diariamente (relação de 1,0g de flor seca/100 ml de água fervida filtrada), infundida por

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foram divididos em Grupo Controle (GC), recebeu água filtrada e dieta comercial, e Grupo Hibisco (GH), que recebeu 15 ml/dia de chá da flor *Hibiscus sabdariffa* L. (preparado 1g da flor/100ml de água), dieta comercial e água filtrada. A infusão da flor de hibisco para preparo do chá foi realizada diariamente (relação de 1,0g de flor seca/100 ml de água fervida filtrada), infundida por

10 minutos, coada e oferecida diariamente aos animais durante 84 dias. Após 84 dias do protocolo experimental, os animais foram eutanasiados e avaliados parâmetros bioquímicos como uréia, creatinina, cálcio, magnésio, fósforo, ferro, proteínas totais, albumina, perfil lipídico e glicemia de jejum. A análise dos componentes antioxidantes foi realizada no chá de hibisco e a atividade antioxidante sérica foram analisados pelos métodos 2,2-difenil-1-picrilhidrazil (DPPH), capacidade de absorção de radicais de oxigênio (ORAC) e atividade antioxidante total por redução de ferro (FRAP). **Resultados:** o chá de hibisco apresentou alto potencial antioxidante, reduziu a glicemia de jejum ($p < 0,05$), sem alterações na capacidade antioxidante sérica, na massa corporal, ingestão hídrica, consumo alimentar, perfil lipídico, e biomarcadores hepáticos e renais. **Conclusão:** o consumo diário do chá de hibisco, apresentou maior potencial antioxidante e redução da glicemia de jejum, sem efeitos tóxicos.

Palavras chaves: *Hibiscus sabdariffa*; antioxidantes; alimentos funcionais; diabetes.

INTRODUCTION

Individual lifestyle, with a balanced diet in macro and micronutrients, combined with an active lifestyle with regular physical activity, seems to be the best strategy for preventing Chronic Noncommunicable Diseases (NCDs) and comorbidities, such as obesity and type 2 Diabetes¹. In addition, it has been suggested that foods rich in bioactive compounds such as polyphenols, sulfur compounds, carotenoids and anthocyanins, for example, present themselves as a good alternative in NCDs prevention or treatment^{2,3}.

*Those bioactive compounds are easily found in vegetables, fruits, cereals and grains. Still, some herbs and plants have been emphasized in the literature that, when incorporated into a regular diet, seem to aid in weight loss and bring other benefits⁴. *Hibiscus sabdariffa* flower is one of those plants that seems to act therapeutically in reducing body weight due to the ability to reduce body fat accumulation by reducing adipogenesis. In addition, it presented a hepatoprotective role and gastrointestinal regulation, improving lipid profile and blood pressure, when adjunct to traditional treatment of the combination of food and physical activity⁵.*

Hibiscus sabdariffa L., commonly known as Roselle, is an annual edible plant of the Malvacea family, widely cultivated in African and Southeast Asian countries, and of great importance in the food industry due to the high nutritional content it gives to preparations such as juices, salads and jams. Aqueous infusions (tea) prepared with plants have a reddish tone associated with the content of anthocyanins present and an acidic flavour related to organic acids content. They are the most common form of consumption worldwide⁶. Its composition is rich in carbohydrates, proteins, fats and fibers, as well as vitamins and minerals (phosphorus, iron, calcium, thiamine, riboflavin, niacin) phytosterols, polyphenols such as flavonoids (quercetin and luteolin), anthocyanins, organic acids (citric, malic, ascorbic acid) and tartaric), phenolic acids, mainly protocatechuic acid and trace elements, which vary according to the part of the plant used and which confer pharmacological and therapeutic properties, and their composition is associated with their benefits and high antioxidant potential⁷.

Hibiscus sabdariffa L. components are related to carcinogenic, anti-inflammatory and mainly adjuvant effects in diabetes control, cardiovascular diseases and metabolic syndrome associated with obesity^{4,8}. However, despite many reports in the scientific literature about its benefits, only some studies still define the dose and

*forms of consumption of *Hibiscus sabdariffa* to access the bioactive compounds present in plants. Studies suggest that the effects of these components can be improved when comparing aqueous, alcoholic and polyphenolic plant extraction, and the dose of extract that must be consumed for these benefits, and in high doses, may even have adverse effects^{9,10}.*

