Scientific Research Publishing

Food and Nutrition Sciences, 2025, 16(9), 1310-1331
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 Meal 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 Meal Form Combination with *Bifidobacterium breve* 3 in Sprague Dawley Rats. *Food and Nutrition Sciences*, **16**, 1310-1331.
https://doi.org/10.4236/fns.2025.169075

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

Abstract

This study evaluated the anti-obesity and anti-diabetic potential of *Hibiscus sabdariffa* (HS) meal, alone and in combination with *Bifidobacterium breve* 3 (BB), in female Sprague Dawley rats. Rats were fed diets containing 2.5% or 5% HS, with or without 0.25% BB, followed by a high-fat, high-sugar (HFD) diet until week 40. Treatment groups showed significantly lower weight gain (e.g., HFDBB5M: 21.5 g vs. HFDBB0M: 31.5 g), reduced triglyceride levels (HFDBB2.5M: 29 mg/dL vs. HFD0M: 45 mg/dL), and elevated HDL. HbA1c was reduced in HS + BB-fed rats (HFDBB5M: 6.6% vs. HFDBB0M: 15.3%), alongside increased adiponectin and lower leptin and ghrelin levels. Antioxidant enzymes, including catalase, superoxide dismutase, glutathione-S-transferase, and glutathione reductase, were significantly upregulated in treated groups, while pro-inflammatory markers (COX-2, IL-1β) were downregulated. These findings indicate that dietary HS and BB synergistically improve metabolic, oxidative, and inflammatory markers, highlighting their potential as complementary dietary strategies for preventing obesity and insulin resistance.

Keywords

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

1. Introduction

This template, obesity is a complex metabolic disorder primarily characterized by two key morphological alterations: an increase in the number of adipocytes (hyperplasia) and the enlargement of individual adipose cells (hypertrophy) [1]. The

increase and expansion of adipose tissue correlates with an increase in exosomes and adipokines that can trigger the development of other complications such as dyslipidemia, non-alcoholic fatty liver disease (NAFLD) and insulin resistance. The World Health Organization in 2016 estimated that about 650 million adults were diagnosed with obesity and the number is still rising [2]. [3] indicated that the increase in obesity is proportionally increasing with the prevalence of diabetes, as obesity affects both insulin secretion and beta cell functionality. Amongst many factors, the Western diet has been singled out as one of the major causes of obesity and diabetes. The Western diet is characteristically high in saturated and trans fats, sodium, and added sugars, while being markedly deficient in the intake of fruits and vegetables, which are primary sources of essential nutrients and vitamins [4]. Research suggests that in addition to fruits and vegetables, edible flowers are increasingly becoming more acceptable to consumers due to their potential of adding nutritional functionality to foods. The main compounds of interest in edible flowers are phenolic compounds, which are structurally characterized by a hydroxyl group bound to an aromatic compound. *Hibiscus sabdariffa*, an edible flower, has been reported to have anti-obesity effects when supplemented at a dosage of 100 μg/ml for 8 days on 3T3-L1 cell lines [5]. In a mice study conducted by [6], *H. sabdariffa* was reported to reduce weight gain in mice fed a high-fat diet (HFD) treatment. Leptin levels and key transcription factors in adipocyte differentiation have also been reported to be significantly affected by extracts of *H. sabdariffa* in 3T3 cell lines [7]. The anti-diabetic properties of phytochemicals in edible flower extracts have also been reported to inhibit the key digestive enzymes in carbohydrate metabolism (pancreatic α-amylase and intestinal α-glucosidase) in a dose-dependent manner [8]. Probiotics, including viable strains of the Bifidobacterium genus (*B. lactis*, *B. breve*), has been reported to have functional benefits if ingested in sufficient concentrations [9]. Therefore, this knowledge creates space to investigate the singular and/or synergistic effects of *H. sabdariffa* in combination with probiotics (*Bifidobacterium breve*-BB) against key metabolic reactions/cascades in the development of obesity and diabetes, utilizing an animal model.

2. Materials and Methods

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

Dry (HS) calyces were obtained from Monterey Bay Spice Company and ground to a fine powder and mixed in the diet at 2.5% and 5% levels. 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%.

2.2. Animal Housing and Conditions

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.

- **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 (Table 1).

Table 1. Rats fed hibiscus in meal form (Ain-93G) diet.

Ingredient (g)	C0M	HFD/HF Control	C2.5 M	HFD/HF 2.5M	C5M	HFD/HF 5M	BB0M	HFD/BB 0M	BB2.5 M	HFD/HF BB2.5M	BB5 M	HFD/HF BB5M
Cornstarch	397.5	257.5	372.5	232.5	297	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	35	35	35	35	35	35	35	35	35	35	35	35
VM	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; M: Meal; 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), 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

A section of liver tissue was excised, rinsed in ice-cold 1.15% KCl solution, blotted, weighed, and homogenized using four volumes of homogenization 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

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 was 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 and Weight Gain

Figure 1 shows the feed conversion ratio (FCR) calculated by dividing the average daily feed intake by weight gain. Regular diets overall had a significantly ($p \leq 0.05$) higher FCR compared to the high-fat diets. When comparing rats fed high-fat diets, rats fed HFDBB (0.49) and HFDBB5M (1.01) had significantly ($p \leq 0.05$)

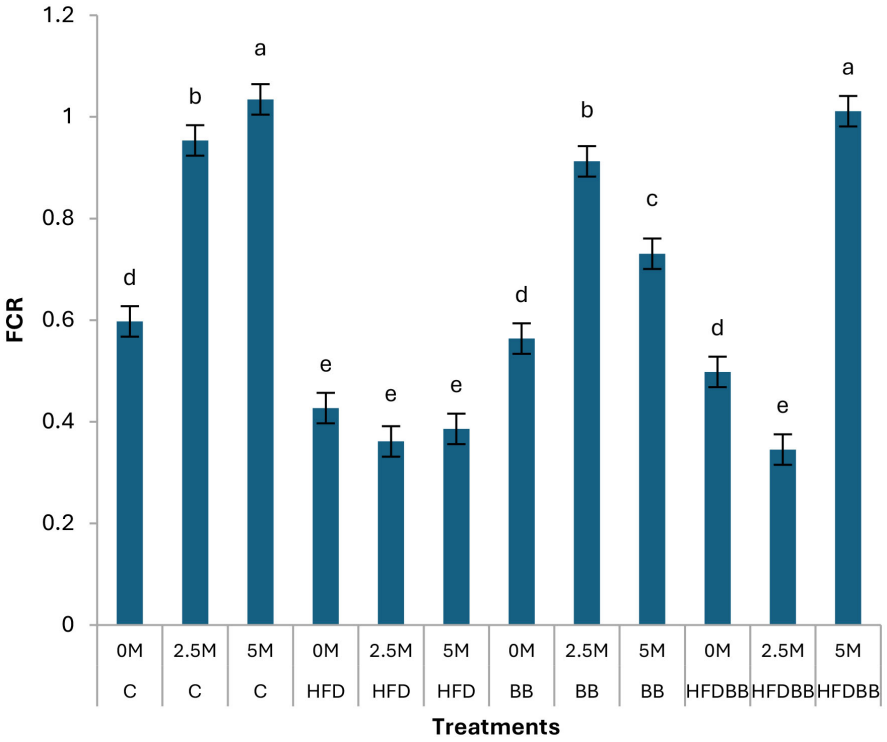

Figure 1. Feed conversion ratio (FCR) of Sprague Dawley rats fed hibiscus meal. Values are means ± SEM. Bars with superscript (abcde) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

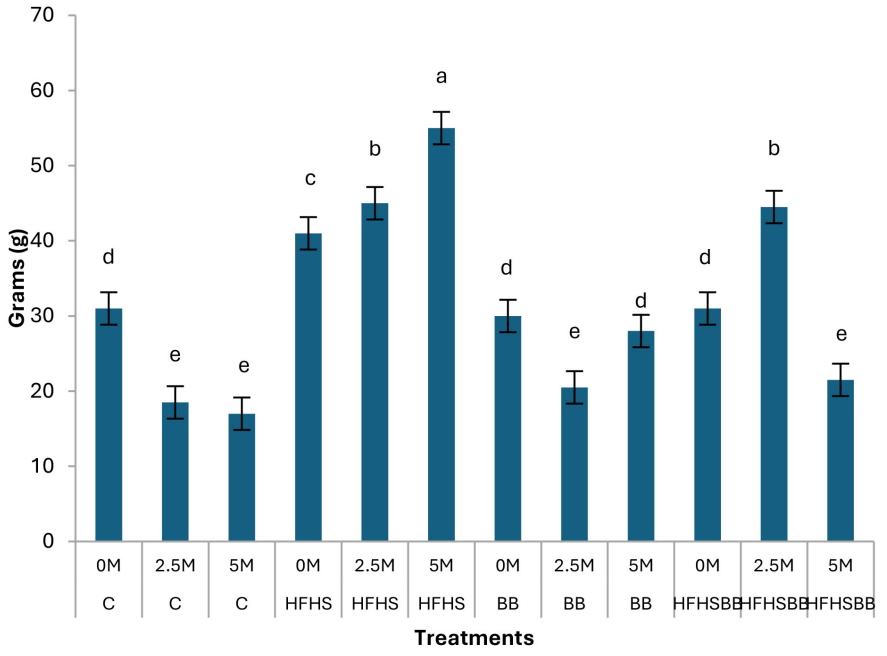

Figure 2. Weight gain in rats fed dietary Hibiscus meal. Values are means ± SEM. Means with control, different superscripts (abcde) are significantly different ($p \leq 0.05$). Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

higher FCR. The weight gain is shown in Figure 2. The rats which were fed the control diets without hibiscus, and probiotics (C0M) (31 g) had a significantly ($p \leq 0.05$) higher weight gain compared to rats fed the (C2.5M) (18.5 g) and (C5M) (17 g). Similarly, the rats fed the high-fat diet (HFDBB5M) (21.5 g) had a significantly ($p \leq 0.05$) lower weight gain compared to (HFDBB0M) (31.5 g). A decreased FCR coupled with the decrease in weight gain could be attributed to the ingestion of available phytochemicals, commonly (flavonoids) present in HS. Studies [10] [11] have shown that reduced food intake via modulation of key appetite hormones, decrease in fat absorption via lipase inhibition and increasing energy expenditure are all common mechanisms associated with feed intake and weight gain modulation in animal models that consume phytochemical-rich treatments.

