Food and Nutrition Sciences, 2025, 16(9), 1288-1309
https://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

The Anti-Obesity and Anti-Diabetic Effect of *Hibiscus sabdariffa* in Juice Form Combination with *Bifidobacterium breve* 3 in Sprague Dawley Rats

Nigel G. Chimbetete, Shantrell Willis, Louis Shackleford, Martha Verghese*

Food and Animal Science Department, Alabama A&M University, Normal, AL, USA
Email: *martha.verghese@aamu.edu

How to cite this paper: Chimbetete, N.G., Willis, S., Shackleford, L. and Verghese, M. (2025) The Anti-Obesity and Anti-Diabetic Effect of *Hibiscus sabdariffa* in Juice Form Combination with *Bifidobacterium breve* 3 in Sprague Dawley Rats. *Food and Nutrition Sciences*, **16**, 1288-1309.
https://doi.org/10.4236/fns.2025.169074

Received: July 24, 2025
Accepted: September 22, 2025
Published: September 25, 2025

Abstract

Hibiscus sabdariffa (HS) is an edible flower that has become a common ingredient in various food matrices, including teas, beverages, and nutraceuticals, due to its functional benefits offered by its inherent phytochemicals. HS originally was grown in Africa, however, because of increased migration from African populations and Latino ethnicities into the United States, alternative medicines, which are affordable and accessible, are increasingly in demand. Increasing research suggests that HS contains a potent phytochemical profile that may prevent or delay chronic illnesses related to obesity and insulin resistance. Furthermore, the usage of probiotics strains because of their functionality in food production and added health benefits, many food production industries are incorporating the usage of microorganisms in their portfolios. Strains such as *Bifidobacterium* have been reported to play a role in insulin signaling pathways and dyslipidemia. The aim of this study was to investigate the anti-obesity and anti-diabetic effect of HS juice from dried calyces in combination with *Bifidobacterium breve* 3 on Sprague Dawley rats. The study showed the possible synergetic effect of probiotics and HS in high-fat diets fed to Sprague Dawley rats, influencing weight control and expression of antioxidative enzymes. In conclusion, this work offers insight into the mechanisms involved in antioxidative systems, endocrine functions and lipid metabolism factors that play key roles in the progression of T2D and obesity.

Keywords

Bifidobacterium breve, Hibiscus, Hypolipidemia, Hypoglycemia, Antioxidant, Adipogenesis, Diabetes

1. Introduction

Obesity is a disease that has become a global epidemic, with over 800 million individuals, including children, diagnosed and expected to increase as of 2015. In the United States, researchers estimate that by 2030, half of the adults will be diagnosed with obesity, further causing a strain on the already overburdened health care system [1] [2]. Obesity pathophysiology is characterized by various factors; however, only a few will be focused on in this study. Positive energy balance and genetic disorders associated with energy imbalance lead to hyperplasia and hypertrophy of adipose cells, which initiate a cascade of biological phenomena that characterize obesity, such as inflammation, immune suppression, hormone insensitivity and dyslipidemia [3].

Hyperplasia and hypertrophy are phenomena that correlate with the increase of exosomes and adipokines, which trigger dyslipidemia, non-alcoholic fatty liver disease (NAFLD) and insulin sensitivity [4].

In the US, the Western diet, which comprises dense calorie foods that are nutrient-deficient, carbonated sodas, high sugars and hydrated fats, is the main culprit for chronic illnesses such as obesity and diabetes. On the contrary, the non-Western diets, such as the Mediterranean diet, are associated with improved lipid metabolism and decreased probability of insulin resistance [5].

The usage of edible flowers is increasing globally due to their culinary and medicinal functionality in many food and beverage industries. In many countries, the incorporation of edible flowers in regular diet has become an alternative tool to fight the global health epidemic, especially centered around chronic illnesses [6]. The main groups of phytochemicals that offer health benefits from edible flowers are phenolic acids, flavonoids and other smaller class bioactives [7]. The edible flower in focus in this study (HS) has become a trending ingredient applied to infused beverages, sauces, carbonated beverages, and ready-to-drink beverages [8]. HS consumed as a juice or extract has been shown to influence management of blood glucose, prevention of dyslipidemia, blood pressure and body mass [9]-[11]. Bioactive compounds such as those found in HS and edible flowers are gaining momentum and are being utilized as metabolism boosters and appetite regulators. The mechanisms of action for functions mentioned are not yet fully elucidated; however, some proposed mechanisms include targeting appetite hormones such as Ghrelin and Leptin and their respective receptors [12]-[14].

Probiotics, specifically *Bifidobacterium* genus (*B. lactis*, *B. breve*), which is the focus of this study, has been reported to have anti-obesity and anti-diabetic effects via improvement of lipid metabolism and improved insulin signal transduction pathways [15]. Based on the work conducted on phytochemicals and probiotics, we can draw parallels between the two groups of bioactive compounds in their similarities involving promoting beneficial effects against chronic illnesses. To draw out inferences to this question, an animal model designed to be against key metabolic reactions/cascades in the development of obesity and diabetes could be utilized.

2. Materials and Methods

2.1. Preparation of (HS) Juice Administered in the Animal Study (Sprague Dawley Rats)

Dry (HS) calyces were obtained from Monterey Bay Spice Company and ground to a fine powder. 200 grams of (HS) powder was added to boiling water (2000 ml) for 10 min. The Hibiscus decoction was cooled, filtered, and diluted to prepare 2.5% and 5% Hibiscus juice solutions. The 2.5% juice corresponds to approximately 11 mg gallic acid equivalents (GAEs) of phenolics per gram of dried *Hibiscus sabdariffa* calyx, while the 5% juice contains about 22 mg GAE per gram according to our preliminary studies. Hibiscus juice was administered via gavage at these concentrations. Daily fluid intake was recorded across groups, and no significant differences were observed, indicating consistent treatment exposure. For the high-fat and sugar diets, the level of fat was increased to 40% of the whole diet while the percentage of probiotics (*Bifidobacterium breve* 3, Morinaga Milk Industry) was maintained at 0.25% (2.5 g per kg of feed), providing a concentration of 1×10^{11} CFU/g. This corresponds to approximately 2.5×10^{11} CFU per kilogram of feed and 2.5×10^{10} CFU per 100 grams of feed. According to supplier data, the strain maintains stability for up to 36 months at room temperature and is recognized for its documented metabolic benefits in adult humans.

2.2. Animal Care

Twenty female Sprague Dawley rats, three weeks old (Harlan, IN), were used in this study. Animals were housed in a temperature- and humidity-controlled facility at 20˚C ± 1˚C and 50% relative humidity, under a 12-hour light/dark cycle. Rats were allowed one week to acclimate to the facility prior to dietary intervention. Only female rats were used, which could restrict how well the results apply to males. Differences between sexes in fat storage, hormone levels, and gut microbiota might lead to varied effects. Future research should include both male and female subjects with larger groups to confirm these initial observations.

- **Phase 1 (Weeks 2 - 15):** Rats were fed their respective experimental diets with or without hibiscus and probiotics. This period corresponds to the growth phase until adulthood.
- **Phase 2 (Weeks 16 - 40)**: Half of the animals within each group were transitioned to a high-fat (HF), high-sugar (HS) diet. During this phase, hibiscus and probiotics were removed from the HF and HS diet and replaced with corn starch in the AIN-93 formulation.
- **Daily Monitoring**: Body weight, feed and water intake, and blood glucose levels were recorded daily throughout the 30-week feeding period.
- **Endpoint Procedures (Week 40)**: At the end of the study, terminal blood samples were collected. Rats were then euthanized, and vital organs relevant to the study were excised, weighed, and stored under appropriate conditions for further analysis.

The animals were handled in accordance with the AAMU guidelines for the protection and care of animals. The Institute of Animal Care and Use Committee (IACUC) approved the protocol for the study before beginning the experiment.

The animals were handled in accordance with the AAMU guidelines for the protection and care of animals. The Institute of Animal Care and Use Committee (IACUC) approved the protocol for the study before beginning the experiment (Table 1).

Table 1. Rats fed hibiscus in juice form (AIN-93G) diet.

Ingredient (g)	C0J	HFD/HF C0J	C2.5 J	HFD/HF 2.5J	C5J	HFD/HF 5J	BB0J	HFD BB0J	BB 2.5J	HFD/HF BB2.5J	BB 5J	HFD/HF BB5J
Cornstarch	397.5	257.5	372	232.5	297.5	207.5	395	255	370	230	345	205
Sucrose	100	100	100	100	100	100	100	100	100	100	100	100
Casein	200	200	200	200	200	200	200	200	200	200	200	200
Fiber	50	50	50	50	100	50	50	50	50	50	50	50
SO	70	105	70	105	70	105	70	105	70	105	70	105
Lard	0	105	0	105	0	105	0	105	0	105	0	105
Dextrose	132	132	132	132	132	132	132	132	132	132	132	132
MM (G)	35	35	35	35	35	35	35	35	35	35	35	35
V M	10	10	10	10	10	10	10	10	10	10	10	10
L-Cysteine	3	3	3	3	3	3	3	3	3	3	3	3
Choline	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Hibiscus	0	0	25	25	50	50	0	0	25	25	50	50
BB	0	0	0	0	0	0	2.5	2.5	2.5	2.5	2.5	2.5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

Abbreviations: SO: Soybean oil; MM: Mineral mix; VM: Vitamin mix; C: Control; J: Juice; HFD: High fat diet; HF: High fat; 0: 0%; 2.5: 2.5%; 5: 5%.

2.3. Plasma and Liver Lipid Profile Examination of Sprague-Dawley Rats

Plasma concentration of ghrelin, glucose, triglycerides, total cholesterol, high-density (HDL) and low-density lipoprotein (LDL) and cholesterol were measured by commercial kits. Alanine aminotransferase (ALT) enzyme, aspartate aminotransferase (AST) enzyme, and hemoglobin A1C levels were carried out by following instructions provided in commercial kits.

2.4. Antioxidant Activity in Liver

Portions from the livers were removed, washed in ice-cold 1.15% KCl solution, blotted, weighed and homogenized in 4 volumes of the homogenizing buffer. Homogenate was centrifuged at 10,000 *g* for 30 min, at 4˚C. Supernatant was used to determine lipid peroxidation (LPO), and antioxidative enzymes (catalase, superoxide dismutase) and detoxification enzymes glutathione-S-transferase (GST) and antioxidant glutathione (GSH) were determined using standard protocols (Cayman Chemicals, MI).