*Thus, the present study aims to evaluate the daily consumption of aqueous infusion (tea) of *Hibiscus sabdariffa* in the body and biochemical parameters (lipid, liver and renal profile) and antioxidant activity in healthy adult Wistar rats to contribute to expanding scientific knowledge and discussion about daily consumption of *Hibiscus sabdariffa* tea. It should also be considered that there is an incentive to consume this plant due to possible benefits in NCDs prevention, particularly type 2 Diabetes Mellitus. However, few studies still describe molecular, functional and metabolic mechanisms and potential adverse effects associated with habitual consumption of this tea.*

METHODOLOGY

Ethics Committee

The present project was submitted to the Ethics Committee in the Use of Animals of the Universidade Federal Fluminense (UFF) and approved under protocol number 1029, which follows guidelines adopted by the National Council for the Control of Animal Experimentation (CONCEA), in accordance with the Law at the 11,794 sanctioned in 2008. All experiments were performed to minimize the number of rats and suffering caused by the procedures following the ethical doctrine of three "Rs" – reduction, refinement and replacement (Reduction, Refinement, Replacement).

Experimental Design

*The study was conducted at the Experimental Nutrition Laboratory of the Fluminense Federal University (LABNE/UFF). Ten female rats (*Rattus Norvegicus*, Wistar albino) were used, with approximately 90 days of life, provided by the Center for Laboratory Animals at UFF.*

*The 90-day-old animals were kept for another 84 days in individual polypropylene cages in an environment with controlled temperature ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and adequate lighting (light and dark cycle 12-12 hours), divided into two groups ($n=5/\text{group}$): 1) Control Group (CG) – received filtered water and commercial diet, *ad libitum*; 2) *Hibiscus* Group (HG) – received 15 ml/day of *Hibiscus sabdariffa* L.*

flower tea (prepared in the ratio of 1g of flower to 100ml of water), commercial diet and filtered water, ad libitum.

All groups received commercial diets (Nuvilab, Quintia LTDA) and filtered water ad libitum during the experimental period. The flower of *Hibiscus sabdariffa* L. was acquired in the local market, with a validity of 4 months from the day of purchase and stored in a glass pot protected from sunlight. Infusion of flower for tea preparation was performed daily by members of the research group, using the ratio of 1.0g of dried flower to 100 ml of filtered, boiled water, infused for 10 minutes, then strained, and offered to animals in small volumes drinkers (packed with aluminum foil to protect the drink from light), in the amount of 15 ml/day/animal to Hibiscus group¹¹.

The animals were weighed weekly on an electronic scale. The beverage consumption control was carried out in a graduated cylinder, and food consumption was determined by weighing the supply and leftover food using an electronic scale weekly. All data on body mass and food consumption were recorded in individual sheets during days of care until the end of the experiment (84 days). At the end of 84 days of study, animals were submitted to the procedure of vaginal smear to identify the estrous cycle phase, and all rats that were in the "estrus" phase fasted for 6 hours for euthanasia. After this period, animals were anesthetized with an intraperitoneal injection of xylazine hydrochloride associated with ketamine (1:1 solution, amount 0.1 ml/200g of body mass) for blood collection through cardiac puncture and tissue collection (liver and adipose tissue) for further analysis.

Blood aliquots were collected and transferred to vacutainer tubes without anticoagulant. After this process, blood was centrifuged at 3000 rpm for 20 minutes to obtain serum, and aliquots were separated into micro tubes and stored (-80°C) for biochemical analyses. Tissues collected were weighed and stored in a freezer (-80°C).

Analysis of antioxidant components of the *Hibiscus sabdariffa* L.

Determination of total phenolic compounds in *Hibiscus sabdariffa* L. tea. The folin-Ciocalteu method was used to analyze tea's total phenolic compounds from the *Hibiscus sabdariffa* L. flower. Gallic acid was used as a standard in the concentration range of 5-40 mg/ml-1 to construct the calibration curve. Aliquots of 0.05, 0.075 and 0.1 ml of *Hibiscus sabdariffa* L. tea infusion were used. Readings were performed in triplicate at a wavelength of 750nm (Turner 340 spectrophotometer). The concentration of total phenolic compounds in tea was expressed in mg of gallic acid/ml.

2,2-difenil-1-picrylhydrazyl (DPPH)

The measurement of DPPH radical scavenging activity. To evaluate antioxidant activity, 100µL of tea was used for reaction with the DPPH radical (0.004g) at a concentration of 0.1 mM in a methanol solution (100 ml).

The radical reduction of DPPH was measured by reading the absorbance at 515nm in 100 minutes of reaction. Antioxidant activity was expressed according to the Equation described below: %AA = 100 - [(Sample Abs - White Abs) X 100] / Control Abs}.