3.2. Lipid Profile—Triglycerides & High-Density Lipoprotein (HDL)

Figure 3 shows the plasma TG levels. The rats that were fed HFD5M (57 mg/dL) had significantly ($p \leq 0.05$) higher TG levels compared to the rats fed HFD2.5M (36.3 mg/dL). Rats that were fed HFD5M (30 mg/dL) and HFDBB2.5M (29 mg/dL) had a lower TG level compared to the rats that were fed HFD0M (45 mg/dL). Hypertriglyceridemia over the decades has become a common factor in the occurrence of type 2 diabetes and obesity, thus TG is becoming a more reliable biomarker in lipid profiles [12]. In a study conducted by [13] using a *Bifidobacterium breve* strain—BBR4401, bile acid levels were elevated. LDL receptors were upregulated, bile acyl-CoA and cholesterol 7 alpha-hydroxylase (CYP7*a*1) also showed an increase. CYP7*a*1 is a key player in the biosynthesis of bile acid and its overexpression

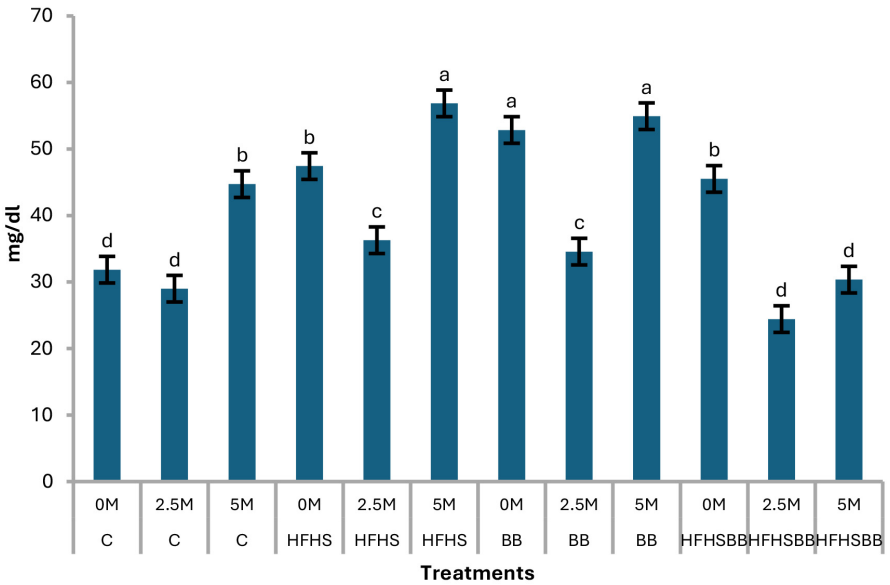

Figure 3. Blood triglycerides levels in rats fed dietary Hibiscus meal. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

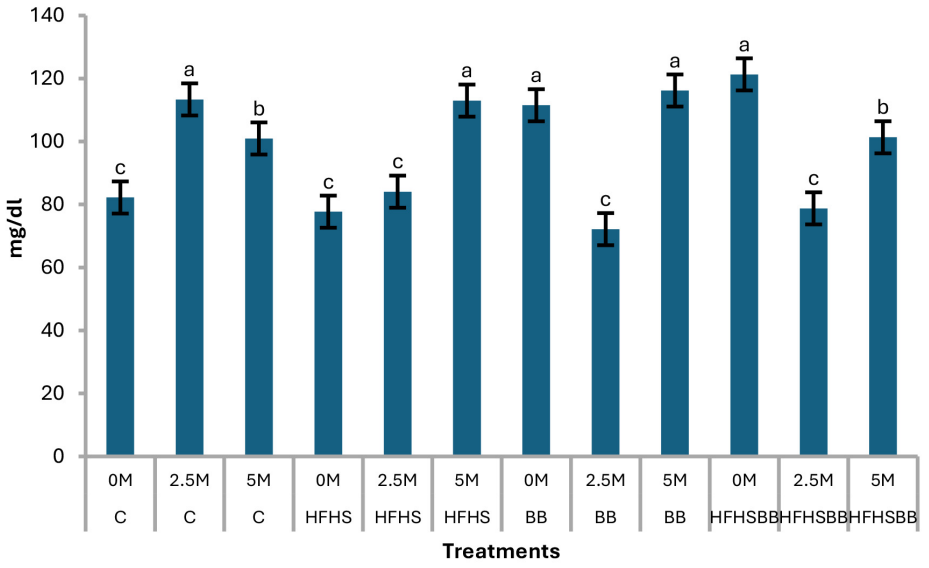

Figure 4. High density lipoprotein Levels in rats fed Hibiscus meal. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

aids in the decrease of triglyceride-rich lipoproteins [14]. Cholesterol (HDL-C) are protein-rich molecules possessing various key biological functions in the progression of CVD, Type-2 Diabetes and Hypertension. HDL functions include cholesterol efflux via reversal cholesterol transport (RCT), antioxidant activity, and anti-glycation activity (reduction of glycated lipoproteins) [15]. **Figure 4** depicts the plasma HDL levels of rats fed hibiscus in meal form. Rats fed treatments containing HS overall had significantly ($p \leq 0.05$) higher HDL levels compared to rats that received control treatments.

3.3. Blood Glucose and HbA1c Level

The blood glucose levels and HbA1C levels are shown in **Figure 5** and **Figure 6**, respectively. Overall, the groups that received probiotics in their feed BB0M, BB2.5M, and BB5M had the lowest blood glucose levels compared to all the other treatment groups. The insignificance of this result correlates to the Meta-analysis work conducted by [16], where 7 studies conducting work on animal studies did not show any significant reduction of blood glucose levels in rats that were considered healthy (no pathophysiological relevance to obesity, MetS or T2D. Overall, the group of rats that were fed a control diet including hibiscus had a lower HbA1c% compared to the rats fed a high-fat diet including hibiscus. Rats fed HFDBB0M (15.3%) had the highest HbA1c% compared to the rats that were fed HFDBB2.5M (7.3%) HFDBB5M (6.6%). In contrast to healthy animals, a decrease in fasted blood glucose and HbA1c has been observed in animal studies where animals are subjected to high-fat diets or STZ treatment to induce diabetes.

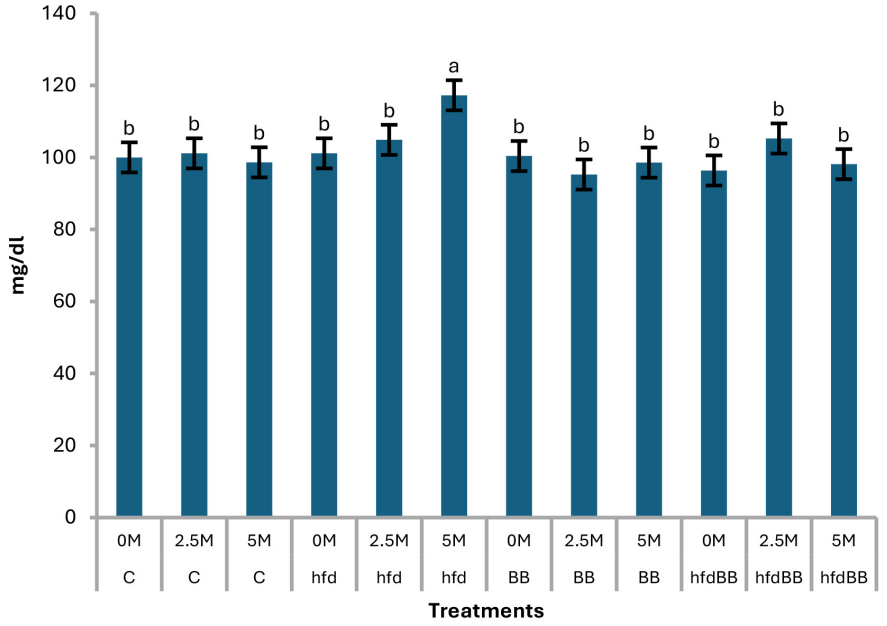

Figure 5. Blood glucose in rats fed dietary Hibiscus meal. Values are means ± SEM. Means with different superscripts (abc) are significantly different ($p \leq 0.05$). Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