2.5. Determination of Selected Hormones and Cytokines

Selected hormones and cytokines (leptin, ghrelin, resistin, Fetuin-A, adiponectin, Interleukin-6 and TNF-*α*) were determined after collection of plasma from rats after euthanization. The protocol for determination of hormones was conducted according to manufacturer's instructions (Thermo Fischer Scientific, NY).

2.6. Determination of Anti-Inflammatory Activity

Cyclooxygenase (COX-II) (Cayman Chemical Company, Ann Arbor, MI) activity was determined according to the manufacturer's instructions.

2.7. Statistical Analysis of Animal Experiment

This experiment was designed as a pilot study to assess the feasibility of dietary intervention using *Hibiscus sabdariffa* and *Bifidobacterium breve*. The small sample size (n = 2 per group) was intended to identify trends and guide the design of future studies. Although statistical power is limited, observed trends provide valuable preliminary insight. Data were expressed as Means ± SEM. Differences were tested for statistical significance using two-way ANOVA. Differences among groups were determined using the Tukey's Studentized range test. (SAS, 2017, Cary, NC). A P value of ≤0.05 was considered to indicate significant differences.

3. Results and Discussion

3.1. Feed Conversion Ratio (FCR) and Weight Gain

Figure 1 shows the feed conversion ratio (FCR), calculated as average daily feed intake divided by weight gain. Rats fed the regular diet C2.5J (1.09) had significantly ($p \leq 0.05$) higher FCR than those fed C0J (0.59) and C5J (0.58). **Figure 2** presents weight gain data, where C2.5J-fed rats (19 g) showed significantly lower gains compared to C5J-fed rats (27 g). Additionally, rats on high-fat control diets gained significantly more weight than those on high-fat diets combined with HS. Effective dosages of hibiscus have been observed to be non-proportional. Rats fed HFHS2.5J (1.03) and HFHS5J (0.80) showed significantly ($p \leq 0.05$) higher FCR compared to HFHS0J (0.42). A similar trend was observed in high-fat diets combined with BB. HS is rich in polyphenols, particularly anthocyanins, which have been linked to weight regulation [16]. These compounds enhance liver enzyme activity, improve lipid metabolism, and upregulate UCP1 expression—a thermogenic gene that boosts energy expenditure—leading to reduced visceral fat around organs such as the liver, heart, and kidneys [11].

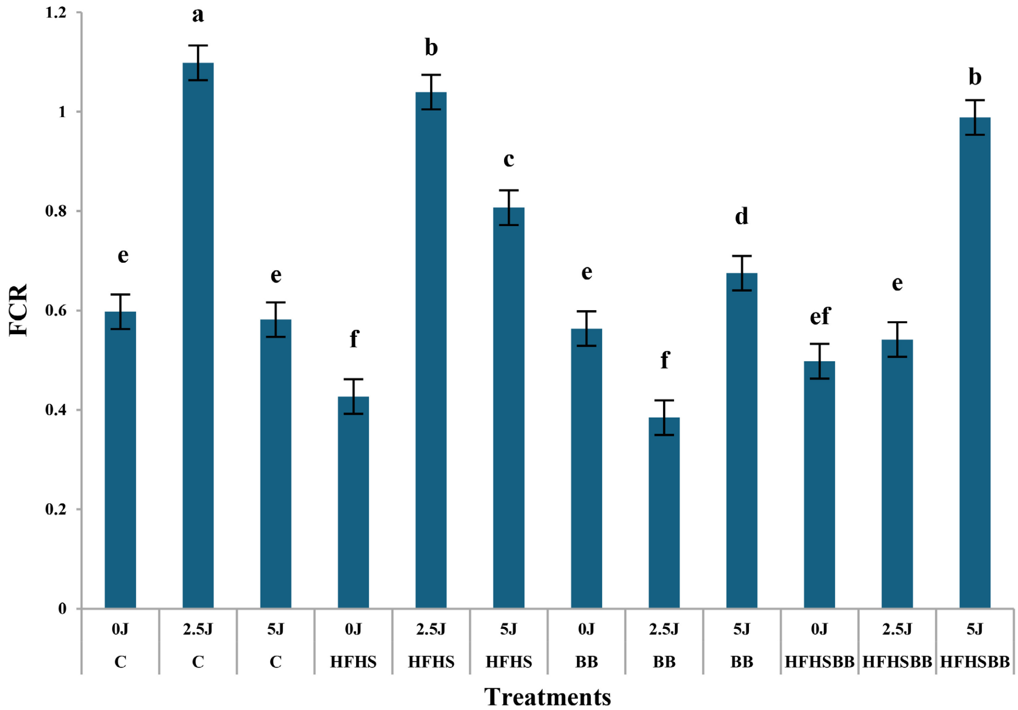

Figure 1. Feed conversion ratio (FCR) of Sprague Dawley rats fed Hibiscus juice. Values are means ± SEM. Bars with superscript (abcdef) indicating meal type effect differ significantly at p ≤ 0.05. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

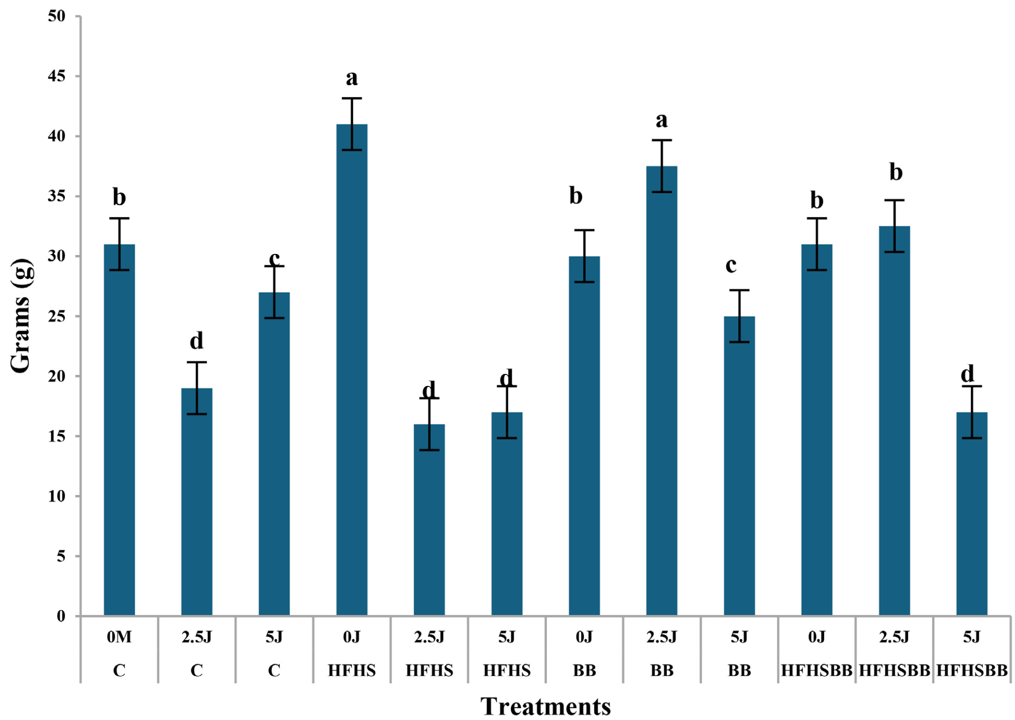

Figure 2. Weight gain in rats fed dietary Hibiscus juice. Values are means ± SEM. Means with different superscripts (abc) are significantly different (p ≤ 0.05). Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

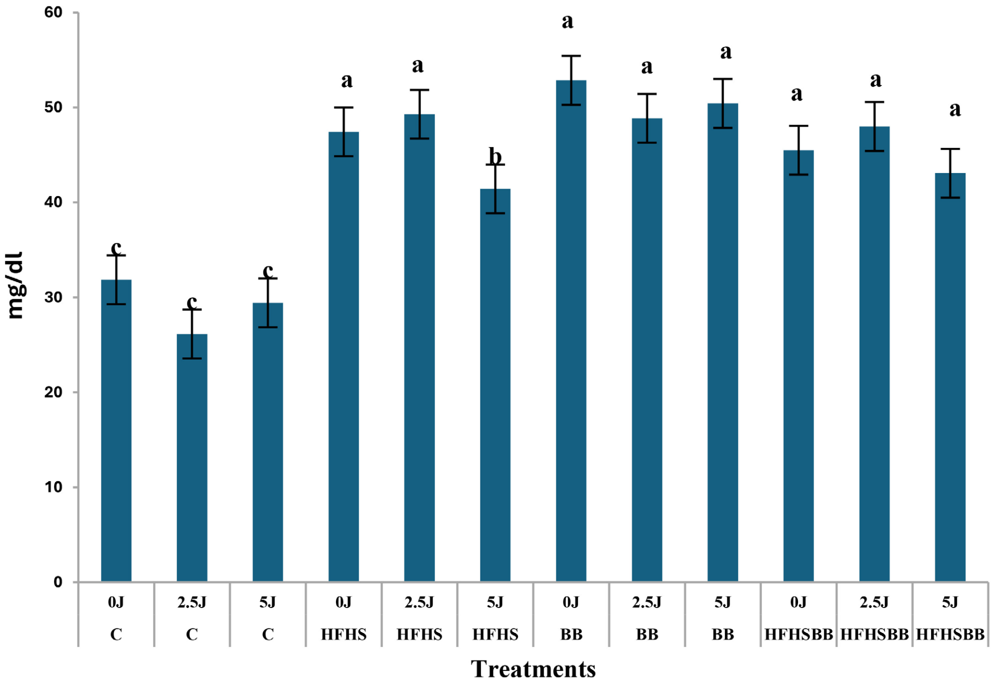

Figure 3. Blood triglycerides levels in rats fed dietary hibiscus juice. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