Biochemical parameters

At the end of the experiment, the animal's blood was centrifuged at 3000 rpm for 20 minutes to obtain serum to determine biochemical analyses. Urea (mg/dl), creatinine (mg/dl), Calcium (mmol/l), Magnesium (mmol/l), Phosphorus (mmol/l), Iron (µmol/l), total proteins (g/dl), albumin (g/dl), lipid profile (Higher Density lipoprotein HDL-c, Low-Density Lipoproteins LDL-c cholesterol, very-low-density lipoproteins cholesterol (VLDL-c), total cholesterol (TC) and triglycerides, mg/dl) and aspartate aminotransferase (TGO/AST) (U/L) and alanine aminotransferase (TGP/ALT) (U/L), were performed by colorimetric method, read in an automated spectrophotometer (BioClin® BS-120 Chemistry Analyzer®). Commercial BioClin® kits and specific wavelengths were used for each biochemical indicator. LDL-c and VLDL-c were determined by mathematical formulas considering TC, triglycerides and HDL-c. VLDL-c was calculated using triglyceride value divided by five, and LDL was calculated using CT values (CT) subtracted from the sum of HDL-c + VLDL-c. The fasting glucose was determined in blood from caudal circulation by an ACCUE CHECH ACTIVE® glucometer and expressed in mg/dl.

Serum antioxidant activities

Oxygen Radical Absorbance Capacity (ORAC)

This method measures the ability of the antioxidant to scavenge peroxy radicals generated by a radical source, AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride), at 37 °C. The peroxy radical, generated by the reaction with atmospheric oxygen with a radical source, interacts with a fluorescent indicator, generating a non-fluorescent product, measured by spectrophotometry with maximum fluorescence emission at 575 nm and 578 nm¹². The antioxidant activity of blood samples was determined through the difference between areas of the sample subtracted by the blank, measured by the fluorescence decay with antioxidant substance addition. Using Trolox of known concentrations, a standard curve was generated, and the ORAC activity of the sample was calculated. The samples were lyophilized, and 0.01g of blood sample was used. The results were expressed in ORAC units or Trolox equivalents per micromol.

Analysis of determination of total antioxidant activity by the iron reduction method (FRAP)

The antioxidant activity of animals' blood was determined by the FRAP method. This method is based on quantifying the reduction capacity of the Fe+3 - TPTZ complex (light blue colour) to the Fe+2 - TPTZ complex

(dark blue colour) in an acid medium, where the reaction by the sample's antioxidant occurs. The method consists of adding 100µl of deproteinized blood samples, using methanol and quantifying it in 2.7ml of FRAP reagent added to 270µl of distilled water. After preparation, the solution was kept in a water bath for 30 minutes, after which the absorbance was read at 595 nm. Results were expressed as µM of ferrous sulfate per gram of sample. The standard curve was produced with a ferrous sulfate solution using four different dilutions¹².

Serum DPPH

The DPPH radical measures the antioxidant activity. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a free radical that is reduced in the presence of an antioxidant. To evaluate antioxidant activity, the blood samples, after deproteinization, using methanol, were added for reaction with the stable radical DPPH in a methanol solution. DPPH has a characteristic absorption at 515 nm in the radical form, which disappears after reduction by hydrogen stripped from an antioxidant compound. The radical reduction of DPPH was measured by reading the absorbance at 515nm in 100 minutes of reaction. Antioxidant activity was expressed according to the equation described below: %AA = 100 - {[(Sample Abs - White Abs) X 100] / Control Abs}.

Statistical Analysis

Data were analyzed using the GraphPad Prism 9 statistical program and expressed as mean ± standard error of the mean. One-way ANOVA analysis of variance was used, and the t-student test was applied as a post-test,

being considered statistically significant when $p < 0.05$.

RESULTS

Hibiscus sabdariffa tea's antioxidant capacity

The analysis of total phenolic compounds performed in Hibiscus sabdariffa tea solution presented 21986.53 mg gallic acid/ml of total phenolics (Table 1), evidencing a high content of phenolic compounds in preparation offered. According to DPPH analysis, the Hibiscus sabdariffa tea presented a 77.24% antioxidant capacity (Table 1).

Table 1 – Hibiscus sabdariffa tea antioxidant capacity

	Hibiscus sabdariffa tea
Total phenolic compounds (mg gallic acid/100ml)	21.98± 6.82
DPPH (%)	2.33

Subtitle: DPPH = 1,1-diphenyl-2-picrylhydrazyl.

Source: research data

Body mass parameters and diets, water and tea intake.

