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Effect of cavendish green banana flour " The Mediterranean " on the improvement of metabolic dysfunctions in diabetic Wistar rats

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Abstract

Green bananas, a rich source of bioactive compounds essential for human health. The objective of this study was to examine the beneficial effects of Cavendish Green Banana Flour "The Mediterranean" consumption on diabetic Wistar rats. The experimental rats were randomly divided into 3 groups: the negative control group (NCG) received a standard diet (SD), the positive control group (PCG) received a high-fat diet (HFD), and the green banana group (GBG) received a HFD enriched with green banana flour (GBF). The diabetic animal model was established through a 10-week HFD followed by a low dose (35 mg/kg) of streptozotocin (STZ). The experimental results after the 4th week revealed that the rats in the GBG began to adjust their daily food intake (both solid and liquid). Their weight gain was significantly inhibited when compared to the PCG. Blood glucose levels decreased, and serum levels demonstrated considerable improvement. Organ weights and histological sections showed significant improvements in the pancreas, liver, kidneys, and testes, along with a notable reduction in body fat mass. The findings of this study demonstrate that green banana flour "The Mediterranean" has facilitated the improvement of blood glucose levels and the complications associated with metabolic disorders due to diabetes in rats. This study supports the use of "The Mediterranean" green banana flour as a functional food for managing blood glucose and metabolic disorders. It provides a natural dietary alternative to improve the health outcomes and quality of life for diabetic patients.

Keywords: Diabetes, Green cavendish banana flour, High-fat diet, Streptozotocin, Wistar rat.

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Transparency: The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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1. Introduction

Diabetes is one of the four major non-communicable diseases, constituting a significant public health problem due to its prevalence and serious long-term complications [1]. According to the latest estimates from the International Diabetes Federation (IDF), 589 million people are living with diabetes. This number is projected to rise to 643 million by 2030 and 853 million by 2050. In Algeria, the prevalence of diabetes is estimated at approximately 17.5% among adults, corresponding to nearly 4.8 million patients [2].

Type 2 diabetes mellitus (T2DM) is often clinically characterized by metabolic syndrome, overweight or obesity, a frequent family history of diabetes, as well as obstetrical history such as gestational diabetes and macrosomia. The management of patients with T2DM aims to reduce morbidity and mortality, notably through proper glycemic control, prevention, screening, and treatment of vascular and renal complications, as well as the improvement of the patient's quality of life [3]. In this context, scientific research has focused on pharmacological and nutritional therapies, particularly plant-based sources, which is devoid of adverse effects [4, 5]. Foods possessing physiological and functional properties are emerging as a key nutritional strategy [6]. Particularly those containing antioxidant compounds [7], probiotics, and prebiotics [8].

Green bananas possess high nutritional value, representing a rich source of fiber, vitamins (C, B6, and provitamin A), minerals (potassium, phosphorus, magnesium, zinc), bioactive compounds such as phenolic compounds, and resistant starch (RS) [9] which are essential for human health [10] thus allowing them to be classified as a functional food [11].

The objective of this study was to investigate the beneficial effect of GBF (Cavendish cultivar) on Wistar rats rendered diabetic. This flour is extracted from a strain of green banana cultivated in Algeria, known as "The Mediterranean".

2. Materials and Methods

2.1. Plant Material

The selected green banana is known as "The Mediterranean". It is a local variety cultivated in the "El-Mazouzi" nursery located in Sidi Fredj (Algiers). It is derived from the Cavendish subgroup, also referred to as the improved Large Dwarf "CV 902".

Banana bunches were harvested at the first stage of maturation, have completely green skin. The bananas are weighed, washed, and manually peeled. They are then sliced, spread separately, and dried in an oven (ARRAS MAXEI) at 55°C with an air flow of 1.0 m/s before being ground. The resulting flour is weighed, then packaged in polyethylene bags and stored at 10°C [12].

2.2. Animal Experimentation

The rat strain used for the experimentation was Wistar. The breeding, conducted according to standard rules and in compliance with regulations, at the animal facility of the Laboratory, Department of Biology, Faculty of Natural and Life Sciences, Ahmed Ben Bella Oran 1 University. The animals were healthy, free from stress and agitation, to avoid compromising the experimental results [13].