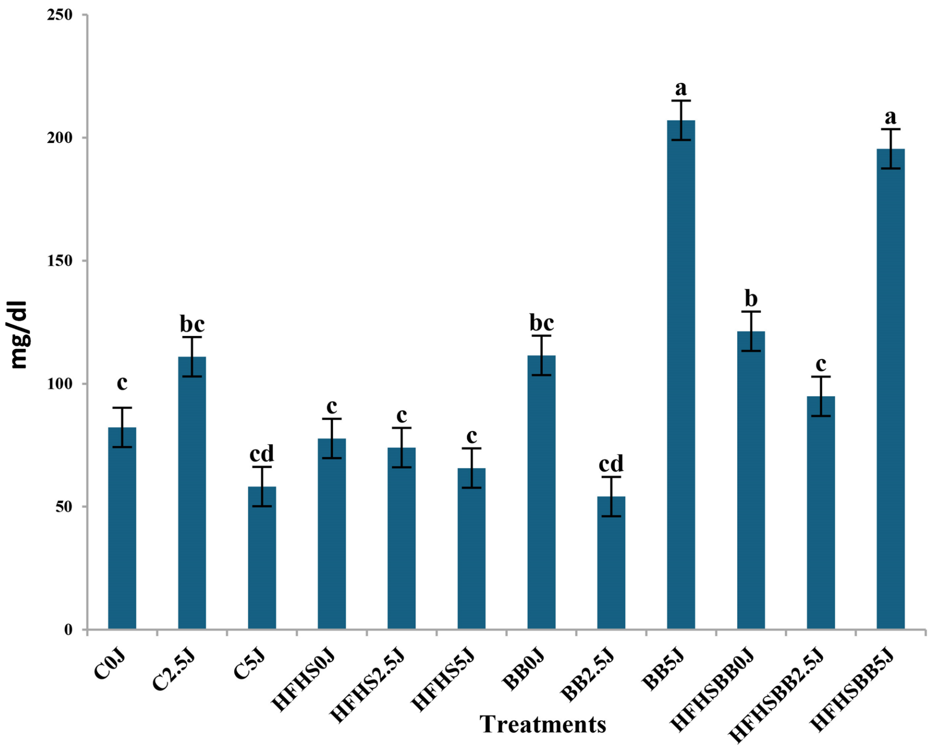

Figure 4. High-density lipoprotein levels in rats fed Hibiscus juice. Values are means ± SEM. Bars with superscript (abcd) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; J: juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

3.2. Blood Triglycerides (TG) and High-Density Lipid (HDL)

Figure 3 shows the levels of TG in the plasma of rats. Overall, rats that received a high-fat diet in combination with hibiscus had higher TG levels. Work conducted by [17], proposed a mechanism that involves hydroxycitric acid in HS, which increases lipolysis while inhibiting activity of citrate lyase, which plays a key role in the adipocyte cell differentiation and synthesis of fatty acids. Figure 4 shows the HDL level of rats. Rats fed the highest concentration of HS BB5J (207 mg/dl) and HFHSBB5J (195.4 mg/dl) had significantly ($p \le 0.05$) higher HDL levels compared to rats fed lower concentrations of HS in their treatment groups. The administration of HS could have played a significant role in the elevated levels of HDL in the rats. In a study conducted by [18] [19], HS administered in beverages, teas and capsules has a much more effective effect on lipid profiles compared to other means of ingestion or food matrices. Moreover, they observed that the administration of 1 Liter of HS juice daily, including other factors such as physical exercise and consuming similar foods that may contain similar polyphenol profiles, such as purple fruits, could aid in improving dyslipidemia. This indicates that our study showed similar positive results in the administration of HS in juice form compared to meal form.

3.3. Blood Glucose and HbA1c Levels

Figure 5 shows the blood glucose levels. The highest blood glucose level was seen in the rats fed high-fat diets HFHSBB2.5J (114 mg/dl), HFHSBB5J (111 mg/dl), and

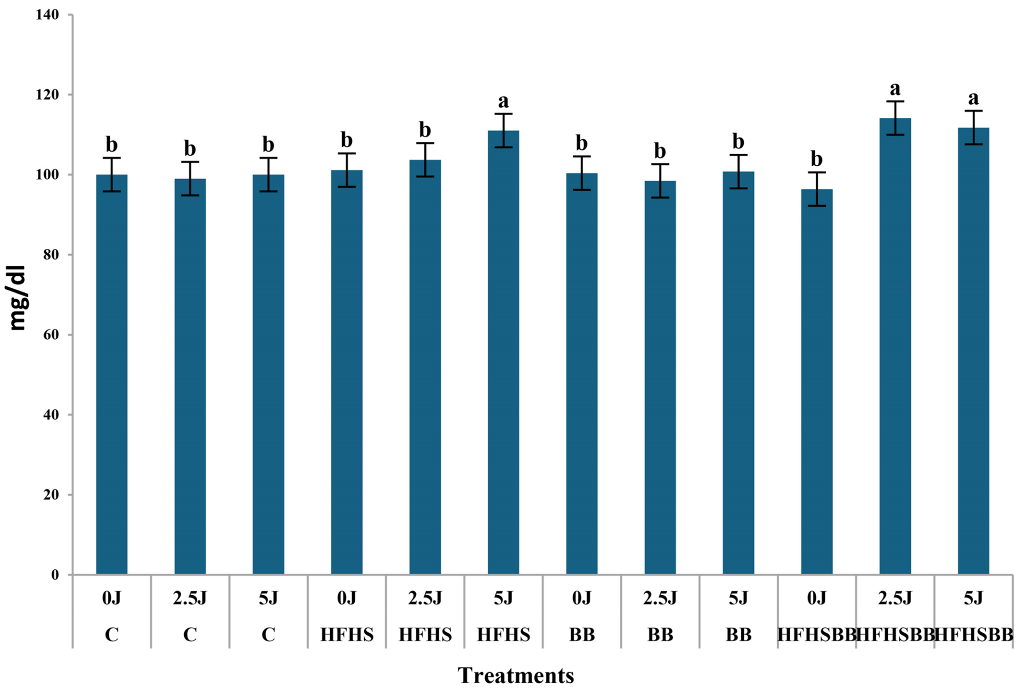

Figure 5. Blood glucose levels in rats fed dietary Hibiscus juice. Values are means ± SEM. Means with different superscripts (abc) are significantly different ($p \le 0.05$). Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

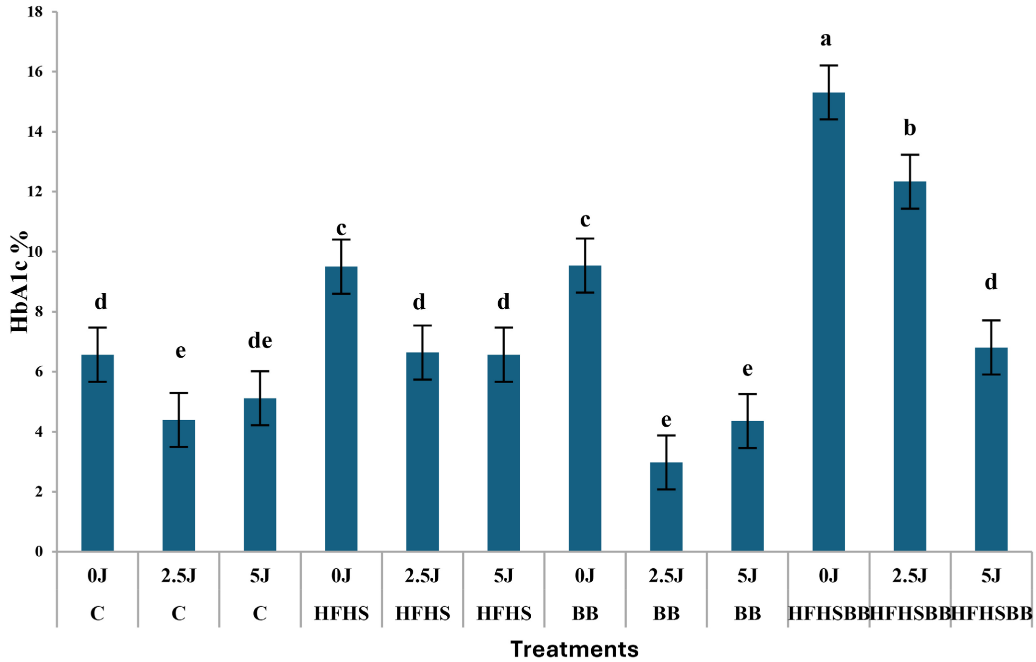

Figure 6. Plasma HbA1c levels in rats fed dietary Hibiscus juice. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

HFHS5J (111 mg/dl). Rats that received high fat in their diet in combination with or without BB or Hibiscus had the highest average blood glucose levels. Figure 6 shows the HbA1c% of rats. Amongst the group of rats that were fed control diets including Hibiscus, C0J (6.5%) had the highest HbA1c% compared to the rats that were fed C2.5J (4.4%) and C5J (5.1%). In the group of rats fed high-fat diets including hibiscus and probiotics, there was a proportional decrease in HbA1c% as the concentration of hibiscus increased in the diets. Most studies involving the administration of HS have shown positive effects in lowering glucose levels in animal models. Some proposed mechanisms include the suppression of genes for DNA and RNA involved in stress signal pathways, improving insulin secretion and circulation and enhanced antioxidant activity [16] [20] [21].