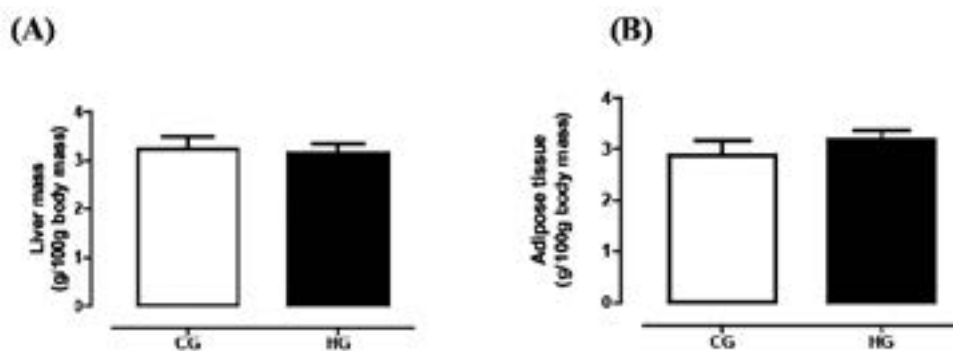
The results of body mass, water and tea intake and food consumption are presented in Table 2. Initial and final body mass and weight gain of group Hibiscus sabdariffa tea did not show a statistical difference compared to the control group (Table 2). When we evaluated HG's food consumption and water intake, we did not observe any statistical difference when compared to CG (Table 2). In the same direction, no changes were observed in liver and adipose tissue mass in HG compared to CG (Figure 1).

Table 2 – Body mass parameters and diets, water and tea intake.

	GC (n=5)	HG (n=5)	P-value
Initial body mass (g)	241.40±19.08	248.40±5.99	$P > 0.05$
Final body mass (g)	262.80±13.77	267.30±3.56	$P > 0.05$
Body mass gain (g)	21.40±6.13	18.90±8.06	$P > 0.05$
Total diet intake (g)	13.37±64.39	13.03±182.90	$P > 0.05$
Daily diet intake (g/100g body mass/day)	6.06 ± 0.32	5.79 ± 0.75	$P > 0.05$
Filter water intake (ml/100g b.m./d)	10.26 ± 1.13	8.21 ± 4.10	$P > 0.05$
Hibiscus sabdariffa tea intake (ml/100g b.m./d)	nd	4.55 ± 0.87	nd

Subtitle: CG = Control group, animals that received commercial diet and filter water during 84 days; HG = Hibiscus group, animals that received commercial diet, filter water and Hibiscus sabdariffa tea during 84 days; nd = no determined. The results were expressed as mean ± standard error of the mean.

Figure 1 - Liver and adipose tissue mass.



Subtitle: CG = Control group, animals that received commercial diet and filter water during 84 days of treatment; HG = Hibiscus group, animals that received commercial diet, filter water and *Hibiscus sabdariffa* tea during 84 days of treatment; nd = no determined. The results were expressed as mean \pm standard error of the mean.

Biochemical parameters

HG presented no changes in total protein, albumin, minerals profile, urea, creatinin, liver enzymes and lipid

profile compared to CG (Table 3 and Figure 2). HG presented lower fasting glucose (-15%, $p < 0.05$) when compared to CG (Figure 3).