2.3. Experimental Design

This study lasted eight months, from 11/2023 to 06/2024. It included a mating phase, an eight-week breeding phase to obtain adult animals, and an experimental phase in which adult rats weighing 160 ± 12 g were selected and grouped as follows:

- A group of rats was subjected to HFD containing 30% sheep fat for 10 weeks. At the end of this period, the selected rats weighed 260 ± 20 g. A solution of streptozotocin (STZ) was freshly prepared (0.1 g dissolved in 5 ml of 0.1 M sodium citrate buffer, pH 4.5). These selected rats were then subjected to a prior fasting period and injected intraperitoneally with a low dose of STZ (35 mg/kg body weight) to induce type 2 diabetes [14, 15].
- Another non-diabetic group was maintained on a SD and given a single dose of saline solution to remain under the same experimental conditions.

One and a half hours later, feeding was reintroduced. Rats with fasting blood glucose levels > 2 g/L and exhibiting signs of glycosuria after 48 hours of STZ induction were considered diabetic. The rats were then allocated into three groups:

- Negative Control Group (NCG): consisting of six healthy rats receiving water and a SD for 28 days;
- Positive Control Group (PCG): consisting of six diabetic rats receiving water and an HFD for 28 days;
- Green Banana Group (GBG): consisting of six diabetic rats receiving water and an HFD enriched with 45% green banana flour for 28 days.

The weight, water and food consumption, and blood glucose levels were measured throughout the duration of the experiment.

2.4. Euthanasia, Organ Collection and Blood Sampling

Following a total duration of fourteen weeks and a twelve-hour fasting period, the rats in each group were weighed and subsequently anesthetized. At the time of euthanasia, a quantity of blood was collected via the abdominal aorta for the determination of biochemical parameters. After blood collection, the target organs of the experiment were carefully excised, rinsed with physiological saline, weighed, and then immediately fixed in 10% buffered formalin to prevent tissue autolysis and ensure the quality of the histological examination results [16].

2.5. Biochemical Analysis

Blood samples were centrifuged at 3500 g for 10 minutes to obtain serum. The concentrations of total tri-glycerides (TTG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), aspartate transaminase (AST), alanine transaminase (ALT), creatinine, and testosterone were measured using specific kits from BIOLABO (France).

2.6. Histological Analysis

The target organs of the experiment (pancreas, kidneys, liver, heart, and testes) were prefixed in formalin. These organs underwent a rigorous standard protocol of dehydration, impregnation, and tissue embedding in paraffin. Histological sections of three microns in thickness were prepared from each paraffin block. The slides were then placed in an oven at 60°C before being stained with hematoxylin and eosin and mounted to ensure protection and preservation of the staining [16]. The slides were interpreted using a microscope (Olympus CX43) equipped with a camera and connected to a computer.

2.7. Statistical Analysis

Analysis was performed using IBM SPSS Statistics software, version 26.0. Results are presented as mean \pm standard error (SE). Analysis of variance (ANOVA) was used for comparisons between group means. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Daily Food and Water Consumption

The estimated daily consumption of food and water during the experimental period on rats was indicated in Figure 1.