3.4. Adiponectin and Resistin Levels

Adiponectin is a hormone that is produced by adipocytes and is responsible for regulating the carbohydrate and lipid metabolism process. Figure 7 shows the adiponectin levels in rats. The rats fed HGHS5J (8.79 µg/ml) and HFHS0J (8.96 µg/ml) had significantly ($p \leq 0.05$) higher adiponectin levels compared to rats fed HFHS2.5J (7.04 µg/ml). Overall, adiponectin levels were significant amongst the group of rats fed high-fat/high-sugar diets. Despite ongoing research to further understand the role of adiponectin, it has been well understood that adiponectin plays a role in the T2D and dyslipidemia. A study conducted on knockout mice concluded that low/reduced levels of adiponectin correlated to the progression or likelihood of insulin resistance [22]. Figure 8 shows the levels of resistin, a protein

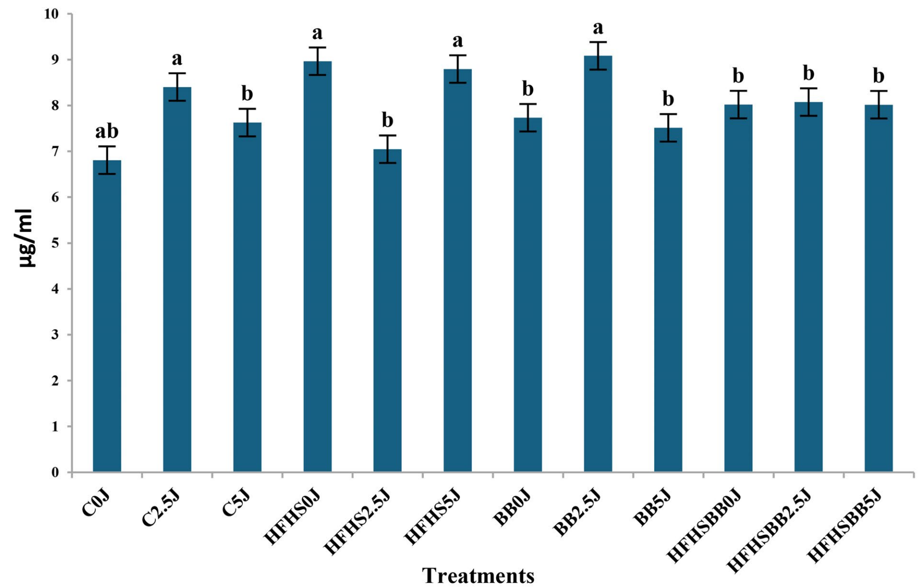

Figure 7. Adiponectin levels in rats fed hibiscus juice. Values are means ± SEM. Bars with superscript (abcd) indicating meal type effect differ significantly at p ≤ 0.05. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

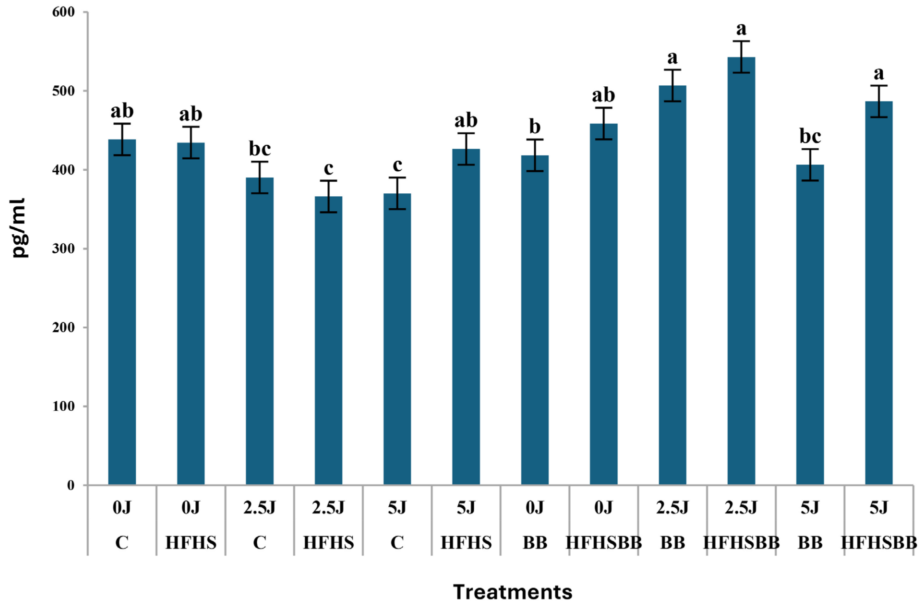

Figure 8. Resistin levels in rats fed dietary Hibiscus juice. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at p ≤ 0.05. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

secreted by adipose tissue, which might have a link to the development of obesity and insulin resistance. The application of collected results from mice studies has been difficult to transcribe and apply to human studies because resistin is produced in various locations in mice compared to humans. Rats fed HFHS2.5J (366 pg/ml) was significantly ($p \leq 0.05$) lower compared to rats fed HFHS0J (434.4 pg/ml). Rats fed BB5J had significantly ($p \leq 0.05$) lower levels of resistin compared to rats fed HFHS5J (486.7 pg/ml) and HFHSBB2.5J (534 pg/ml). Increased knowledge of resistin and adiponectin has outlined a link between the two adipokines in regulating glucose and lipid metabolism; therefore, exploring the resistin/adiponectin ratio could be a tool in understanding progression or state of disease related to obesity and diabetes [23].

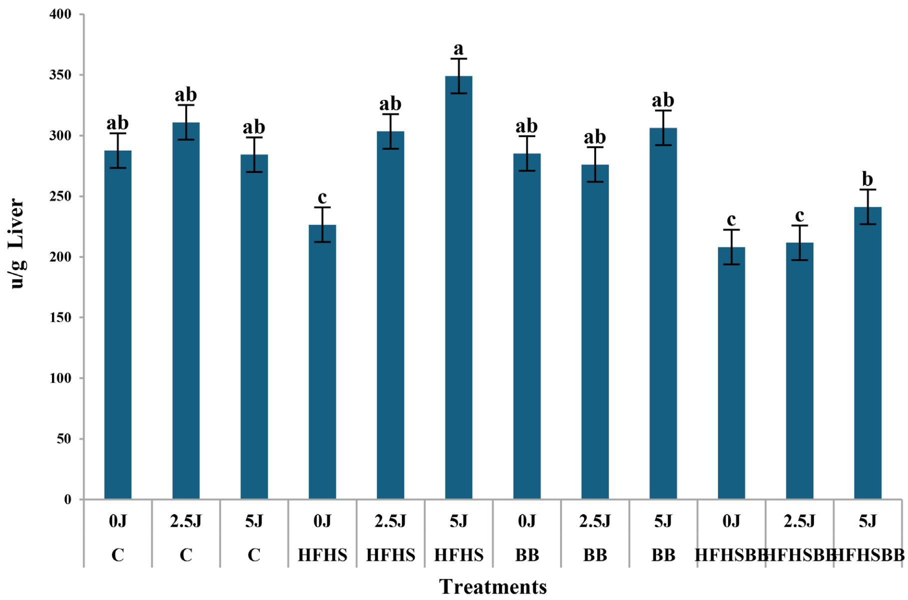

Figure 9. Hepatic catalase activity in rats fed dietary Hibiscus juice. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \le 0.05$. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

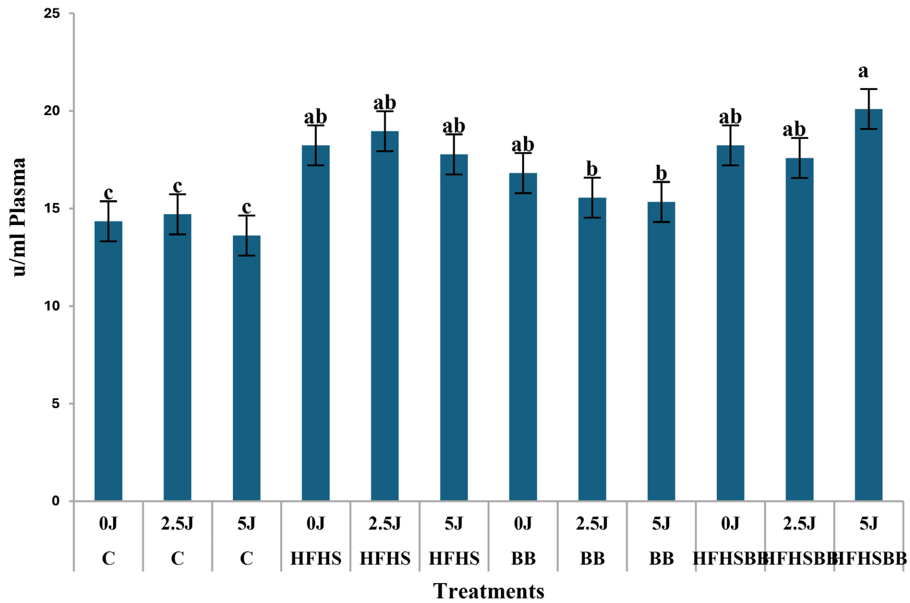

Figure 10. Plasma catalase activity in rats fed dietary Hibiscus juice. Values are means ± SEM. Bars with superscript (ab) indicating meal type effect differ significantly at $p \le 0.05$. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

3.5. Hepatic and Plasma Catalase Activity

Figure 9 and Figure 10 show the hepatic and plasma levels, respectively. Hepatic catalase activity was not significant across treatments as compared to circulating plasma levels. Plasma catalase results showed a significant ($p \le 0.05$) difference between rats that were fed regular diets as compared to the rats that received HF diets and regular diets that include BB.

3.6. Hepatic Glutathione and Glutathione Reductase Activity

Glutathione (GSH) is a major antioxidant in the body that protects cells from reactive oxygen species such as free radicals, peroxides and heavy metals. Glutathione can serve as an important biomarker to determine the antioxidant potential. In the groups of rats that were fed a regular diet including hibiscus only, the range of glutathione levels slightly increased in rats that were fed C0J, C2.5J and C5J (30.2 u/g, 30.6 u/g, 30.9 u/g). Hepatic glutathione reductase (GR), as shown in Figure 11 and Figure 12, is an important enzyme in the biological system that plays a critical role in the reduction of GSH disulfide (GSSG) back to glutathione in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) [24]. The GR enzyme is an important biomarker that helps maintain oxidative damage. Rats, which were fed the control diet, C0J (0.77u/g), had a significantly ($p \le 0.05$) lower GR activity compared to the rats fed C2.5J (1.37 u/g). In the groups of rats fed a high-fat diet including probiotics and hibiscus, the rats fed HFHSBB5J (1.19 u/g) had a significantly ($p \le 0.05$) higher GR activity compared to the rats