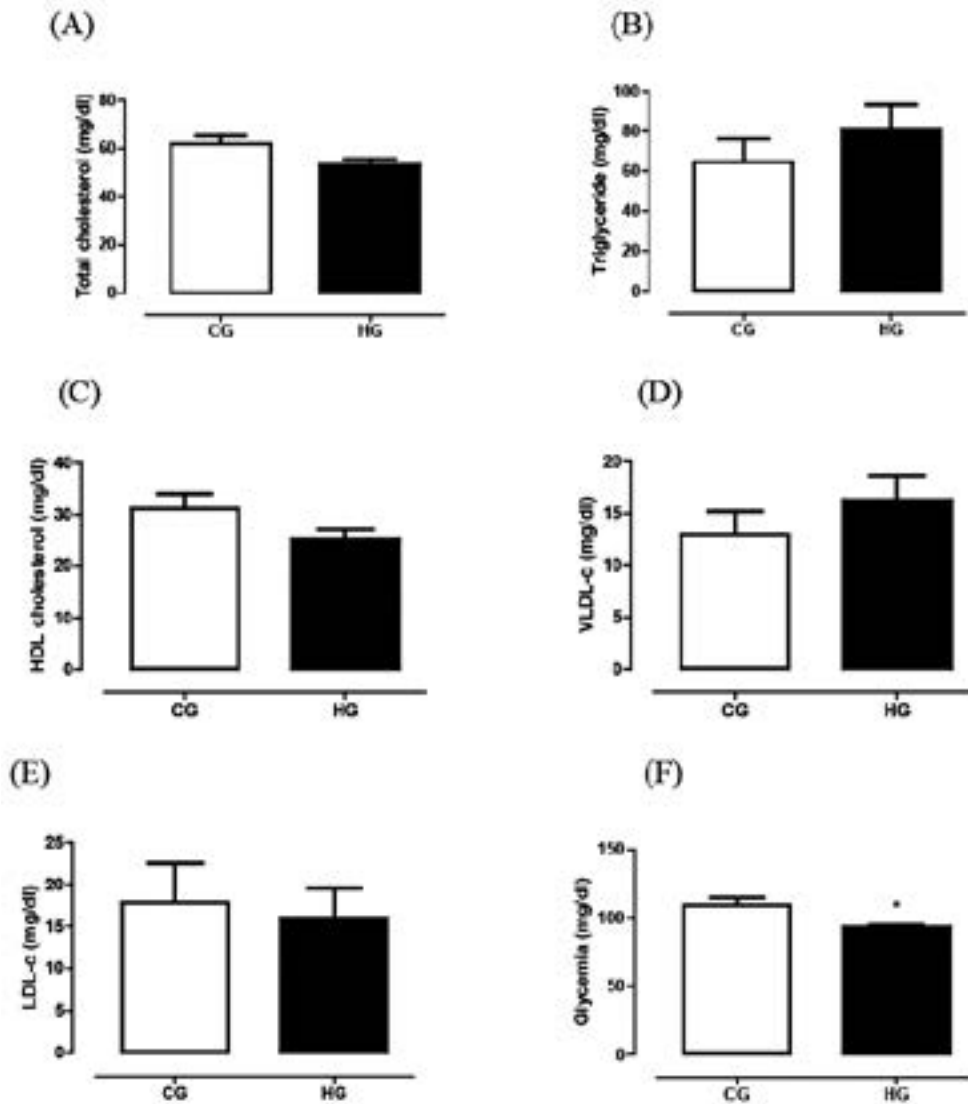
Table 3-Serum biochemical parameters.

	CG (n=5)	HG (n=5)	P-value	References values (Diniz et al. 2006)
Total protein (g/dl)	6.60 \pm 0.52	5.94 \pm 0.97	0.21	5.00 – 7.7 mg/dl
Albumin (g/dl)	2.66 \pm 0.32	2.56 \pm 0.26	0.60	1.3 – 3.8 mg/dl
Calcium (mg/dl)	9.52 \pm 1.96	9.38 \pm 2.36	0.92	6.7 – 11.0 mg/dl
Phosphor (mg/dl)	4.34 \pm 0.55	4.42 \pm 0.60	0.83	3.00 -11.00 mg/dl
Magnesium (mg/dl)	1.62 \pm 0.39	1.68 \pm 0.18	0.76	1.60 – 4.40 mg/dl
Iron (μ g/dl)	160.80 \pm 33.83	160.6 \pm 30.83	0.99	nd
Urea (mg/dl)	49.40 \pm 3.43	45.80 \pm 10.62	0.49	24 – 49 mg/dl
Creatin (mg/dl)	0.66 \pm 0.05	0.68 \pm 0.08	0.66	0.28 – 1.10 mg/dl
Aspartate aminotransferase (U/L)	168.00 \pm 21.37	145.80 \pm 13.02	0.09	nd
Alanine Aminotransferase (U/L)	56.00 \pm 6.68	49.80 \pm 11.78	0.38	nd

Subtitle: CG = Control group, animals that received commercial diet and filter water during 84 days of treatment; HG = Hibiscus group, animals that received commercial diet, filter water and *Hibiscus sabdariffa* tea during 84 days of treatment; nd = no determined. The results were expressed as mean \pm standard error of the mean.