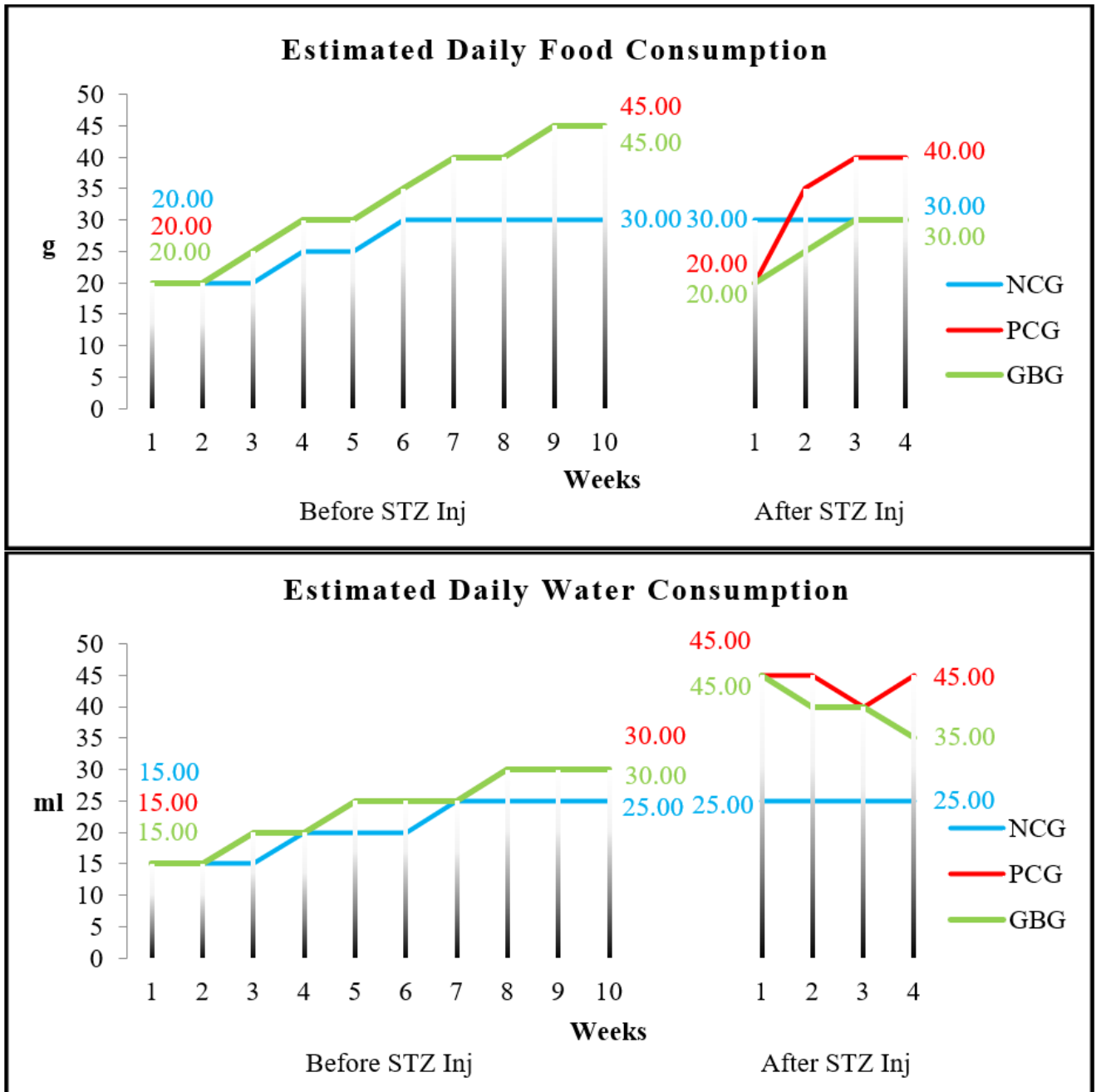


Figure 1. Estimated Daily Food and Water Consumption in Wistar Rats.
 Note: Inj: Injection; STZ: Streptozotocin;
 NCG: Negative Control Group; PCG: Positive Control Group; GBG: Green Banana Group

During the first six weeks of the experiment, the estimated daily food consumption for all three groups showed a progressive increase. From the sixth week onward, the consumption for the NCG plateaued at approximately 30 g/day/rat, while it continued to increase in the other two groups, stabilizing at 45 g/day/rat by the ninth week. Immediately following the diabetes modeling in the rats, food consumption in the PCG and GBG increased until the second week, then decreased to stabilize between the third and fourth weeks, with values of 40 g/day/rat for the PCG and 30 g/day/rat for the GBG.

The estimated daily water consumption for the three groups (NCG, PCG, GBG) demonstrated a progressive increase over the first seven weeks. It reached approximately 25 mL/day/rat in the NCG and remained stable until the end of the experiment. In contrast, consumption in the PCG and GBG was maintained at about 30 mL/day/rat until the 8th week. Following the STZ injection, the consumption in the PCG surged significantly to 45 mL/day/rat, whereas the GBG exhibited a moderate increase, reaching approximately 35 mL/day/rat.

3.2. Body Weight Progression

The results of the variation between initial and final body weight, along with the body weight monitoring throughout the experimental duration, are presented in illustrations (Figure 2 and Table 1).