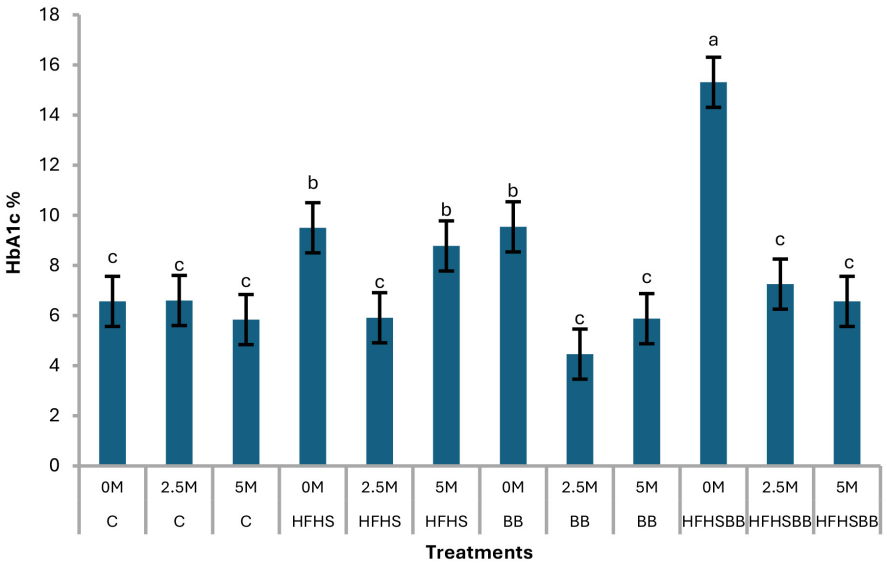

Figure 6. Plasma HbA1c levels in rats fed dietary Hibiscus meal. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%;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

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** below shows adiponectin levels. The adiponectin levels of rats BB2.5M (9.3 µg/ml) were signif-

icantly ($p \leq 0.05$) higher than all rats in the group fed BB, including hibiscus only. Rats fed HFDBB5M (8.96 μg/ml) have significantly higher adiponectin levels compared to rats fed HFDBB0M (8.01 μg/ml) and HFDBB2.5M (7.91 μg/ml). Adiponectin plays a critical role in decreasing hepatic glucose production via decreasing the expression of two key gluconeogenesis enzymes—phosphoenolpyruvate carboxykinase and glucose-6-phosphatase [17]. **Figure 8** shows the levels of resistin, a protein secreted by white adipose tissue in rodents. The link between resistin and diseased states (diabetes and obesity) is continuously criticized because resistin gene expression and circulating resistin levels do not correlate and can have opposing biological functions [18]. The resistin levels of rats fed the HFDBB5M diet (482.7 pg/ml) were significantly higher ($p \leq 0.05$) compared to those in the BB5M and HFDBB2.5M groups. This result is paradoxical, given that the HFDBB5M group also showed favorable metabolic outcomes like reduced weight gain and increased antioxidant enzyme activity. One possible explanation could be a species-specific hormonal response or an endocrine adjustment due to prolonged metabolic stress, especially at the higher dose of *Hibiscus sabdariffa*. It's also possible that the elevated resistin reflects a compensatory feedback mechanism. More work is needed to understand this dynamic and how different doses of HS, especially when combined with probiotics, may influence hormonal regulation in metabolic pathways. [19] conducted to elucidate the mechanisms of action related to resistin levels and gene expression and elucidated that high

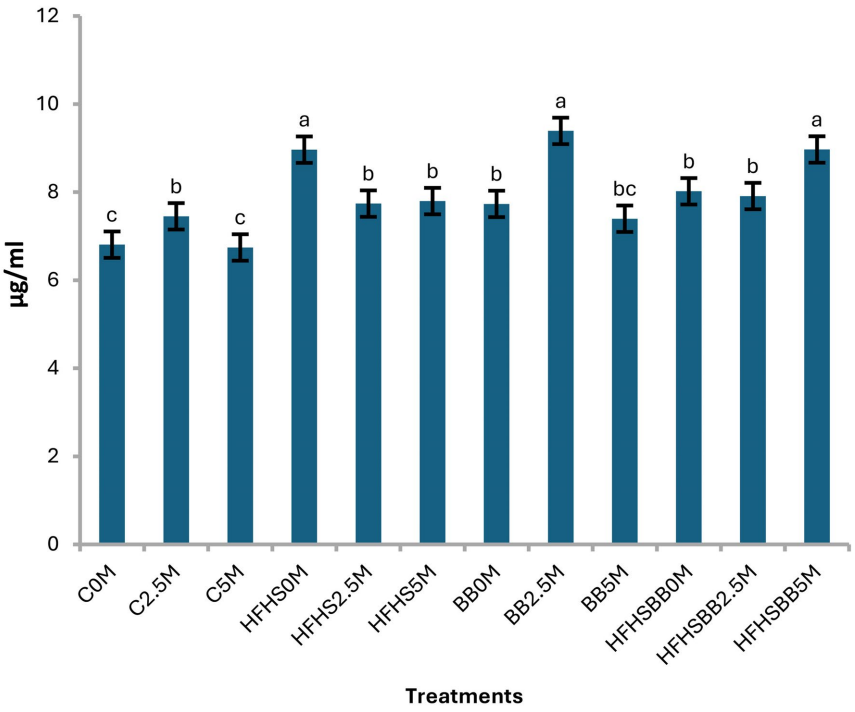

Figure 7. Adiponectin levels in rats fed Hibiscus meal. Values are means ± SEM. Bars with superscript (abcd) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

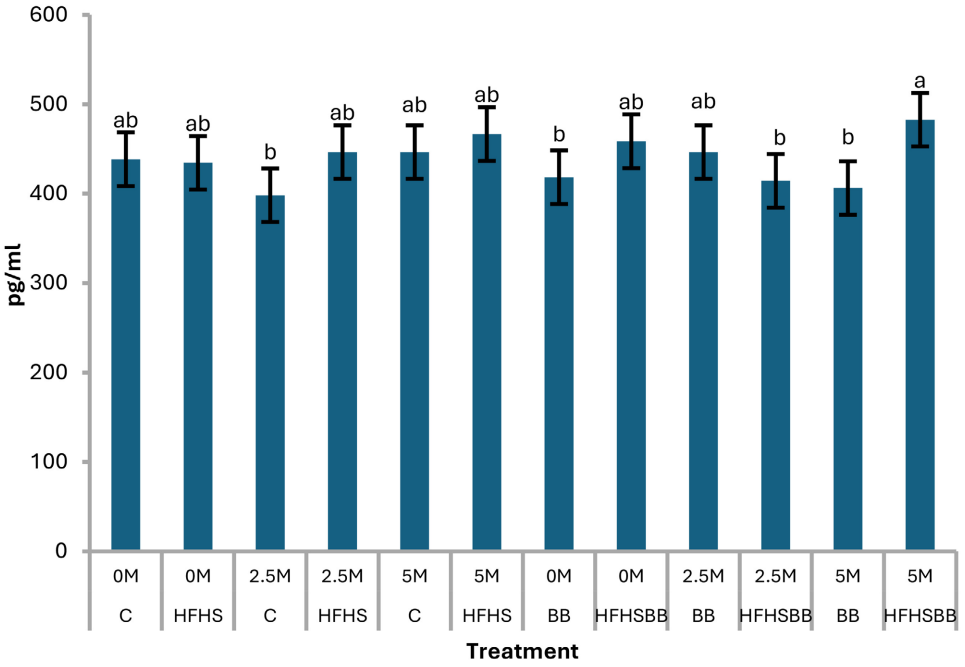

Figure 8. Blood resistin levels in rats fed dietary Hibiscus meal. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

resistin circulating in obese mice could be possible despite reduced expression in mRNA levels in the adipose tissue. Moreover, the high circulating levels could be attributed to the extended protein half-life due to binding factors in the serum or tertiary changes that have also been observed in other adipokines such as leptin and TNF-alpha [20] [21].

3.5. Hepatic and Plasma Catalase

Figure 9 and Figure 10 show the Hepatic and plasma levels, respectively. Catalase 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. In Figure 10, rats fed C5M (18.2 u/ml) had a significantly ($p \leq 0.05$) higher catalase activity compared to rats that received C0M (14.3 u/ml), which shows a concentration effect of the treatment. In a study by [22], rats administered a phytochemical treatment with compounds similar to HS exhibited significantly higher levels of SOD in the control groups compared to the groups fed a high-fat diet. These results correlated with our study, whereby overall rats that received a regular diet had significantly ($p \leq 0.05$) higher catalase levels compared to the high-fat diet groups.

3.6. Hepatic Glutathione, Glutathione Reductase and Glutathione-S-Transferase

Glutathione is a major antioxidant in the body that protects cells from reactive oxygen species such as free radicals, peroxides and heavy metals. Glutathione can

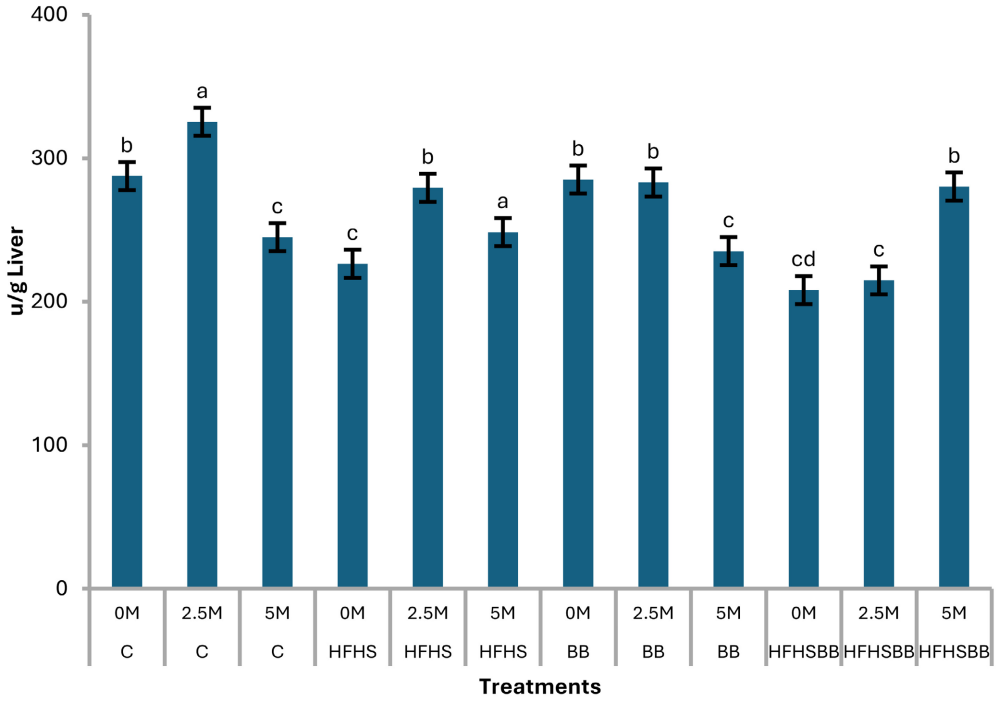

Figure 9. Hepatic catalase activity in rats fed dietary Hibiscus meal. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