Source: research data

Figure 2 – Lipid profile and fasting glucose

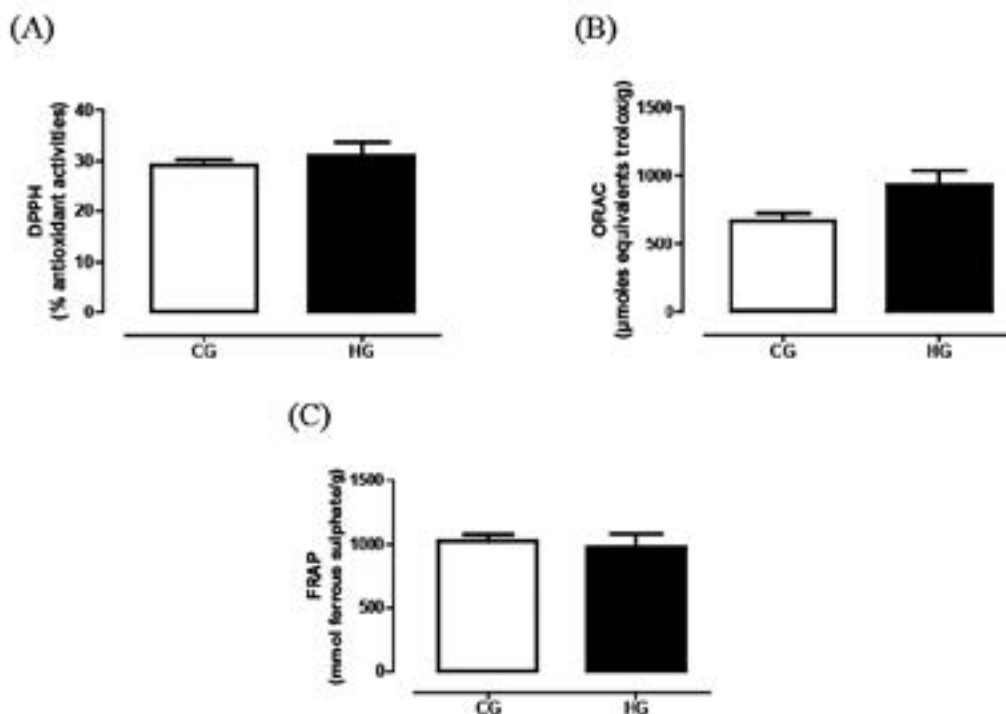


Subtitle: CG = Control group, animals that received commercial diet and filter water during 84 days of treatment; HG = Hibiscus group, animals that received commercial diets, filter water and Hibiscus *sabdariffa* tea during 84 days of treatment; HDL-c = High-Density Lipoproteins; LDL-c = Low-Density Lipoproteins; VLDL-c = very-low-density lipoproteins. The results were expressed as mean \pm standard error of the mean, * vs C, with $p < 0.05$.

Antioxidant activities (DPPH, ORAC e FRAP)

According to serum analysis of antioxidant activities, no changes were observed in HG when compared to CG for DPPH, ORAC and FRAP analysis (Figure 3, $p > 0.05$).

Figure 3 – Antioxidant activity in serum after 84 days of treatment.



Subtitle: CG = Control group, animals that received commercial diet and filter water during 84 days of treatment; HG = Hibiscus group, animals that received commercial diet, filter water and Hibiscus sabdariffa tea during 84 days of treatment; DPPH= 2,2-diphenyl-1-picrylhydrazyl; ORAC= Oxygen Radical Absorbance Capacity; FRAP= Ferric-Reducing Ability of serum. The results were expressed as mean \pm standard error of the mean.

DISCUSSION

The present study demonstrated that oral consumption of *Hibiscus Sabdariffa* tea for 84 days to healthy adult female rats did not show significant changes in body mass, food and water consumption, or biochemical parameters. However, we observed a significant reduction in fasting blood glucose after treatment with *Hibiscus sabdariffa* tea.

Hibiscus sabdariffa tea already had its medicinal consumption commonly known in some Western countries. It has gained popularity in tropical countries due to its diuretic functional properties, which can reduce body fat mass and blood glucose, and its high antioxidant potential¹³.

In the present study, we analyzed the antioxidant capacity of *Hibiscus sabdariffa* tea offered to healthy female rats for 84 days, and antioxidant activity in that hibiscus concentration and way of tea preparation was considered satisfactory and equivalent to values found in scientific literature¹⁴. The concentration of *Hibiscus sabdariffa* used in the infusion (1g/100ml of boiled water) presented a total of 21.98 ± 6.82 mg/100ml gallic acid equivalents of total phenolic, evidencing a high content of phenolic compounds in preparation, with an index of 77.24% of antioxidant activity measured by the DPPH method. Preciado-Saldanã et al.¹⁵ (2019) demonstrated that the tea preparation with 4.9 g of hibiscus in 100ml warm water presented 14.80 ± 1.4 mg/100ml gallic acid equivalents of total phenolic content.