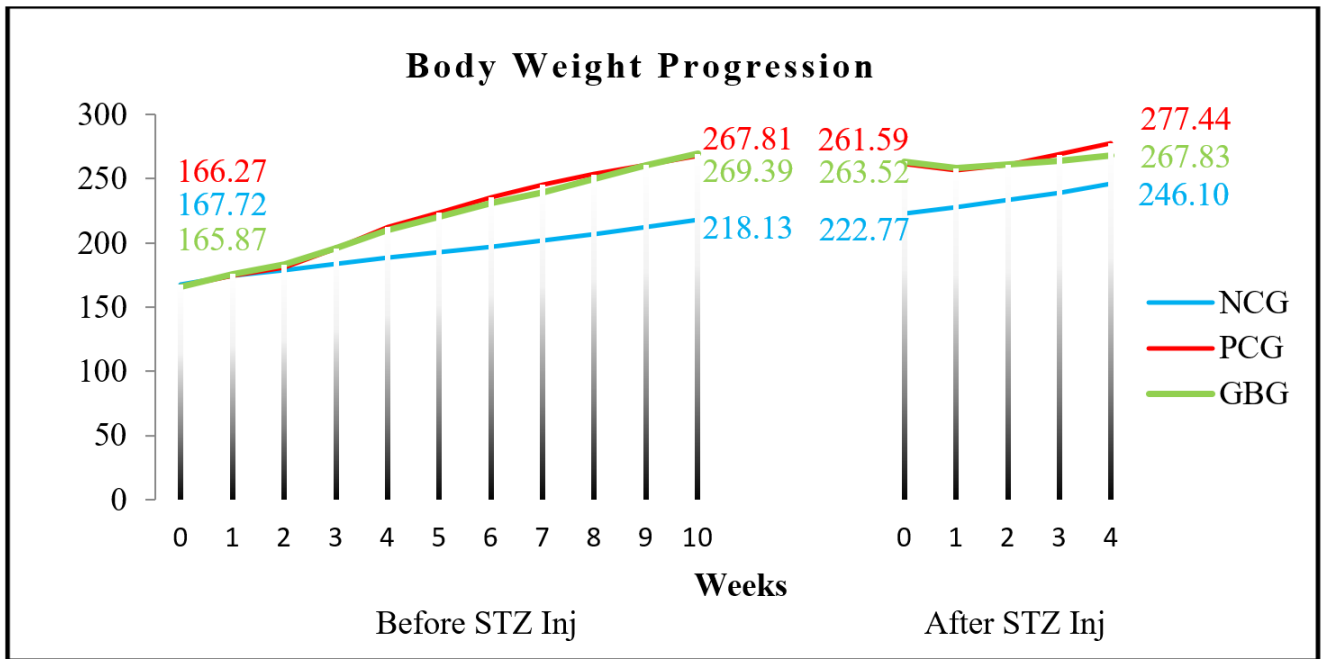


Figure 2. Weight Progression of Wistar Rats During the Experiment.
 Note: Inj: Injection; STZ: Streptozotocin;
 NCG: Negative Control Group; PCG: Positive Control Group; GBG: Green Banana Group

Table 1. Weight variations in Wistar rats (initial weight and final weight).

	Weight variation (g)			Weight variation (%)		
	NCG	PCG	GBG	NCG	PCG	GBG
RHL.P	+50.40 ± 0.88 ^a	+101.54 ± 1.64 ^b	+103.52 ± 1.33 ^b	+30.05 ± 0.75 ^a	+61.07 ± 1.48 ^b	+62.41 ± 1.23 ^b
STZ.P	+23.33 ± 0.55 ^a	+15.85 ± 1.08 ^b	+04.31 ± 0.58 ^c	+10.47 ± 0.28 ^a	+06.06 ± 0.44 ^b	+01.64 ± 0.22 ^c
T.E.P	+78.37 ± 1.24 ^a	+111.17 ± 1.47 ^b	+101.96 ± 1.23 ^c	+46.78 ± 1.11 ^a	+66.95 ± 1.60 ^b	+61.53 ± 1.25 ^b

Note: Mean ± Standard Error values within the column followed by different letters, differ significantly according to Tukey's post-hoc test (p<0.05). (HFD.P: High-Fat Diet Period; STZ.P: Streptozotocin Period; T.E.P: Total Experiment Period; NCG: Negative Control Group; PCG: Positive Control Group; GBG: Green banana Group).