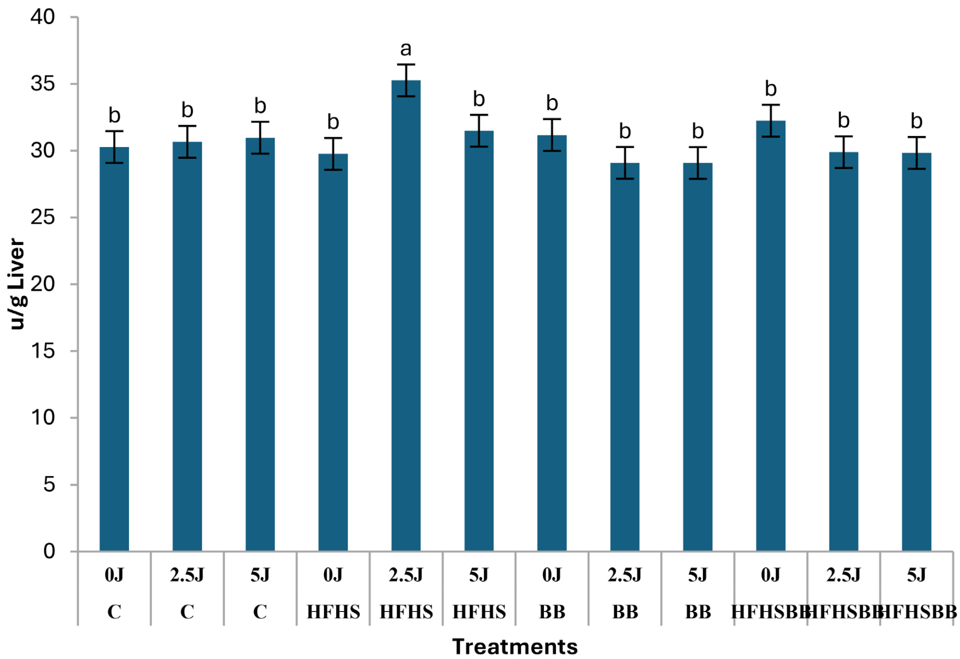

Figure 11. Hepatic glutathione levels in rats fed dietary hibiscus juice. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \le 0.05$. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

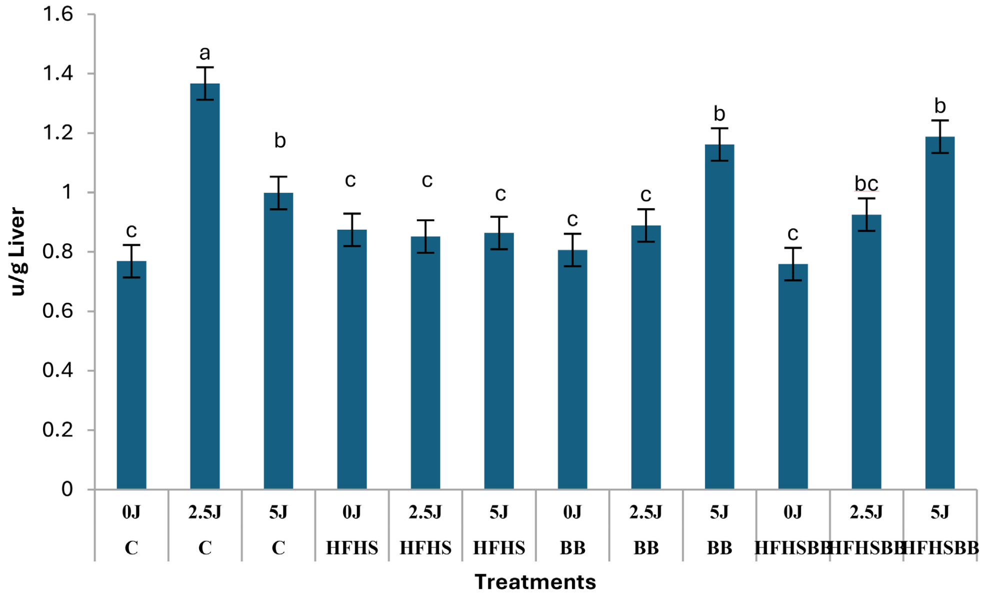

Figure 12. Hepatic glutathione reductase activity in rats fed dietary hibiscus juice. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at p ≤ 0.05. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

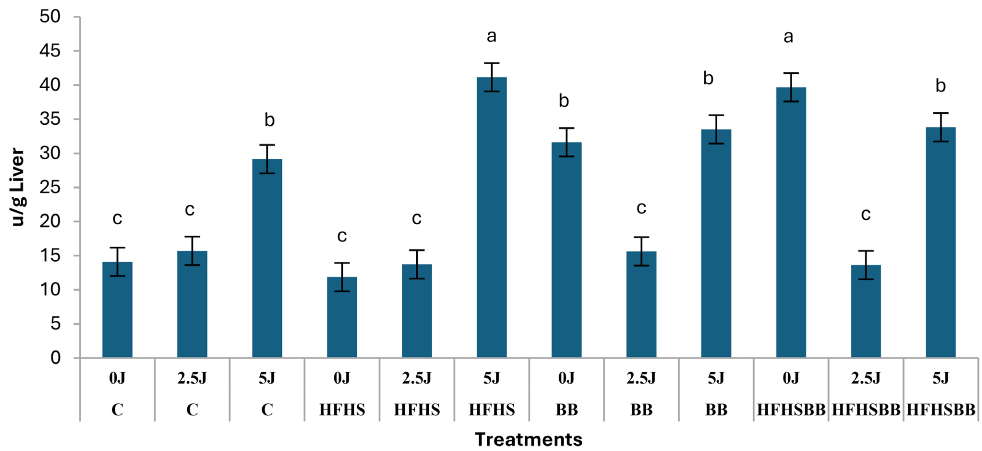

Figure 13. Hepatic GST activity in rats fed dietary Hibiscus juice. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at p ≤ 0.05. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

fed the control diet HFHSBB0J (0.76 u/g). These results indicate that HS treatments may have played a role in increasing GSH and GR was upregulated in HFD, including BB and HS, compared to rats fed control HFD. GST is a phase-2 enzyme that plays a significant role in the conjugation of glutathione to xenobiotics. Figure 13 shows Hepatic GST levels. Rats fed C0J (14.1 u/g) and C2.5J (15.7 u/g) had significantly ($p \leq 0.05$) lower GST activity compared to the rats fed C5J (29.1 u/g). Rats fed HFHSBB5J (33.8 u/g) had significantly ($p \leq 0.05$) higher GST levels compared to rats fed HFHSBB2.5J (13.7 u/g). In this study, we observe that increased

GST is prevalent in higher dosages of HS, thus indicating the beneficial effect of GST in binding to xenobiotics and excretion [25].

3.7. Hepatic and Plasma Superoxide Dismutase (SOD)

Figure 14 and Figure 15 show the Hepatic and the Plasma levels of SOD, respectively. Figure 15 shows the plasma SOD activity in rats. Rats fed HFHS2.5J (6.5

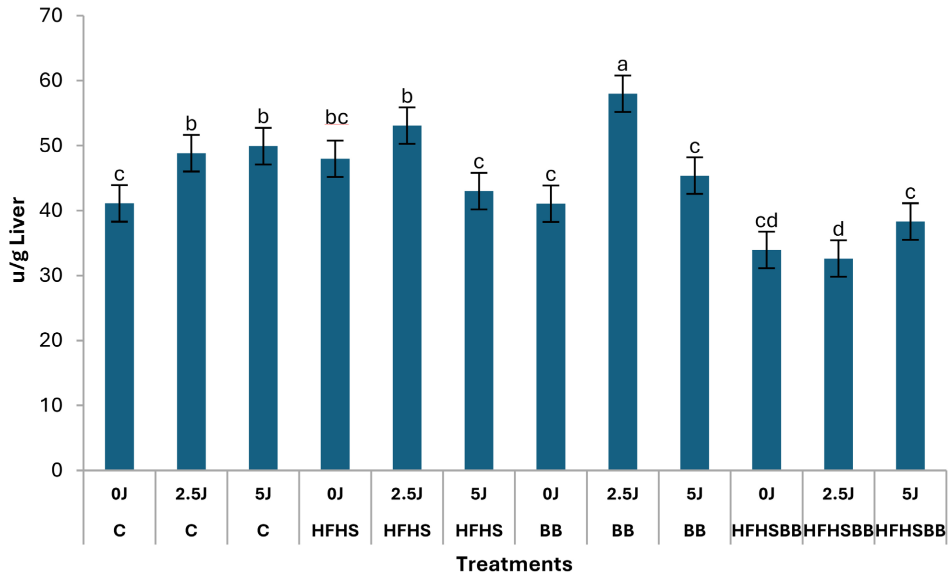

Figure 14. Hepatic SOD activity in rats fed dietary Hibiscus juice. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \le 0.05$. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

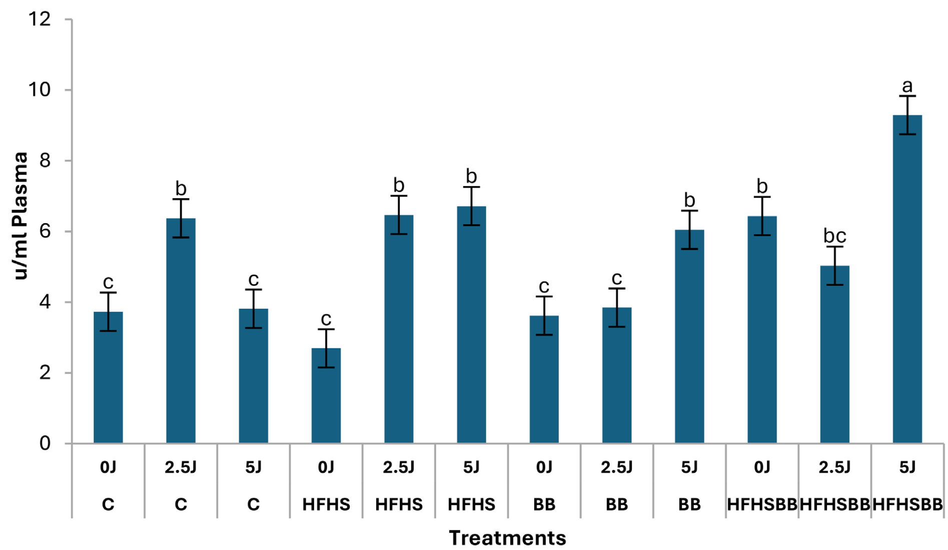

Figure 15. Plasma SOD activity in rats fed dietary Hibiscus juice. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \le 0.05$. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

u/g) and HFHS5J (6.7 u/g) had a significantly ($p \leq 0.05$) higher SOD activity compared to the rats that were fed the HFHS0J (2.7 u/g). During oxidative stress that occurs in obese and diabetic individuals, reactive oxygen species (ROS) are constantly generated. The catalase (CAT) enzyme is a hemoprotein that plays an important role in the decomposition of hydrogen peroxide to aqueous and oxygen and thus can be a useful biomarker to detect oxidative stress. These findings suggest rats fed a regular diet containing HS had a low induction of CAT and in low and medium doses of HS had a high induction of SOD in the plasma.