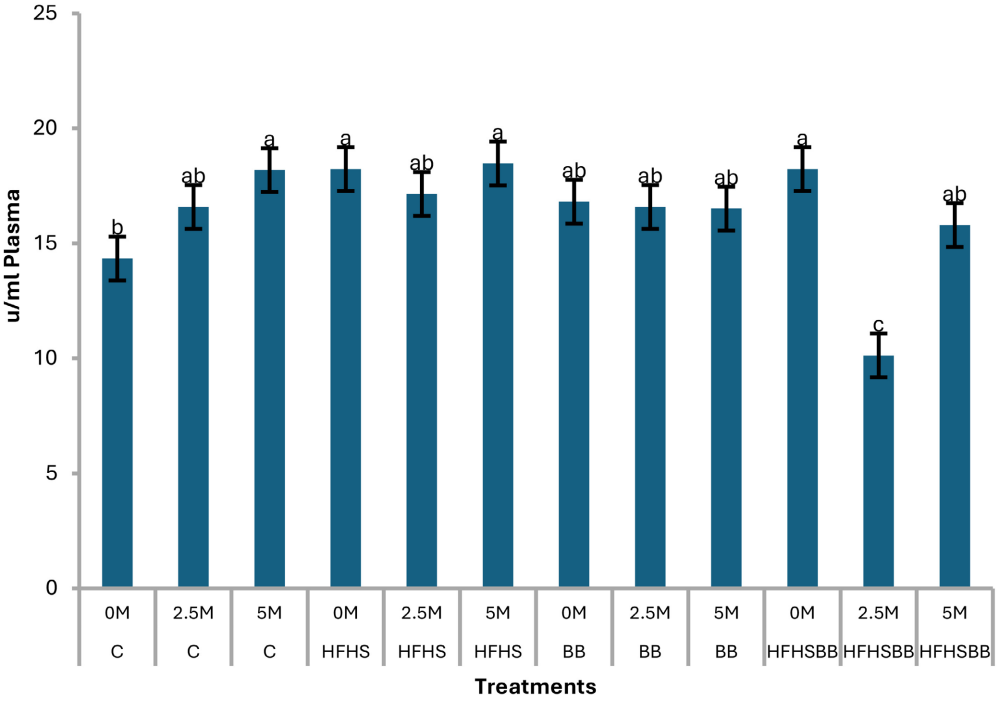

Figure 10. Plasma catalase activity in rats fed dietary Hibiscus meal. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

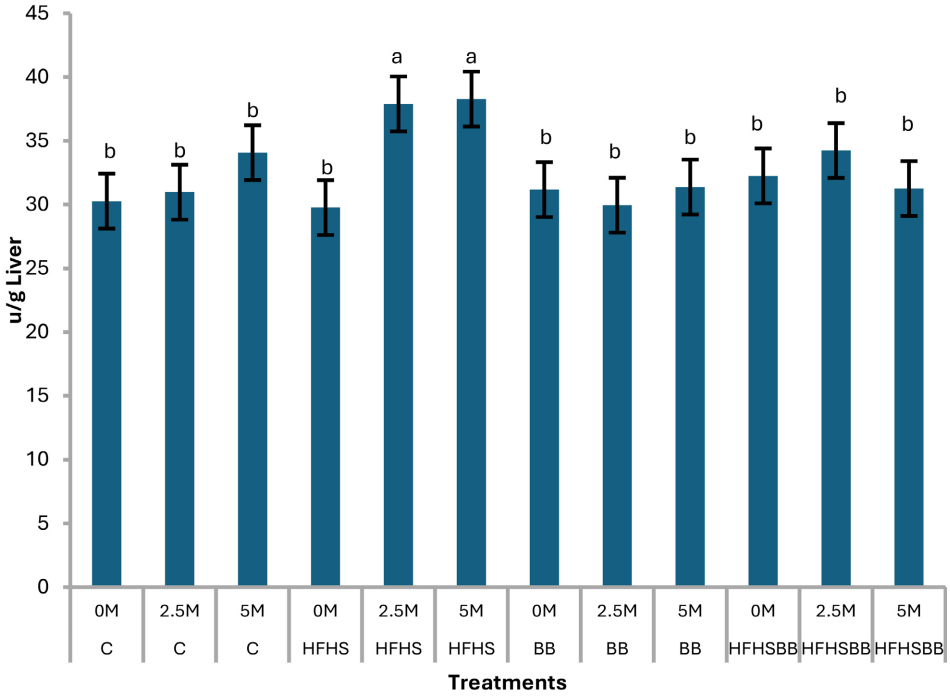

Figure 11. Hepatic glutathione levels in rats fed dietary Hibiscus meal. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

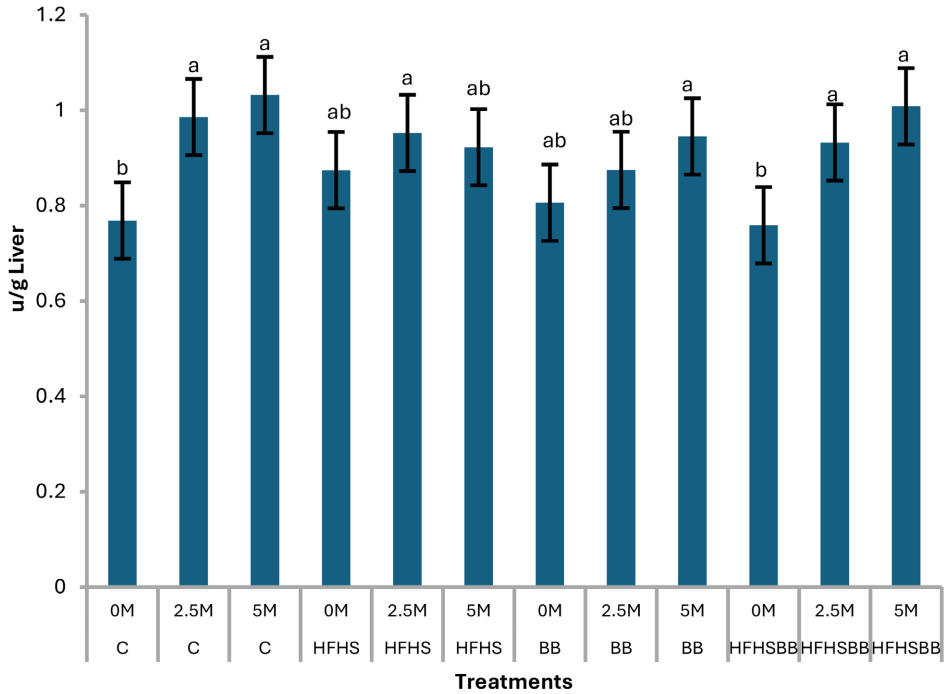

Figure 12. Hepatic glutathione reductase activity in rats fed dietary Hibiscus meal. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

serve as an important biomarker to determine the antioxidant potential in the livers of rats fed hibiscus either in meal or juice form. Figure 11 shows the glutathione levels. Rats fed the high-fat diets, including hibiscus and probiotics had slightly higher GR activity. The rats which were fed HFDBB2.5M (34.2 u/g) had higher glutathione levels compared to rats fed HFDBB0M (32.3 u/g).

Glutathione reductase, shown in Figure 12, is an important enzyme in the biological system that plays a critical role in the reduction of glutathione disulfide (GSSG) back to glutathione. The GR enzyme is an important biomarker that helps maintain oxidative damage. Overall, the rats that were fed the high-fat diets, including probiotics and hibiscus had lower GR activity compared to the rats fed the regular diet, including probiotics and hibiscus.

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 that were fed control diets including hibiscus only, rats fed C0M (14.1 u/g) and C2.5M (13.0 u/g) had significantly ($p \leq 0.05$) lower GST activity compared to rats fed C5M (26.6 u/g). Overall, the rats fed high-fat diets had significantly ($p \leq 0.05$) higher GST levels compared to rats fed control diets.