Throughout the experimental period, the body weight of the NCG rats increased at a relatively moderate rate, reaching an average weight of 218.13 ± 1.84 g by the 10th week and 246.10 ± 1.82 g at the end of the experiment.

Regarding the other two groups, weight increased at a relatively rapid rate until the 10th week, where the recorded average weight was 267.81 ± 1.64 g for the PCG and 269.39 ± 1.75 g for the GBG, with no significant difference observed between them (p = 0.80).

Following STZ injection, a decrease in body weight was observed in both PCG and GBG in the first week. Starting from the second week, an increase was noted, reaching an average at the end of the experiment of 277.44 ± 0.66 g for the PCG and 267.83 ± 1.45 g for the GBG.

Table 01 presents the body weight changes measured before and after diabetes modeling, as well as the cumulative variation over the entire experimental period.

3.3. Monitoring of Blood Glucose Levels

The blood glucose measurements and the corresponding calculated variations for the three experimental groups are presented in Figure 2.

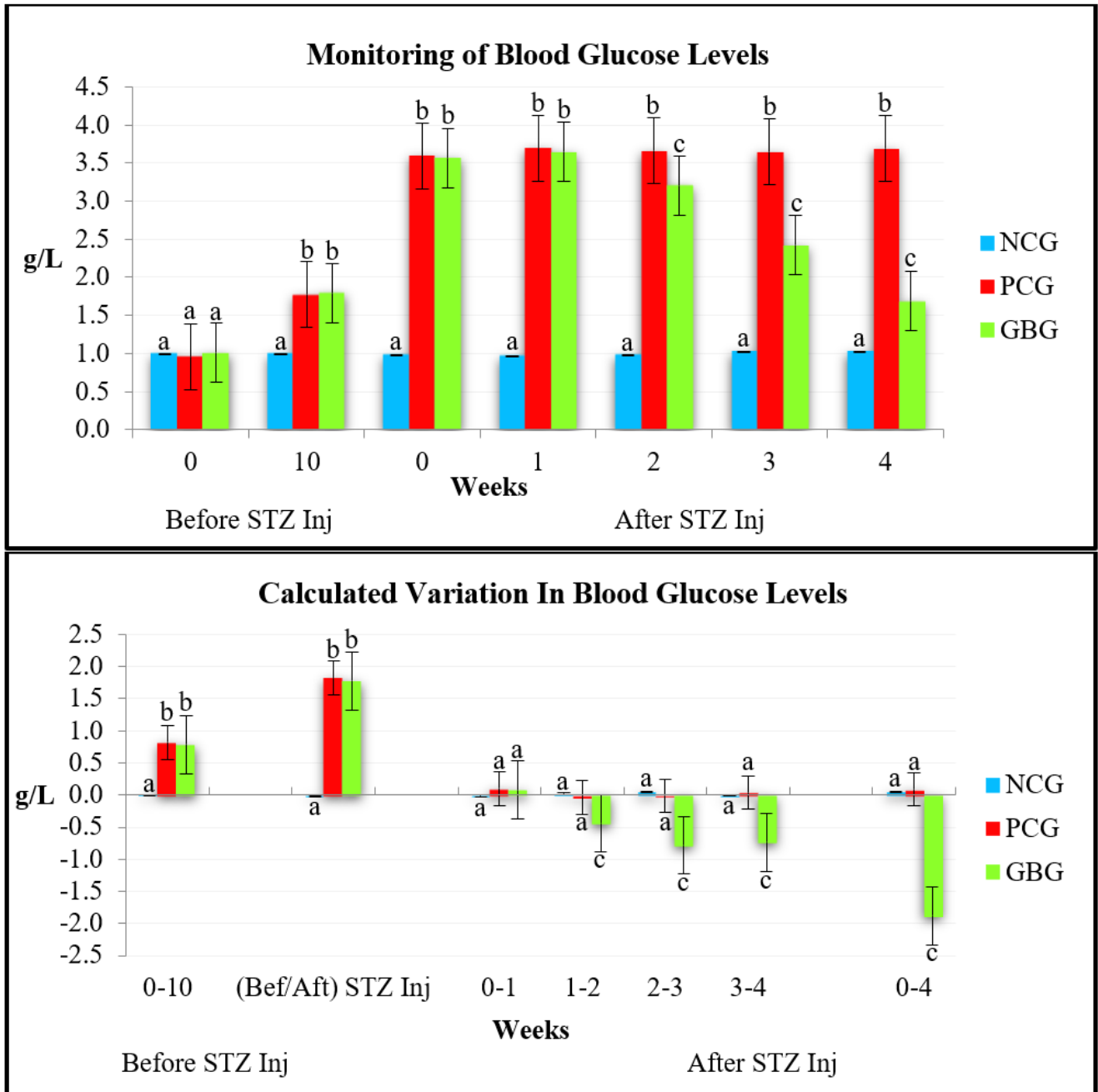


Figure 3. Blood Glucose Monitoring and Calculated Variations for the Three Wistar Rats Groups.
 Note: Mean ± Standard Error values represented in the graph with different letters indicate significant differences according to Tukey's post-hoc test (p<0.05).
 Inj: Injection; STZ: Streptozotocin; Bef: Before; Aft: After
 NCG: Negative Control Group; PCG: Positive Control Group; GBG: Green Banana Group

At the beginning of the experiment (Figure 3) it was observed that all rats had a blood glucose level of approximately 1 g/L. The NCG maintained the same value throughout the entire duration of the experiment. In contrast, the PCG and GBG exhibited a slight significant increase after 10 weeks of HFD.

Following the injection of streptozotocin (STZ), diabetes was confirmed with blood glucose levels exceeding 3.5 g/L in these two groups. In the first week post-diabetes induction, no notable changes were observed in the blood glucose levels of both groups. However, by the second week, the blood glucose levels of the GBG group began to decrease significantly, although they did not return to normal values, reaching a level of 1.69 ± 0.09 g/L at the end of the experiment.