In the same direction, the antioxidant capacity of *Hibiscus sabdariffa* extracts is well described in the literature due to its rich composition in phenolic compounds and plant antioxidants for disease prevention. Sobota, Pinho, Oliveira¹⁶ (2016) demonstrated the dosage of total polyphenols, flavonoids and antioxidant activity against the DPPH radical of extracts obtained by aqueous decoction, ethanolic decoction, aqueous infusion and ethanolic infusion; they observed that ethanolic extracts had higher levels of polyphenols, and there is no difference to infusion and decoction. However, the aqueous method, commonly used by the population that consumes *Hibiscus sabdariffa* tea, the decoction method showed a 44.59% increase in yield¹⁶. It also demonstrated a more significant antioxidant activity of aqueous extract solution obtained by decoction (measured by the DPPH method), followed by ethanolic extracts and a lower index in aqueous extract obtained by infusion¹⁶. Despite the infusion presenting lower antioxidant activity to decoction and ethanolic extraction methods, our study showed that tea infusion presented high antioxidant activity, which corroborates the literature. It is essential to highlight that phenolic compound content and antioxidant activity directly related to extracting the components and tea preparing techniques.

Chang et al.¹⁷ (2014) performed a clinical trial in which one group received two capsules made with 450 mg of *Hibiscus sabdariffa* extract (HSE) and 50 mg of starch, and the other group received two capsules of 500mg of starch three times a day, after meals. After 12 weeks of

supplementation, it was observed that 70% of the individuals in the group that received HSE supplementation showed a reduction in body mass and BMI, accompanied by a reduction in body fat and waist circumference. Villalpando-Arteaga et al.¹⁸ (2013) also observed a reduction in body weight in an experimental study with obese mice treated with 33mg/kg of dry extract of *Hibiscus sabdariffa* three times a week for eight weeks. However, in our study, no changes were observed in body parameters, which can be related to the hibiscus used to prepare the tea, techniques and duration of treatment.

Hibiscus sabdariffa seems to be an excellent adjuvant against obesity due to polyphenols composition, that act directly on proteins related to intermediary metabolism such as peroxisome proliferator-activated receptor (PPAR), fatty acid synthase (FASN), lipase, adiponectin, leptin, monocyte chemoattractant Protein-1 (MCP-1), adenosine monophosphate-activated protein kinase (AMPK), nuclear factor kappa B (NF- κ B) and superoxide dismutase (SOD). In addition, *Hibiscus sabdariffa* extract can act in inflammation signalling pathways and energy metabolism, restoration and cellular function.⁷ Our study observed no difference between the groups in visceral fat mass, liver tissue weight and markers. Several studies demonstrated that *Hibiscus sabdariffa* extract led to a reduction in body mass and body fat mass^{19,20}. It is known that AST and ALT, present in the liver, kidneys, and skeletal muscle, are two transaminases that, when altered, indicate initial acute liver injury²¹. Thereby, both groups received the same diet. We suggest that hibiscus tea at the concentration used did not lead to liver toxicity, probably due to the high presence of antioxidant compounds.

Kao et al.²² (2016) demonstrated the benefits of *Hibiscus sabdariffa* extract in the hepatic system, which obese rats that received *Hibiscus sabdariffa* tea showed dose-dependent AST and ALT reduction. Njinga et al.²³ (2020) evaluated acute (300 and 2000mg/kg), subacute (for 28 days) and subchronic (for 90 days) of *Hibiscus sabdariffa* L. extract administered in 3 different doses (125mg, 250mg and 500 mg of aqueous extracts of *Hibiscus sabdariffa* L.), respectively prepared in cold distilled water and demonstrated that extract was relatively less toxic with subacute than subchronic administrations.

Recently, Manzano-Pech et al.²⁴ (2022) evaluated the toxicity of *Hibiscus sabdariffa* L. (HSL) supplementation in drinking water in different concentrations such as 15, 30, and 60 g/ liter of the HSL calyxes for Wistar rats during four weeks and no toxicity effects were observed in the concentration of 15g/ liter. In our study, we aimed to reproduce the most usual form of consumption of *Hibiscus sabdariffa* L. by the population through aqueous infusion with a lower amount of *Hibiscus*, which the concentrations used represented 10g/ liter in boiled water and no toxicity in rats was observed. Therefore, more studies are necessary to determine the security dose of *Hibiscus sabdariffa* by population.

According to urea and creatinine serum concentrations, which are glomerular filtration (GFR) markers, we demonstrated that both groups presented similar concentrations within normal parameters for rats. Thus, we suggest that oral consumption of *Hibiscus sabdariffa* L. tea did not cause renal overload in these animals. The same was observed regarding proteins and minerals serum concentrations in our study. On the other hand, Njinga et al.²³ (2020) demonstrated that oral administration of *Hibiscus sabdariffa* L. significantly increased total protein, albumin, globulin, sodium, chlorine and reduction of uric acid, creatinine, hemoglobin and serum lipids. However, our study observed opposite results, probably due to the low concentration of *Hibiscus sabdariffa* tea in the oral solution offered to the animals and maybe because they are healthy animals.