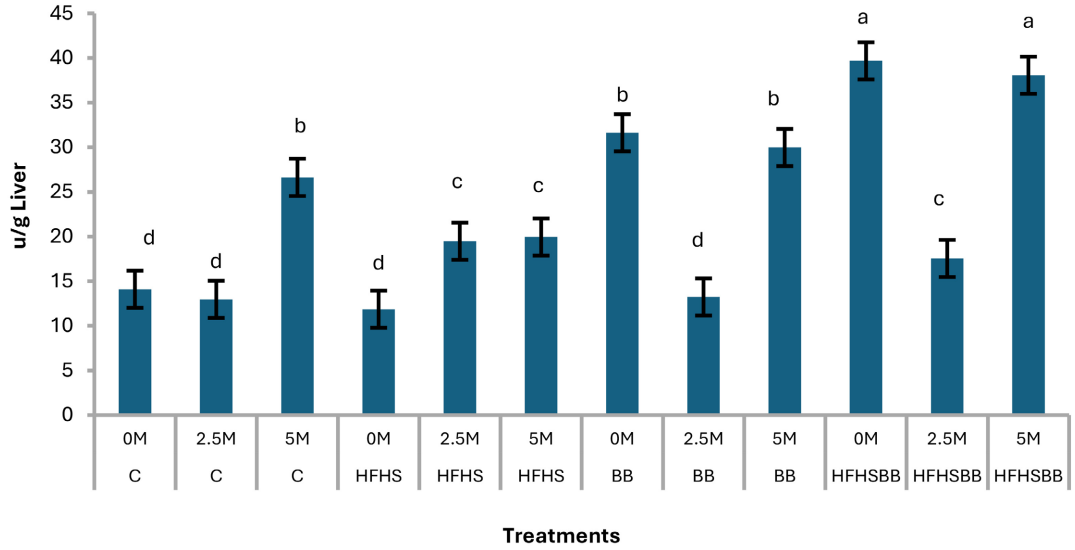

Figure 13. Hepatic GST activity in rats fed dietary Hibiscus meal. Values are means ± SEM. Bars with superscript abc indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

[23] conducted a study whereby rats a dietary supplement containing antioxidant phytochemicals, which decreased the activation of antioxidative enzymes such as GST, glutathione reductase and reduction of oxidized glutathione, thus improving the Total GSH/GSSG ratio.

3.7. Hepatic Plasma and Hepatic Superoxide Dismutase (SOD)

Figure 14 and Figure 15 illustrate hepatic and plasma superoxide dismutase (SOD) activity, respectively. SOD is a critical antioxidant enzyme that catalyzes the

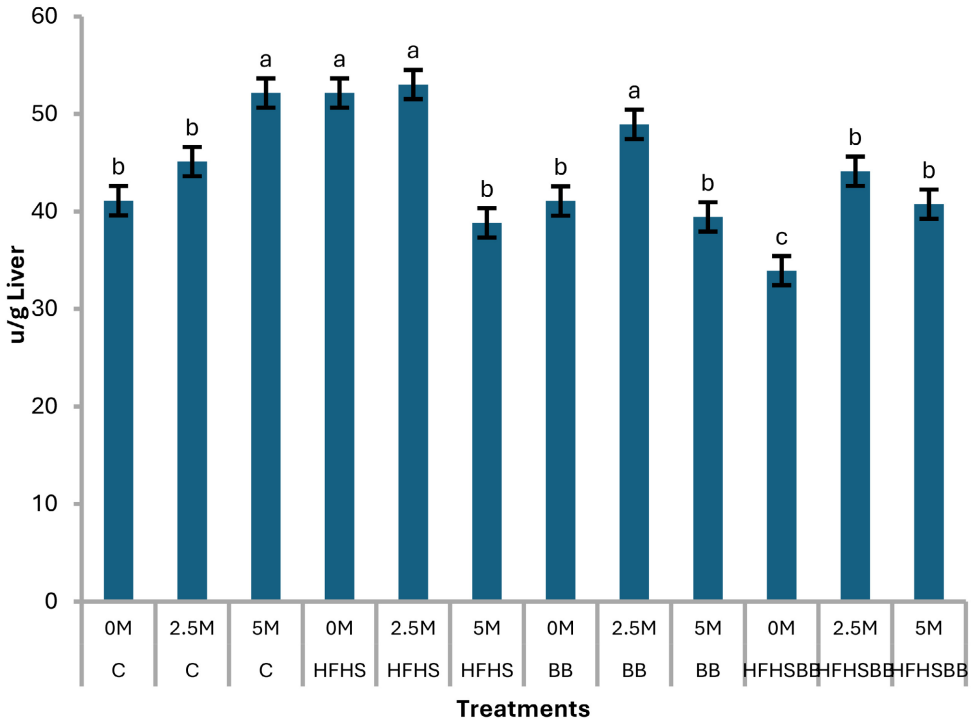

Figure 14. Hepatic SOD activity in rats fed dietary Hibiscus meal. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

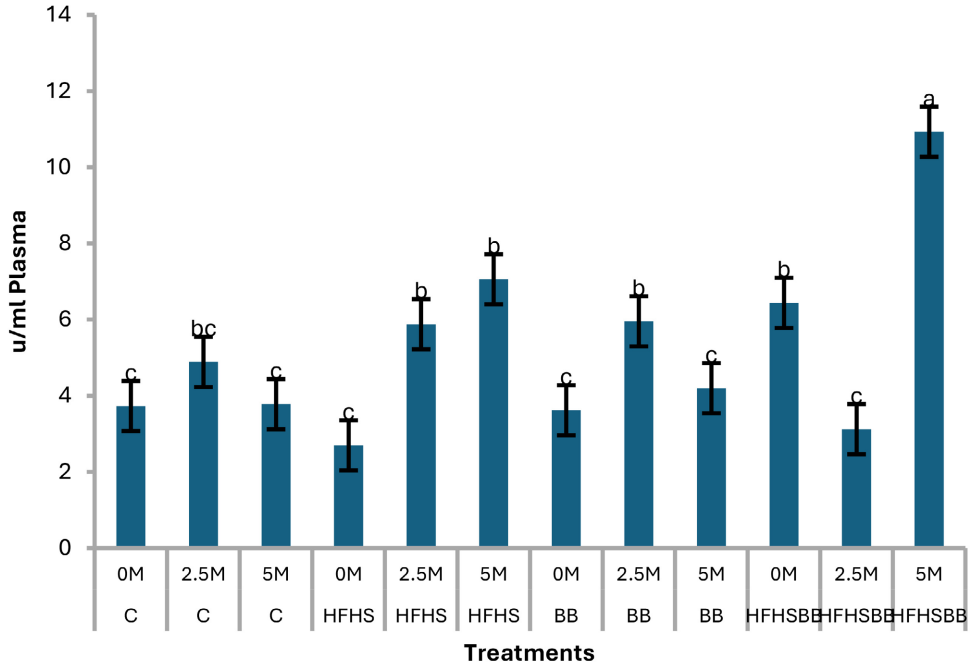

Figure 15. Plasma SOD activity in rats fed dietary Hibiscus meal. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

dismutation of superoxide radicals into hydrogen peroxide or oxygen. In Figure 14, rats on the control diet (C0M, 41.1 u/g) exhibited significantly lower hepatic SOD activity compared to those on C2.5M (45.1 u/g) and C5M (52.1 u/g). Rats fed HFDBB2.5M (44.1 u/g) and HFDBB5M (40.7 u/g) also showed elevated hepatic SOD levels relative to HFDBB0M (33.9 u/g). These results align with [24], who reported increased hepatic SOD with phytochemicals in high-fat diets, though our study showed more pronounced treatment differences.

Figure 15 shows plasma SOD activity. Rats on C2.5M (4.9 u/g) had significantly higher levels ($p \leq 0.05$) than those on C0M (3.7 u/g). Rats fed HFDBB5M (10.9 u/g) had significantly greater plasma SOD activity than those on HFDBB2.5M (31.2 u/g) and HFDBB0M (64.3 u/g), indicating differential antioxidant responses across treatments.

3.8. Hepatic COX, Interleukin-1β, and Interleukin-10

Figure 16 shows the COX activity. The COX enzyme is an enzyme that plays a key role in the production of prostanoids, which ultimately play a role in inflammation. There were no significant differences in the group of rats that were fed the high-fat diets, including hibiscus, however, rats fed high-fat diets HFD0M (10.9), HFD2.5M (1.2 u/g) and HFD5M (1.5 u/g) had significantly ($p \leq 0.05$) higher COX activity compared to the control diets mentioned above.

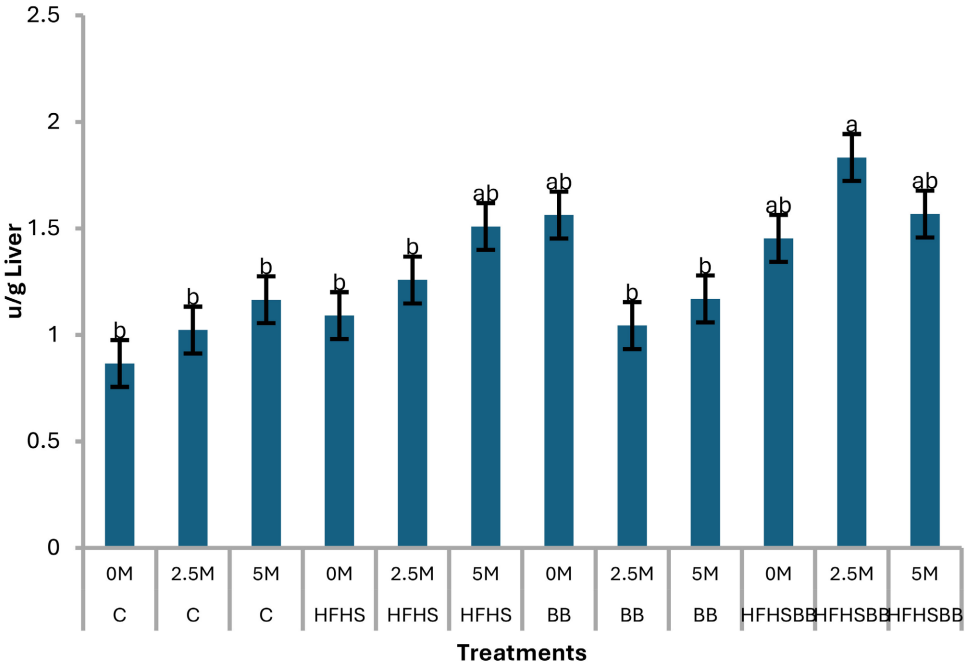

Figure 16. Hepatic COX activity in rats fed dietary Hibiscus meal. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: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