3.4. Biochemical Parameters

Table 2 displays the biochemical profiles of the rat groups following the experimental period.

Table 2.
Results of biochemical analyses.

Parameters	NCG	PCG	GBG
TC g/L	0.71 ± 0.04 ^a	1.13 ± 0.17 ^b	0.78 ± 0.04 ^{ab}
LDL-C g/L	0.33 ± 0.05 ^a	0.73 ± 0.04 ^b	0.57 ± 0.03 ^c
HDL-C g/L	0.16 ± 0.03 ^a	0.16 ± 0.01 ^a	0.15 ± 0.01 ^a
TTG g/L	0.56 ± 0.03 ^a	1.37 ± 0.09 ^b	0.89 ± 0.01 ^c
Créatinine mg/L	7.05 ± 0.26 ^a	8.43 ± 0.20 ^b	6.69 ± 0.19 ^a
AST UI/L	98.78 ± 1.34 ^a	122.85 ± 3.21 ^b	101.52 ± 2.25 ^a
ALT UI/L	49.86 ± 0.83 ^a	55.22 ± 2.49 ^a	53.72 ± 1.43 ^a
Testostérone ng/mL	8.09 ± 0.07 ^a	2.35 ± 0.22 ^b	5.75 ± 0.09 ^c

Note: Mean ± Standard Error values within the column followed by different letters differ significantly according to Tukey's post-hoc test (p<0.05). (TC: Total Cholesterol; HDL: High-Density Lipoproteins Cholesterol; LDL: Low-Density Lipoproteins Cholesterol; TG: Total Tri-Glycerides; AST: Aspartate Transaminase; ALT: Alanine Transaminase; NCG: Negative Control Group; PCG: Positive Control Group; GBG: Green banana Group)

Based on the results presented in Table 2 the total serum cholesterol levels were significantly higher in the PCG compared to the NCG, whereas no significant difference was observed for the GBG when compared to these two groups. The levels of LDL-C and triglycerides noted for the PCG and GBG were significantly increased in comparison to the NCG. However, these levels were significantly higher in the PCG than in the GBG. For HDL-C and ALT, no significant differences were noted among the three groups. The creatinine and AST levels were significantly higher in the PCG compared to both the NCG and GBG, while no difference was observed between the latter two. The serum testosterone levels were significantly lower in both the PCG and GBG compared to the NCG, while it was significantly less reduced in GBG than in PCG.