3.8. Hepatic COX Activity and Interleukin-1β Levels

Figure 16 shows the COX-2 activity. The COX enzyme is an enzyme that plays a key role in the production of prostanoids from eicosanoids and induces a hormone—like effect in paracrine and or autocrine systems—which ultimately play a role in inflammation. The rats that were fed the control diet, C0J (0.87 u/g), had significantly ($p \leq 0.05$) lower COX activity compared to the rats fed C2.5J (1.3 u/g) and C5J (1.1 u/g). In the group of rats that were fed diets including probiotics, BB0J (1.56 u/g) had significantly ($p \leq 0.05$) higher COX compared to BB2.5J (1.24 u/g) and BB5J. Rats that were fed high-fat diets including probiotics, the COX activity of HFHSBB5J (1.69 u/g) and HFHSBB0J (1.45 u/g) was significantly ($p \leq 0.05$) higher than that of rats fed HFHSBB2.5J (1.02 u/g). These results show an increased expression of COX enzyme in the hepatic system. Hepatic COX-2 activity has been identified in playing a protective role against diet-induced fat retention, obesity and found to play a role in rat studies, expression was found to protect against diet-induced steatosis, obesity, and insulin insensitivity [26].

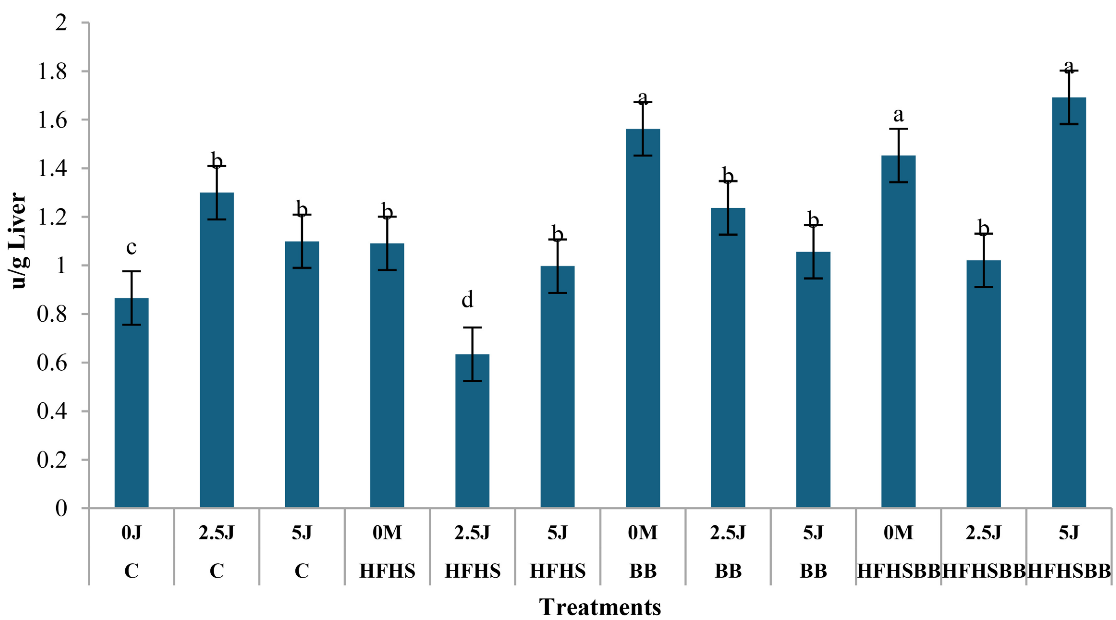

Figure 16. Hepatic COX activity in rats fed dietary Hibiscus juice. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

Interleukin-1β (IL-1β) is a member of the interleukin family of cytokines that play a major role in the inflammatory response due to injury or illness. Figure 17 shows IL-1b levels. The study showed that trend could not be established within the doses administered, however, IL-B expression in treatments including regular diets in combination with BB and HS showed significantly ($p \leq 0.05$) lower IL-B levels compared to BB0.

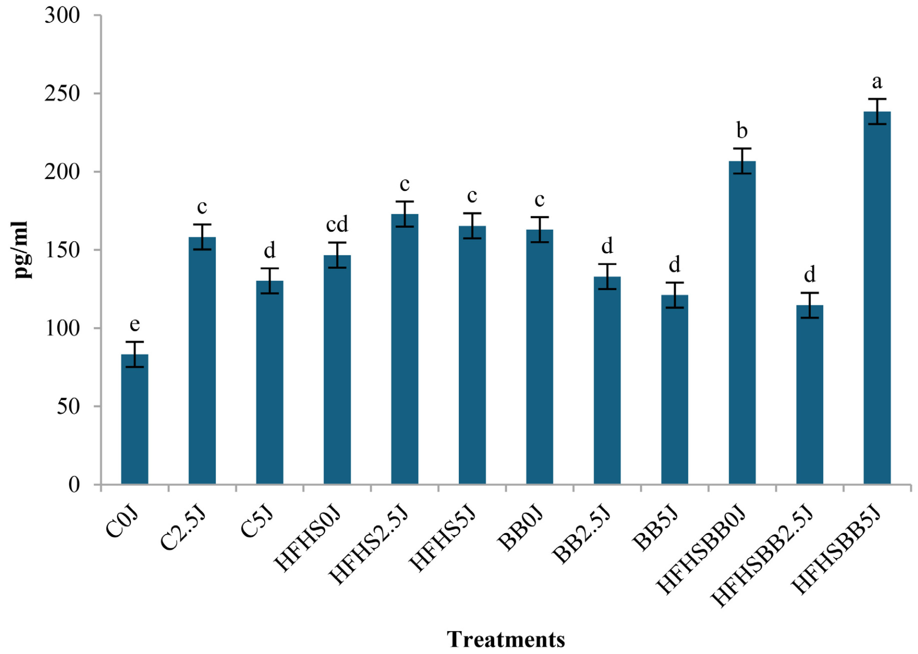

Figure 17. Interleukin-1β levels in rats fed Hibiscus juice. Values are means ± SEM. Bars with superscript (abcde) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

3.9. Interleukin-10 and Fetuin-A Levels

Interleukin-10 (IL-10) is another member of the Interleukin cytokine family and plays a major role as an anti-inflammatory agent in the biological system due to illness or injury/stress. Figure 18 shows the IL-10 levels of rats. Rats that were fed HFD diets, had significantly higher IL-10 levels compared to rats fed regular diets including HS and HFD diets including HS and BB. These results indicate that the administration of HS could have played a role in increasing the levels of IL-10 produced in monocytes and lymphocytes [27]. Figure 19 shows the levels of Fetuin-A. Fetuin-A is a negatively charged glycoprotein that is responsible for the transportation of various elements and substances in the blood, including the pro-inflammatory cytokine Nf-κb. The presence of elevated levels of Fetuin-A has been associated with the prevalence of T2D and the metabolic syndrome [28] [29]. In the group of rats fed HFD in combination with HS, the control diet HFD0J had a significantly ($p \leq 0.05$) higher Fetuin-A level compared to HFD diets that included HS.

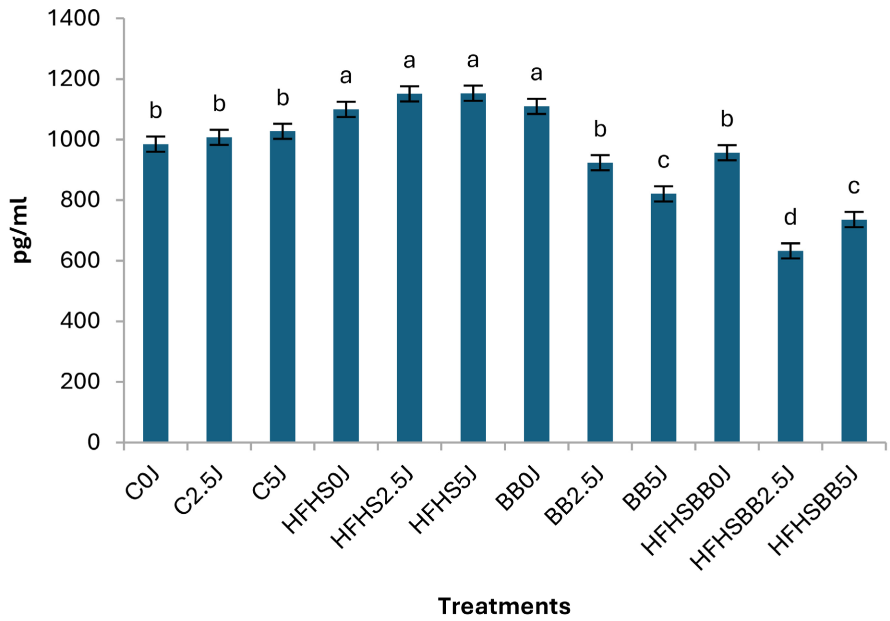

Figure 18. Interleukin-10 levels in rats fed Hibiscus juice. Values are means ± SEM. Bars with superscript (abcd) indicating meal type effect differ significantly at p ≤ 0.05. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