Interleukin-1β (IL-1β) is a cytokine antagonist that plays a key role in the reg-

ulation of inflammatory responses due to injury or illness. Figure 17 shows IL-1β levels. Amongst the group of rats fed high-fat diets, the IL-1β levels of rats fed HFD5M (236 pg/ml) were significantly ($p \leq 0.05$) higher than levels of rats fed HFD0M and HFD2.5M. Rats fed HFHSBB2.5J and HFHSBB5J had significantly ($p \leq 0.05$) lower IL-1β levels compared to rats fed HFDBB0M (206.8 pg/ml). It has been reported that overexpression of IL-1β could potentially lead to the impairment of adipose tissue to store TGs via the inhibiting hyperplasia in adipose tissue, inducing lipolysis and ultimately promoting liver steatosis [25]. The link between upregulated levels of IL-1β in obese states and diabetes is the ability of IL-1β in downregulating the production of key insulin-regulating proteins (GLUT-4 IRS1, PI3K p85a and pAkt), which can lead to insulin resistance.

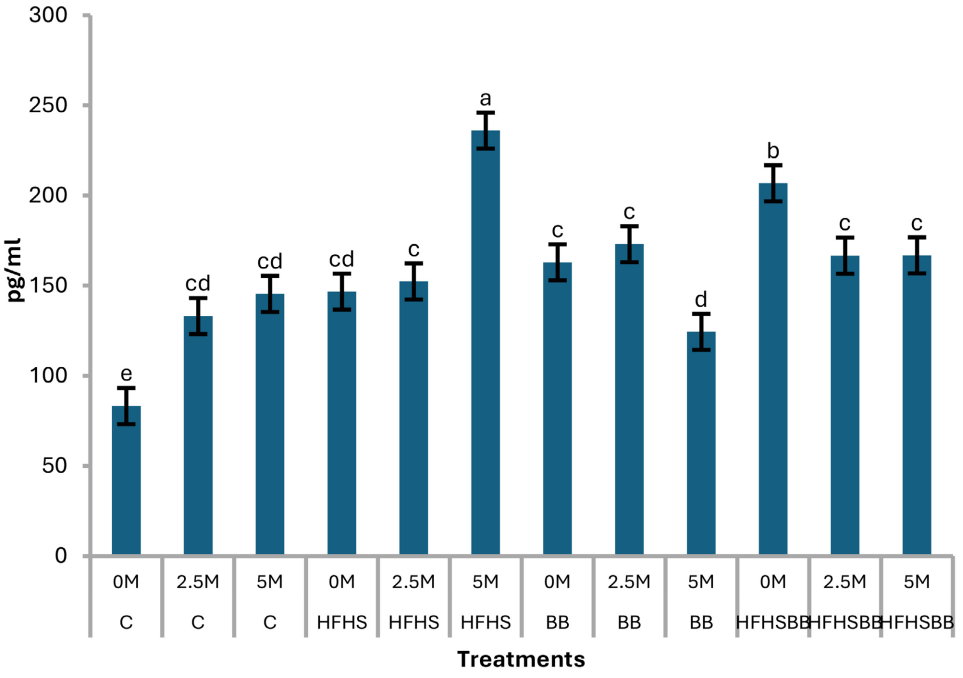

Figure 17. Interleukin-1B levels in rats fed Hibiscus meal. Values are means ± SEM. Bars with superscript (abcd) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

Interleukin-10 (IL-10) is a cytokine and, unlike IL-1β, it plays a critical role as an anti-inflammatory agent and controls inflammatory responses. Moreover, the introduction of IL-10 has been shown to improve insulin sensitivity in high-fat-induced animal models [26]. Figure 18 shows the IL-10 levels of rats. The rats fed C2.5M (841 pg/ml) and C5M (861.6 pg/ml) had significantly ($p \leq 0.05$) lower levels of IL-10 compared to rats fed C0M (985.7 pg/ml). In the group of rats fed high-fat diets, rats fed HFD0M (1099.7 pg/ml) and HFD2.5M (11300.3 pg/ml) had significantly ($p \leq 0.05$) lower levels of IL-10 compared to rats fed HFD5M (1255.3 pg/ml). In this study, we observed elevated IL-10 levels in control diets compared to treatments, which may indicate the role of HS, BB or a combination in both

downregulating circulating IL-10 levels.

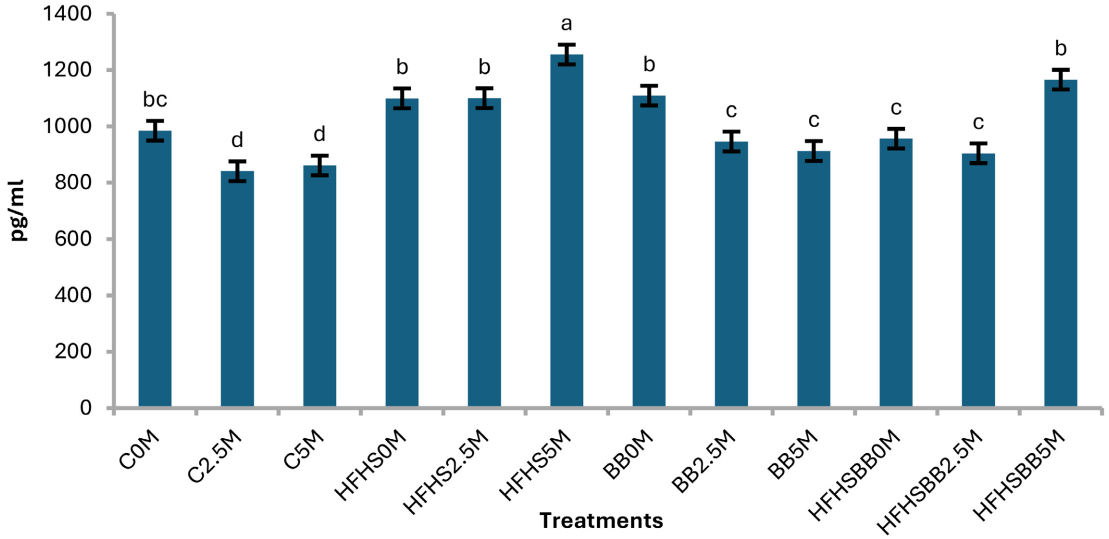

Figure 18. Interleukin-10 levels in rats fed Hibiscus meal. Values are means ± SEM. Bars with superscript (abcd) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

3.9. Fetuin-A, Leptin and Ghrelin Hormones

Fetuin-A is a glycoprotein that is synthesized in hepatic and adipose tissue. In an obese and T2D states, animal models indicate high Fetuin-A levels. Various sources of studies conducted on Fetuin-A have supported its evidence in the occurrence of impaired glucose metabolism, loss of function of insulin receptors, dyslipidemia and hepatic inflammation [27]. **Figure 19** shows the levels of Fetuin-A. Amongst the rats fed high-fat diets including hibiscus only, rats fed HFD0M (4.5 ng/ml) had significantly ($p \leq 0.05$) higher Fetuin-A levels compared to rats fed HFD2.5M (2.19) and HFD5M (1.1 ng/ml). This result was in correlation with the work conducted by [28]. Moreover, in our study, there was a concentration-dependent effect of administered HS only in regulating Fetuin-A levels in rats fed a high-fat diet.

Leptin, a hormone that is transcribed from the ob gene in adipocytes, is responsible for various metabolic factors that occur in normal and diseased states. Besides its common function of appetite control, leptin plays a significant role in inflammatory signals, weight management, angiogenesis and food intake [28]. **Figure 20** shows the levels of leptin. Rats fed C2.5M (12.5 ng/ml) had significantly ($p \leq 0.05$) lower leptin levels compared to rats fed C0M (15.4 ng/ml). Overall, rats fed a high-fat diet including hibiscus had significantly ($p \leq 0.05$) higher leptin levels compared to rats fed a regular diet. The group of rats fed HFDBB0M (17 ng/ml) had significantly ($p \leq 0.05$) higher levels of leptin compared to rats fed HFDBB2.5M (14.4 ng/ml) and HFDBB5M (13.5 ng/ml). High levels of leptin levels in groups fed a high-fat diet are commonly classified as leptin resistance. Hyperleptinemia due to its overexpression in adipose tissue and weight gain are common hallmarks for obe-

sity. In our study, a concentration dependent effect of HS was observed in rats fed a high-fat diet.