In our study, *Hibiscus sabdariffa* L. tea was able to reduce fasting glucose in healthy adult animals, even in low concentrations; hibiscus tea can help control blood glucose since the present study did not perform any pathological induction or intervention in the usual diet therapy of the animals. Thus, *Hibiscus sabdariffa* L. can be an important adjuvant in preventing and treating type 2 Diabetes. Adeyemi et al.²¹ (2014) showed in diabetes animals, the administration of *Hibiscus* extract restored liver markers, reduced levels of antioxidant enzymes such as glutathione, catalase and superoxide dismutase and reduced AST and ALT. *Hibiscus sabdariffa* L. presents higher amounts of anthocyanins, which seem to have the potential to inhibit the DPP-4 enzyme, which influences the catalytic pathway and reduces GLP-1 activity^{25,26}. GLP-1 concentrations seem to be lower under normal conditions of type 2 diabetes. Kartinah et al.²⁷ (2019) offered two doses of 200mg/kg and 500mg/kg of body weight, respectively, of aqueous extract of *Hibiscus sabdariffa* L. to streptozotocin-induced diabetic and non-diabetic rats and observed that diabetic rats that received the dose of 500mg/kg of body weight did not show a reduction in GLP-1 enzyme as expected by the pathological condition. Thus, hibiscus extract was able to maintain GLP-1 secretion due to its content of polyphenols and anthocyanins. Mohamed et al.²⁸ (2013) showed that streptozotocin-induced diabetic rats, a diabetic rats group treated with *Hibiscus sabdariffa* L., improved glycemic parameters and lipid profile, followed by an increase superoxide dismutase, suggesting a reduction in oxidative damage caused by streptozotocin. Several studies demonstrated the positive effects of *Hibiscus sabdariffa* L. on lipid profile due to higher concentration of anthocyanins, polyphenols and soluble fibers, which can reduce triglycerides and increase HDL, with consequent reduction of LDL^{6,29-31}.

Carvajal-Zarrabal et al.¹⁹ (2005) demonstrated in a study with male Sprague-Dawley rats with atherogenic characteristics induced by a high-fat diet were divided into four groups that received different doses of ethanol extract at concentrations of 5%, 10% and 15% of *Hibiscus sabdariffa*, during four weeks, and was observed

changes in triacylglycerol, cholesterol, total lipids, and LDL inversely proportional to the concentrations of the extract offered, demonstrating that a higher concentration improve serum benefits. In a clinical study (30 healthy subjects aged 21-55), where subjects received an aqueous extract of *Hibiscus sabdariffa* at a concentration of 1% and sweetened at a concentration of 5% of sugar which received 200 ml of this two times a day preparation for 30 consecutive days associated with physical exercises, was able to reduce abdominal circumference and systolic and diastolic pressure, increase high-density lipoproteins and reduce low-density lipoproteins, triglyceride levels and cholesterol lipoproteins, even being offered in a low concentration compared to other studies and with added sugar³². Unfortunately, in our study, the hibiscus group did not present significant changes in lipid profile compared to the control group, possibly because we used healthy adult animals and lower concentrations of hibiscus.

Antioxidant compounds in hibiscus tea act as defense mechanisms against free radicals, inhibiting and reducing possible damage³³⁻³⁴. Our study found no influence of this antioxidant capacity in serum, especially when compared with antioxidant activity in sample analysis. However, some limitations were observed in our study, such as the number of animals used per group, duration of experiment treatment and concentration of hibiscus used in tea preparation.

Thus, science literature presented several studies with *Hibiscus sabdariffa* L. However, studies are controversial about the best way of tea consumption in home preparation. Furthermore, some studies suggest hepatotoxic effects of the plant when used in extract concentration²³, especially in higher doses.

CONCLUSION

The present study suggests that daily intake of *Hibiscus sabdariffa* L. tea, prepared by infusion, was able to reduce fasting glucose but had no influence on lipid profile, body parameters, kidneys and liver markers. *Hibiscus sabdariffa* L. flower infusion presented high antioxidant activity and phenolic compounds, which can be an essential nutritional strategy in type 2 Diabetes. However, more studies are needed to elucidate molecular and cellular mechanisms involved in the modulation of antioxidant response and effects in intermediary metabolism.

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