3.5. Macroscopic and Histopathological Anatomopathological Aspects

3.5.1. Macroscopic Aspects: Comparative Organ Weights

Table 3 details the comparative analysis of absolute and relative organ weights across all experimental groups.

Table 3.
Comparative and relative organ weights.

Organs	Organ weight (g)			Relative organ weights (%)		
	NCG	PCG	GBG	NCG	PCG	GBG
Right kidney	0.86 ± 0.21 ^a	0.90 ± 0.22 ^b	0.88 ± 0.22 ^{ab}	0.35 ± 0.08 ^a	0.32 ± 0.08 ^b	0.33 ± 0.08 ^b
Left kidney	0.84 ± 0.21 ^a	0.88 ± 0.22 ^b	0.87 ± 0.22 ^{ab}	0.34 ± 0.08 ^a	0.32 ± 0.08 ^b	0.32 ± 0.08 ^b
Pancreas	0.65 ± 0.16 ^a	0.44 ± 0.10 ^b	0.56 ± 0.14 ^c	0.26 ± 0.06 ^a	0.16 ± 0.04 ^b	0.21 ± 0.05 ^c
Liver	9.98 ± 2.42 ^a	10.26 ± 2.40 ^a	9.81 ± 2.35 ^a	4.06 ± 0.96 ^a	3.70 ± 0.87 ^b	3.66 ± 0.87 ^b
Heart	0.86 ± 0.21 ^a	0.85 ± 0.20 ^a	0.84 ± 0.20 ^a	0.35 ± 0.08 ^a	0.31 ± 0.07 ^b	0.31 ± 0.07 ^b
Right testis	1.84 ± 0.44 ^a	1.83 ± 0.42 ^a	1.87 ± 0.45 ^a	0.75 ± 0.18 ^a	0.66 ± 0.15 ^b	0.70 ± 0.16 ^b
Left testis	1.89 ± 0.46 ^a	1.82 ± 0.42 ^a	1.86 ± 0.44 ^a	0.77 ± 0.18 ^a	0.66 ± 0.15 ^b	0.70 ± 0.17 ^b
A. A. T.	1.24 ± 0.38 ^a	3.80 ± 1.76 ^b	1.44 ± 0.61 ^a	0.50 ± 0.16 ^a	1.37 ± 0.64 ^b	0.54 ± 0.23 ^a
V. A. T.	1.29 ± 0.38 ^a	4.69 ± 1.10 ^b	2.32 ± 0.63 ^c	0.53 ± 0.15 ^a	1.69 ± 0.39 ^b	0.87 ± 0.24 ^c
E. A. T.	1.38 ± 0.37 ^a	3.14 ± 1.05 ^b	1.93 ± 0.71 ^a	0.56 ± 0.15 ^a	1.13 ± 0.38 ^b	0.72 ± 0.26 ^a

Note: Mean ± Standard Error values within the column followed by different letters differ significantly according to Tukey's post-hoc test (p<0.05). (AAT: Abdominal Adipose Tissue; VAT: Visceral Adipose Tissue; EAT: Epididymal Adipose Tissue; NCG: Negative Control Group; PCG: Positive Control Group; GBG: Green banana Group)

Based on the results presented in Table 3 we noted a significant increase in the weight of both kidneys in PCG compared to NCG. However, no significant difference was reported when comparing these two groups with GBG. It was also observed that there was a significant decrease in the total and relative weight of the pancreas in PCG (severe) and in GBG (moderate) when each was compared to NCG. No difference was observed regarding the weight of the liver, heart, or both testes among the three groups. Nevertheless, a significant decrease in the relative weight of these latter organs was noted when comparing PCG and GBG to NCG. Furthermore, no difference was observed between PCG and GBG.

Regarding the NCG, a significant increase was noted in both total weight and relative weight of abdominal, visceral, and epididymal adipose tissues in PCG. In contrast, no differences were observed for GBG, except for visceral adipose tissue, which showed a moderate significant increase. However, a significant decrease was observed in GBG in relation to PCG.

A significant increase was observed in both total weight and relative weight of abdominal, visceral, and epididymal adipose tissues in the PCG. No differences were noted for GBG, except for visceral adipose tissue, which showed a moderate significant increase. A significant decrease was observed in the GBG compared to the PCG.