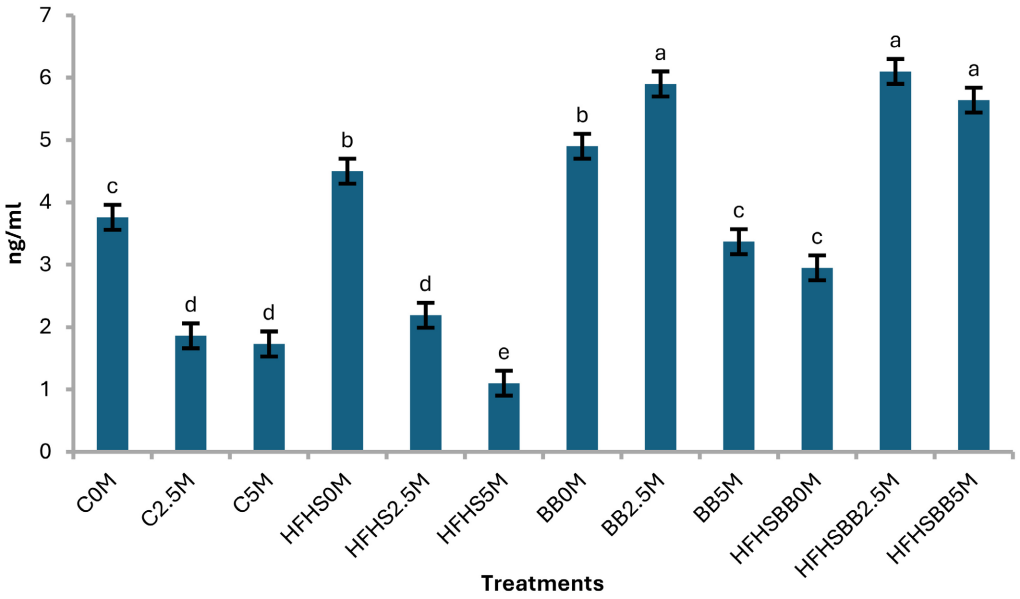

Figure 19. Fetuin-A levels of rats fed Hibiscus meal. Values are means ± SEM. Bars with superscript (abcde) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

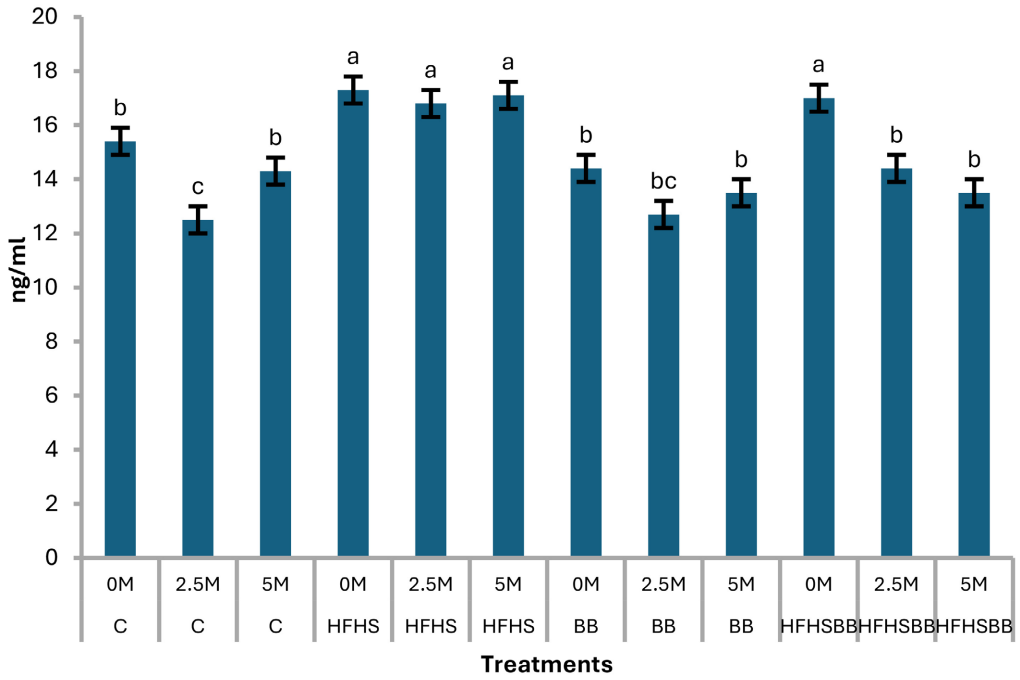

Figure 20. Leptin levels of rats fed Hibiscus meal. Values are means ± SEM. Bars with superscript (abc) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

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 below shows the ghrelin levels of rats. Rats fed HFD0M (502 pg/ml) overall had a significantly ($p \leq 0.05$) higher level of ghrelin compared to all treatment groups. Rats fed BB2.5M (455.3 pg/ml) had significantly ($p \leq 0.05$) lower levels of ghrelin compared to rats fed HFDBB2.5M (479.1 pg/ml). Lastly, rats fed HFDBB0M (479 pg/ml) had a significantly ($p \leq 0.05$) higher level of ghrelin compared to rats fed BB0M (455.4 pg/ml). Like leptin resistance, ghrelin resistance is also a hallmark of obesity, which is caused by the downregulation of NPY/AgRP response to ghrelin and suppression of the ghrelin axis in the neuroendocrine system [29].

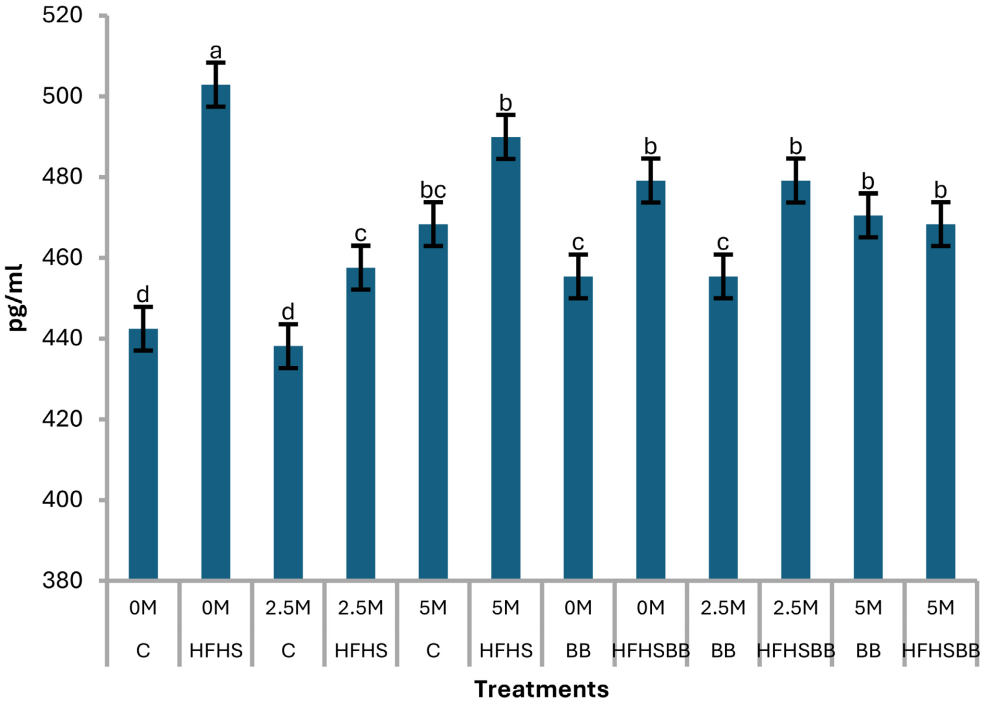

Figure 21. Ghrelin levels of rats fed Hibiscus meal. Values are means ± SEM. Bars with superscript (abcd) indicating meal type effect differ significantly at $p \leq 0.05$. Abbreviations: C: Control; M: Meal; HFD: High fat diet; HFDBB: High fat diet + *Bifidobacterium breve*; 0: 0%; 2.5: 2.5%; 5: 5%.

4. Conclusions

The 40-week animal study of the combination of *Hibiscus sabdariffa* (HS) and *Bifidobacterium breve* (BB) demonstrated significant potential in mitigating key biomarkers of the metabolic syndrome associated with obesity and type 2 diabetes.

While the study design included both high-fat and high-sugar components, it's important to note that metabolic disturbances like insulin resistance, hepatic steatosis, and weight gain are often worsened by excess dietary sugars. Sugars are known to drive lipogenesis and oxidative stress, which may have heightened the need for antioxidant defense in this model. As a result, the sugar component likely played a role in the severity of the metabolic profile and influenced the overall therapeutic effect seen with *Hibiscus sabdariffa* and *B. breve* treatment. Rats, which were ad-

ministered HS at higher concentrations, showed lower feed conversation ratios, reduced weight gain and improved lipid profiles (elevated HDL levels & decreased TGs). The combination of HS and BB also exhibited a reduction in HbA1c levels, increased adiponectin levels and lower circulation of ghrelin and leptin hormones. The regulation of key appetite hormones and glycated hemoglobin can highlight improved insulin sensitivity and appetite regulation—key indicators of healthy metabolic function. Significant elevation of key antioxidative enzymes such as catalase, SOD and glutathione also indicates an improved hepatic oxidative stress response and defense. In addition, pro-inflammatory markers such as IL-1β and COX-2 were decreased and Hepatic enzymes such as GST and glutathione reductase showed improved detoxification potential. The increase in hepatic glutathione-S-transferase (GST) and glutathione reductase activity points to enhanced detoxification and antioxidant response. These enzymes play a key role in managing oxidative stress, which is known to drive insulin resistance and liver dysfunction. Their activation in the treated groups supports the idea that antioxidant pathways may be contributing to the improvements seen in both glucose regulation and lipid metabolism. This data supports the synergetic interaction of HS and BB in improving metabolic, inflammatory and oxidative stress markers in Sprague-Dawley rats. Moreover, these findings suggest that dietary supplementation of polyphenol-rich botanicals such as HS, and in combination with probiotics, may be a potential preventative measure against obesity and insulin insensitivity.