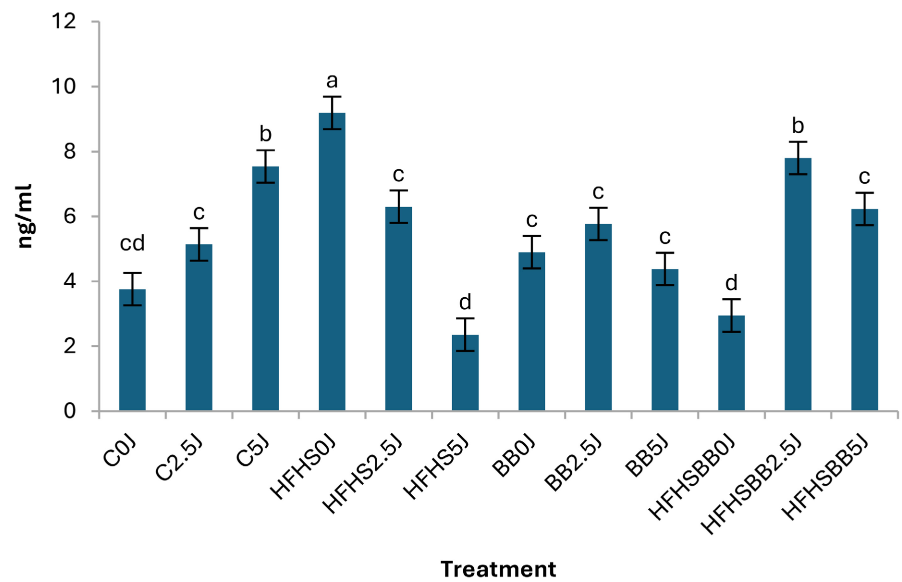

Figure 19. Fetuin-A levels of rats fed Hibiscus juice. Values are means ± SEM. Bars with superscript (abcd) indicating meal type effect differ significantly at p ≤ 0.05. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

3.10. Leptin and Ghrelin Levels

Leptin is a hormone that is responsible for the regulation of feed intake via inducing the feeling of satiety after a meal. Figure 20 shows the levels of leptin. Levels of leptin in HFD diets including HS were significantly (p ≤ 0.05) higher compared

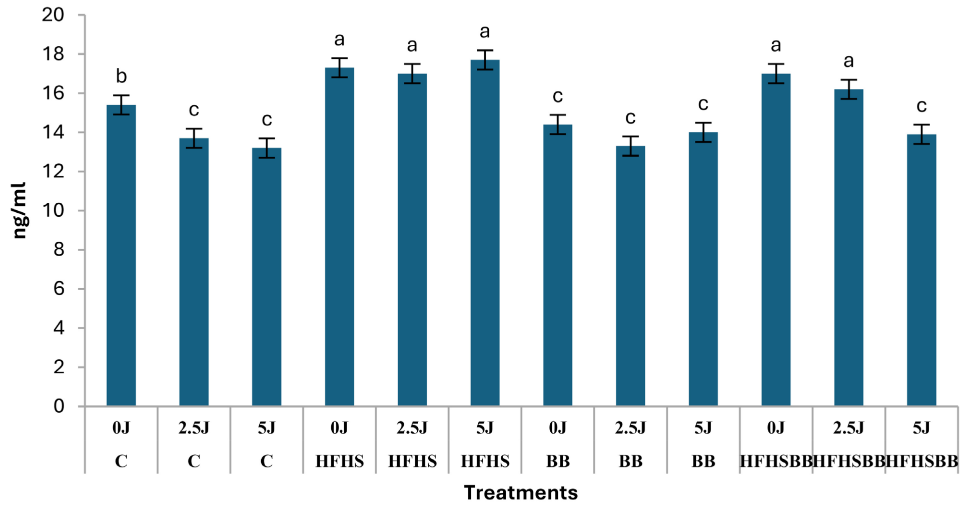

Figure 20. Leptin levels of rats fed Hibiscus juice. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

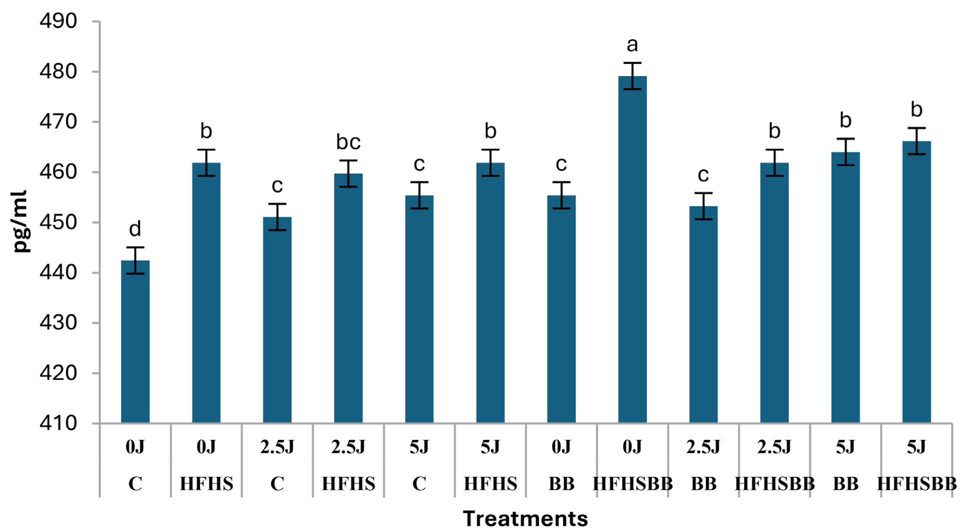

Figure 21. Ghrelin levels of rats fed Hibiscus juice. Values are means ± SEM. Bars with superscript (abcd) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; J: Juice; HFHSBB: High fat/high sugar diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

to regular diets and regular diets including BB. Leptin resistance is characterized by various factors, including the failure of leptin to reach the hypothalamus, a decrease in receptors in target cells or abnormal leptin signaling [30] [31]. Our study shows that despite the administration of HS or BB in HFD, significantly ($p \leq 0.05$) higher levels of leptin were still present. This may present an opportunity of administering other means of ingesting or sample preparation of HS to maintain leptin levels in HFD comparable to regular diets. Ghrelin, also called the hunger hormone, is responsible for signaling the host to consume a meal. Levels of ghrelin are usually upregulated before a meal and gradually decrease as the host reaches the point of satiety. **Figure 21** shows the ghrelin levels in rats. Rats fed

HFHS0J (461.8 pg/ml) had significantly ($p \leq 0.05$) higher levels of ghrelin compared to the group fed a regular diet C0J (442.2 pg/ml). The group of rats fed HFHSBB0J (479.1 pg/ml) had significantly ($p \leq 0.05$) higher ghrelin levels compared to rats fed BB2.5J (453.2 pg/ml). Our study may suggest that not only HFD showed to increase the level of ghrelin, in addition the administration of HS may aid in the suppression of circulating ghrelin levels. In a research study conducted by [32] [33], herbal supplements were identified to have suppressive effects on ghrelin levels.

Emerging research studies acknowledge the complex interactions between dietary polyphenols and the gut microbiota, suggesting that polyphenols are not only metabolized by intestinal microbes but also act as prebiotic modulators of microbial composition [34] [35]. These interactions may enhance the production of short-chain fatty acids, improve gut barrier integrity, and reduce systemic inflammation, which are key mechanisms in the prevention of metabolic syndrome. Similarly, probiotics such as *Bifidobacterium breve* have demonstrated the ability to modulate lipid metabolism, enhance glucose uptake, and downregulate pro-inflammatory cytokines [36] [37]. The observed improvements in glycemic control, antioxidant enzyme activity, and adipokine profiles in this study may therefore be attributed, in part, to the synergistic effects of polyphenol-microbiota interactions and probiotic-mediated signaling.

4. Conclusion

HS has been a widely used edible flower for culinary purposes in beverages, desserts, jams and snacks. The polyphenols present in this flower, most commonly anthocyanins, could affect various metabolic pathways that can develop into chronic illnesses. Currently, HS is a common ingredient in the US market as a tea beverage; however, its inclusion in other categories of foods is still yet to be explored fully. In this study, we examined the effect of HS as juice. Our studies showed that HS singularly and in combination with Probiotics influences antioxidative enzymes, appetite-regulating hormones and weight control. With the increase in the rise of obesity and diabetes across all demographics, there is a need to investigate synergetic effects of various bioactive groups as shown in this study. Human trials could be the next step in this work to provide public health recommendations.

Acknowledgements

This research is supported by a USDA NIFA grant studying functional evaluation of health-promoting properties of selected spices and plant extracts to aid against obesity (anti-oxidative, anti-obesity, anti-diabetic).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Ward, Z.J., Bleich, S.N., Cradock, A.L., Barrett, J.L., Giles, C.M., Flax, C., *et al.* (2019)

Projected U.S. State-Level Prevalence of Adult Obesity and Severe Obesity. *New England Journal of Medicine*, **381**, 2440-2450. https://doi.org/10.1056/nejmsa1909301

[2] Afshin, A., Reitsma, M. B. and Murray, C. J. (2017) Health Effects of Overweight and Obesity in 195 Countries. *The New England Journal of Medicine*, **377**, 1496-1497.

[3] Busebee, B., Ghusn, W., Cifuentes, L. and Acosta, A. (2023) Obesity: A Review of Pathophysiology and Classification. *Mayo Clinic Proceedings*, **98**, 1842-1857. https://doi.org/10.1016/j.mayocp.2023.05.026

[4] Rosso, C., Kazankov, K., Younes, R., Esmaili, S., Marietti, M., Sacco, M., *et al.* (2019) Crosstalk between Adipose Tissue Insulin Resistance and Liver Macrophages in Non-Alcoholic Fatty Liver Disease. *Journal of Hepatology*, **71**, 1012-1021. https://doi.org/10.1016/j.jhep.2019.06.031

[5] Clemente-Suárez, V.J., Beltrán-Velasco, A.I., Redondo-Flórez, L., Martín-Rodríguez, A. and Tornero-Aguilera, J.F. (2023) Global Impacts of Western Diet and Its Effects on Metabolism and Health: A Narrative Review. *Nutrients*, **15**, Article 2749. https://doi.org/10.3390/nu15122749

[6] Prabawati, N.B., Oktavirina, V., Palma, M. and Setyaningsih, W. (2021) Edible Flowers: Antioxidant Compounds and Their Functional Properties. *Horticulturae*, **7**, Article 66. https://doi.org/10.3390/horticulturae7040066

[7] Pinakin, D.J., Kumar, V., Suri, S., Sharma, R. and Kaushal, M. (2020) Nutraceutical Potential of Tree Flowers: A Comprehensive Review on Biochemical Profile, Health Benefits, and Utilization. *Food Research International*, **127**, Article ID: 108724. https://doi.org/10.1016/j.foodres.2019.108724

[8] Maciel, L.G., do Carmo, M.A.V., Azevedo, L., Daguer, H., Molognoni, L., de Almeida, M.M., *et al.* (2018) *Hibiscus sabdariffa* Anthocyanins-Rich Extract: Chemical Stability, *in Vitro* Antioxidant and Antiproliferative Activities. *Food and Chemical Toxicology*, **113**, 187-197. https://doi.org/10.1016/j.fct.2018.01.053

[9] Sini, J. M., Umar, I. A. and Inuwa, H. M. (2011) The Beneficial Effects of Extracts of *Hibiscus sabdariffa* Calyces in Alloxan-Diabetic Rats: Hypoglycaemic and Hypolipidae-Mic Activities. *Journal of Medicinal Plants Research*, **5**, 2182-2186.