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] Alili, L., Robert, M., Diene, G., Valette, M. and Tauber, M. (2023) Adipose Tissue Hyperplasia and Hypertrophy in Common and Syndromic Obesity—The Case of BBS Obesity. *Frontiers in Cell and Developmental Biology*, **11**, Article 1227950. https://doi.org/10.3389/fcell.2023.1227950

[2] World Health Organization (2021) Obesity and Overweight. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

[3] Klein, S., Gastaldelli, A., Yki-Järvinen, H. and Scherer, P.E. (2022) Why Does Obesity Cause Diabetes? *Cell Metabolism*, **34**, 11-20.

[4] Dinu, M., Pagliai, G., Angelino, D., Rosi, A., Dall'Asta, M., Bresciani, L., Martini, D. and Sofi, F. (2023) Global Impacts of Western Diet and Its Effects on Metabolism and Health: A Narrative Review. *Nutrients*, **15**, Article No. 622.

[5] Kim, M.S., Kim, J.K., Kim, H.J., Moon, S.R., Shin, B.C., Park, K.W., *et al.* (2003) Hibiscus Extract Inhibits the Lipid Droplet Accumulation and Adipogenic Transcription

Factors Expression of 3T3-L1 Preadipocytes. *The Journal of Alternative and Complementary Medicine*, **9**, 499-504. https://doi.org/10.1089/107555303322284785

[6] Alarcon-Aguilar, F.J., Zamilpa, A., Perez-Garcia, M.D., Almanza-Perez, J.C., Romero-Nuñez, E., Campos-Sepúlveda, E.A. and Roman-Ramos, R. (2007) Effect of *Hibiscus sabdariffa* on Obesity in MSG Mice. *Journal of Ethnopharmacology*, **114**, 66-71.

[7] Shen, C.Y., Wan, L., Wang, T.X. and Jiang, J.G. (2019) *Citrus aurantium* L. var. *amara* Engl. Inhibited Lipid Accumulation in 3T3-L1 Cells and *Caenorhabditis elegans* and Prevented Obesity in High-Fat Diet-Fed Mice. *Pharmacological Research*, **147**, Article ID: 104347. https://doi.org/10.1016/j.phrs.2019.104347

[8] Bragueto Escher, G., Cardoso Borges, L.d.C., Sousa Santos, J., Mendanha Cruz, T., Boscacci Marques, M., Araújo Vieira do Carmo, M., *et al.* (2019) From the Field to the Pot: Phytochemical and Functional Analyses of *Calendula officinalis* L. Flower for Incorporation in an Organic Yogurt. *Antioxidants*, **8**, Article No. 559. https://doi.org/10.3390/antiox8110559

[9] Pimentel, T.C., Costa, W.K.A.d., Barão, C.E., Rosset, M. and Magnani, M. (2021) Vegan Probiotic Products: A Modern Tendency or the Newest Challenge in Functional Foods. *Food Research International*, **140**, Article ID: 110033. https://doi.org/10.1016/j.foodres.2020.110033

[10] Boschmann, M. and Thielecke, F. (2007) The Effects of Epigallocatechin-3-Gallate on Thermogenesis and Fat Oxidation in Obese Men: A Pilot Study. *Journal of the Ameri-can College of Nutrition*, **26**, 389S-395S.

[11] Luo, Y., Zeng, Y., Peng, J., Zhang, K., Wang, L., Feng, T., *et al.* (2023) Phytochemicals for the Treatment of Metabolic Diseases: Evidence from Clinical Studies. *Biomedicine & Pharmacotherapy*, **165**, Article ID: 115274. https://doi.org/10.1016/j.biopha.2023.115274

[12] Truthmann, J., Schienkiewitz, A., Busch, M.A., Mensink, G.B.M., Du, Y., Bosy-Westphal, A., *et al.* (2016) Changes in Mean Serum Lipids among Adults in Germany: Results from National Health Surveys 1997-99 and 2008-11. *BMC Public Health*, **16**, Article No. 378. https://doi.org/10.1186/s12889-016-2826-2

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

[14] Li, T., Chanda, D., Zhang, Y., Choi, H.S. and Chiang, J.Y.L. (2010) Glucose Stimulates Cholesterol 7α-Hydroxylase Gene Transcription in Human Hepatocytes. *Journal of Lipid Research*, **51**, 832-842. https://doi.org/10.1194/jlr.m002782

[15] Cho, K.H. (2022) The Current Status of Research on High-Density Lipoproteins (HDL): A Paradigm Shift from HDL Quantity to HDL Quality and HDL Functionality. *International Journal of Molecular Sciences*, **23**, Article No. 3967. https://doi.org/10.3390/ijms23073967

[16] Van Syoc, E.P., Damani, J., DiMattia, Z., Ganda, E. and Rogers, C.J. (2023) The Effects of Bifidobacterium Probiotic Supplementation on Blood Glucose: A Systematic Review and Meta-Analysis of Preclinical Animal Models and Clinical Evidence. *Advances in Nutrition*, **15**, Article ID: 100137.

[17] Kadowaki, T. and Yamauchi, T. (2005) Adiponectin and Adiponectin Receptors. *Endocrine Reviews*, **26**, 439-451. https://doi.org/10.1210/er.2005-0005

[18] Banerjee, R.R., Rangwala, S.M., Shapiro, J.S., Rich, A.S., Rhoades, B., Qi, Y., *et al.* (2004) Regulation of Fasted Blood Glucose by Resistin. *Science*, **303**, 1195-1198. https://doi.org/10.1126/science.1092341

[19] Rajala, M.W., Qi, Y., Patel, H.R., Takahashi, N., Banerjee, R., Pajvani, U.B., *et al.* (2004)

Regulation of Resistin Expression and Circulating Levels in Obesity, Diabetes, and Fasting. *Diabetes*, **53**, 1671-1679. https://doi.org/10.2337/diabetes.53.7.1671

[20] Huang, L., Wang, Z. and Li, C. (2001) Modulation of Circulating Leptin Levels by Its Soluble Receptor. *Journal of Biological Chemistry*, **276**, 6343-6349. https://doi.org/10.1074/jbc.m009795200

[21] Winkler, G., Kiss, S., Keszthelyi, L., Sápi, Z., Öry, I., Salamon, F., *et al.* (2003) Expression of Tumor Necrosis Factor (TNF)-Alpha Protein in the Subcutaneous and Visceral Adipose Tissue in Correlation with Adipocyte Cell Volume, Serum TNF-Alpha, Soluble Serum TNF-Receptor-2 Concentrations and C-Peptide Level. *European Journal of Endocrinology*, **149**, 129-135. https://doi.org/10.1530/eje.0.1490129

[22] El-Shehawi, A.M., Alkafafy, M., El-Shazly, S., Sayed, S., Farouk, S., Alotaibi, S., *et al.* (2021) *Moringa oleifera* Leaves Ethanolic Extract Ameliorates High Fat Diet-Induced Obesity in Rats. *Journal of King Saud University—Science*, **33**, Article ID: 101552. https://doi.org/10.1016/j.jksus.2021.101552

[23] Fernández-Iglesias, A., Pajuelo, D., Quesada, H., Díaz, S., Bladé, C., Arola, L., *et al.* (2013) Grape Seed Proanthocyanidin Extract Improves the Hepatic Glutathione Metabolism in Obese Zucker Rats. *Molecular Nutrition & Food Research*, **58**, 727-737. https://doi.org/10.1002/mnfr.201300455

[24] Ragab, S.M., Omar, H.M., Sary, K., Abd Elghaffar, S.K. and El-Metwally, T.H. (2014) Hypolipidemic and Antioxidant Effects of Phytochemical Compounds against Hepatic Steatosis Induced by High-Fat High-Sucrose Diet in Rats. *Archives of Biomedical Science*, **2**, 1-10.

[25] Nov, O., Shapiro, H., Ovadia, H., Tarnovscki, T., Dvir, I., Shemesh, E., *et al.* (2013) Interleukin-1β Regulates Fat-Liver Crosstalk in Obesity by Auto-Paracrine Modulation of Adipose Tissue Inflammation and Expandability. *PLOS ONE*, **8**, e53626. https://doi.org/10.1371/journal.pone.0053626

[26] Joyce, D.A., Gibbons, D.P., Green, P., Steer, J.H., Feldmann, M. and Brennan, F.M. (1994) Two Inhibitors of Pro-Inflammatory Cytokine Release, Interleukin-10 and Interleukin-4, Have Contrasting Effects on Release of Soluble p75 Tumor Necrosis Factor Receptor by Cultured Monocytes. *European Journal of Immunology*, **24**, 2699-2705.

[27] Trepanowski, J.F., Mey, J. and Varady, K.A. (2015) Fetuin-A: A Novel Link between Obesity and Related Complications. *International Journal of Obesity*, **39**, 734-741.

[28] Obradovic, M., Sudar-Milovanovic, E., Soskic, S., Essack, M., Arya, S., Stewart, A.J., *et al.* (2021) Leptin and Obesity: Role and Clinical Implication. *Frontiers in Endocrinology*, **12**, Article 585887. https://doi.org/10.3389/fendo.2021.585887

[29] Briggs, D.I., Enriori, P.J., Lemus, M.B., Cowley, M.A. and Andrews, Z.B. (2010) Diet-induced Obesity Causes Ghrelin Resistance in Arcuate NPY/AgRP Neurons. *Endocrinology*, **151**, 4745-4755. https://doi.org/10.1210/en.2010-0556