[10] Joven, J., March, I., Espinel, E., Fernández-Arroyo, S., Rodríguez-Gallego, E., Aragonès, G., *et al.* (2014) *Hibiscus sabdariffa* Extract Lowers Blood Pressure and Improves Endothelial Function. *Molecular Nutrition & Food Research*, **58**, 1374-1378. https://doi.org/10.1002/mnfr.201300774

[11] Lee, Y., Yang, W., Kim, H.Y., Min, B., Caturla, N., Jones, J., *et al.* (2018) Metabolaid® Combination of Lemon Verbena and Hibiscus Flower Extract Prevents High-Fat Diet-Induced Obesity through Amp-Activated Protein Kinase Activation. *Nutrients*, **10**, Article 1204. https://doi.org/10.3390/nu10091204

[12] Smeets, A.J., Janssens, P.L.H.R. and Westerterp-Plantenga, M.S. (2013) Addition of Capsaicin and Exchange of Carbohydrate with Protein Counteract Energy Intake Restriction Effects on Fullness and Energy Expenditure. *The Journal of Nutrition*, **143**, 442-447. https://doi.org/10.3945/jn.112.170613

[13] Balaji, M., Ganjayi, M.S., Hanuma Kumar, G.E.N., Parim, B.N., Mopuri, R. and Dasari, S. (2016) A Review on Possible Therapeutic Targets to Contain Obesity: The Role of Phytochemicals. *Obesity Research & Clinical Practice*, **10**, 363-380. https://doi.org/10.1016/j.orcp.2015.12.004

[14] van Avesaat, M., Troost, F.J., Westerterp-Plantenga, M.S., Helyes, Z., Le Roux, C.W., Dekker, J., *et al.* (2016) Capsaicin-Induced Satiety Is Associated with Gastrointestinal Distress but Not with the Release of Satiety Hormones. *The American Journal of Clinical Nutrition*, **103**, 305-313. https://doi.org/10.3945/ajcn.115.123414

[15] Joo, I.H., Kim, M.G., Park, J.M., Min, G.Y., Han, S.H., Lee, S.B., *et al.* (2020) *Bifidobacterium breve* Strain IDCC 4401 Improves Dyslipidemia in Rat Model. *International Journal of Clinical and Experimental Medicine*, **13**, 4137-4144.

[16] Jeffery, T.D. and Richardson, M.L. (2021) A Review of the Effectiveness of Hibiscus for Treatment of Metabolic Syndrome. *Journal of Ethnopharmacology*, **270**, Article ID: 113762. https://doi.org/10.1016/j.jep.2020.113762

[17] Carvajal-Zarrabal, O., Hayward-Jones, P.M., Orta-Flores, Z., Nolasco-Hipólito, C., Barradas-Dermitz, D.M., Aguilar-Uscanga, M.G., *et al.* (2009) Effect of *Hibiscus sabdariffa* L. Dried Calyx Ethanol Extract on Fat Absorption-Excretion, and Body Weight Implication in Rats. *BioMed Research International*, **2009**, Article ID: 394592. https://doi.org/10.1155/2009/394592

[18] Ghislain, M.T., Gisele, E.L., Joel Bertr, P.M., Mathieu, F., Honore, F.K., Felicite, T.M., *et al.* (2011) Effect of "Folere" Juice (Calyx of *Hibiscus sabdariffa* Lin) on Some Biochemical Parameters in Humans. *Pakistan Journal of Nutrition*, **10**, 755-759. https://doi.org/10.3923/pjn.2011.755.759

[19] Tazoho, G.M., Gouado, I., Ndomou, M., Bonsi, S.T., Wamba, Y.M. and Agbor, E.E. (2016) Clinical, Hematological and Biochemical Health Benefit Effects of *Hibiscus sabdariffa* Lin Dried Calyces Beverage in Human. *Food and Nutrition Sciences*, **7**, 383-395. https://doi.org/10.4236/fns.2016.75040

[20] Pillai, S.S. and Mini, S. (2016) Polyphenols Rich *Hibiscus rosa sinensis* Linn. Petals Modulate Diabetic Stress Signalling Pathways in Streptozotocin-Induced Experimental Diabetic Rats. *Journal of Functional Foods*, **20**, 31-42. https://doi.org/10.1016/j.jff.2015.10.007

[21] Moqbel, F.S., Naik, P.R., Najma, H.M. and Selvaraj, S. (2011) Antidiabetic Properties of *Hibiscus rosa sinensis* L. Leaf Extract Fractions on Nonobese Diabetic (NOD) Mouse. *Indian Journal of Experimental Biology*, **49**, 24-29.

[22] Shehzad, A., Iqbal, W., Shehzad, O. and Lee, Y.S. (2012) Adiponectin: Regulation of Its Production and Its Role in Human Diseases. *Hormones*, **11**, 8-20. https://doi.org/10.1007/bf03401534

[23] Abudalo, R., Alqudah, A., Qnais, E., Athamneh, R.Y., Oqal, M. and Alnajjar, R. (2024) Interplay of Adiponectin and Resistin in Type 2 Diabetes: Implications for Insulin Resistance and Atherosclerosis. *Pharmacia*, **71**, 1-8. https://doi.org/10.3897/pharmacia.71.e114863

[24] Losada-Barreiro, S., Sezgin-Bayindir, Z., Paiva-Martins, F. and Bravo-Díaz, C. (2022) Biochemistry of Antioxidants: Mechanisms and Pharmaceutical Applications. *Biomedicines*, **10**, Article 3051. https://doi.org/10.3390/biomedicines10123051

[25] Angelucci, F., Baiocco, P., Brunori, M., Gourlay, L., Morea, V. and Bellelli, A. (2005) Insights into the Catalytic Mechanism of Glutathione S-Transferase: The Lesson from Schistosoma Haematobium. *Structure*, **13**, 1241-1246. https://doi.org/10.1016/j.str.2005.06.007

[26] Chan, P., Liao, M. and Hsieh, P. (2019) The Dualistic Effect of COX-2-Mediated Signaling in Obesity and Insulin Resistance. *International Journal of Molecular Sciences*, **20**, Article 3115. https://doi.org/10.3390/ijms20133115

[27] Said, E.A., Dupuy, F.P., Trautmann, L., Zhang, Y., Shi, Y., El-Far, M., *et al.* (2010) Programmed Death-1-Induced Interleukin-10 Production by Monocytes Impairs CD4+ T Cell Activation during HIV Infection. *Nature Medicine*, **16**, 452-459. https://doi.org/10.1038/nm.2106

[28] Ketteler, M., Bongartz, P., Westenfeld, R., Wildberger, J.E., Mahnken, A.H., Böhm, R., *et al.* (2003) Association of Low Fetuin-A (AHSG) Concentrations in Serum with Car-

diovascular Mortality in Patients on Dialysis: A Cross-Sectional Study. *The Lancet*, **361**, 827-833. https://doi.org/10.1016/s0140-6736(03)12710-9

[29] Mori, K., Emoto, M., Yokoyama, H., Araki, T., Teramura, M., Koyama, H., *et al.* (2006) Association of Serum Fetuin-A with Insulin Resistance in Type 2 Diabetic and Nondiabetic Subjects. *Diabetes Care*, **29**, 468-468. https://doi.org/10.2337/diacare.29.02.06.dc05-1484

[30] Scarpace, P.J., Matheny, M. and Tümer, N. (2001) Hypothalamic Leptin Resistance Is Associated with Impaired Leptin Signal Transduction in Aged Obese Rats. *Neuroscience*, **104**, 1111-1117. https://doi.org/10.1016/s0306-4522(01)00142-7

[31] El-Haschimi, K., Pierroz, D.D., Hileman, S.M., Bjørbæk, C. and Flier, J.S. (2000) Two Defects Contribute to Hypothalamic Leptin Resistance in Mice with Diet-Induced Obesity. *Journal of Clinical Investigation*, **105**, 1827-1832. https://doi.org/10.1172/jci9842

[32] Rayalam, S., Dellafera, M. and Baile, C. (2008) Phytochemicals and Regulation of the Adipocyte Life Cycle. *The Journal of Nutritional Biochemistry*, **19**, 717-726. https://doi.org/10.1016/j.jnutbio.2007.12.007

[33] Avula, B., Wang, Y., Pawar, R.S., Shukla, Y.J., Schaneberg, B. and Khan, I.A. (2006) Determination of the Appetite Suppressant P57 in *Hoodia gordonii* Plant Extracts and Dietary Supplements by Liquid Chromatography/Electrospray Ionization Mass Spectrometry (LC-MSD-TOF) and LC-UV Methods. *Journal of AOAC International*, **89**, 606-611. https://doi.org/10.1093/jaoac/89.3.606

[34] Plamada, D. and Vodnar, D.C. (2022) Polyphenols—Gut Microbiota Interrelationship: A Transition to a New Generation of Prebiotics. *Nutrients*, **14**, Article 137. https://doi.org/10.3390/nu14010137

[35] Li, Z., Kanwal, R., Yue, X., Li, M. and Xie, A. (2024) Polyphenols and Intestinal Microorganisms: A Review of Their Interactions and Effects on Human Health. *Food Bioscience*, **62**, Article ID: 105220. https://doi.org/10.1016/j.fbio.2024.105220

[36] Wang, X., Liu, Y. and Zhang, Z. (2023) Impact of Bifidobacterium Species on Lipid and Glucose Metabolism in Metabolic Disease Models: A Systematic Review. *Journal of Nutritional Biochemistry*, **120**, Article ID: 109888.

[37] Chen, X., Yan, L., Yang, J., Xu, C. and Yang, L. (2025) The Impact of Probiotics on Oxidative Stress and Inflammatory Markers in Patients with Diabetes: A Meta-Research of Meta-Analysis Studies. *Frontiers in Nutrition*, **12**, Article 1552358. https://doi.org/10.3389/fnut.2025.1552358