3.5.2. Histopathological Aspects of Organs

Figure 4 illustrates the histology of the pancreas, kidneys, liver, heart, and testes across the three rat groups.

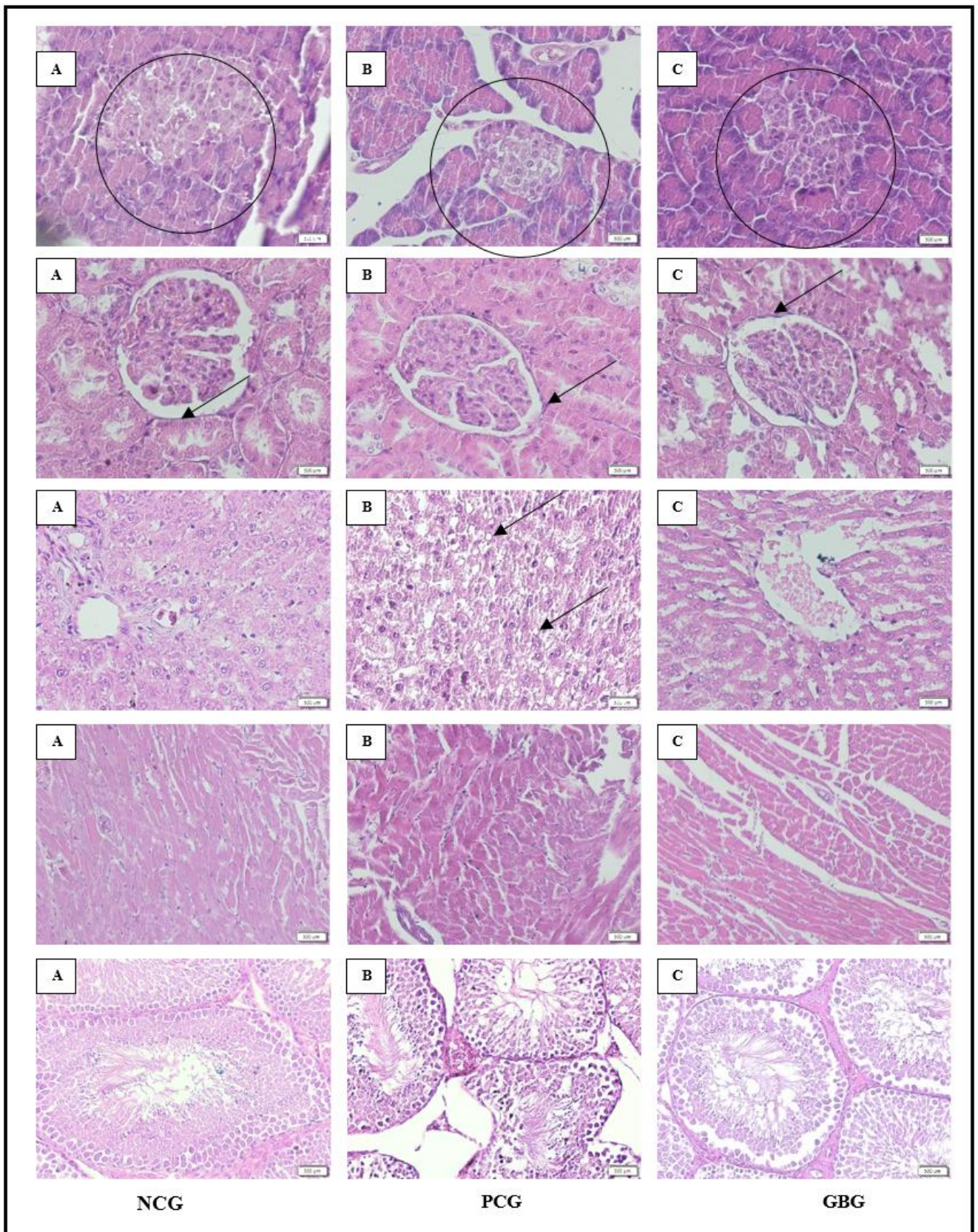


Figure 4. Photomicrographs of the pancreas, kidneys, and liver (magnification $\times 400$), and the heart and testes (magnification $\times 200$) of Wistar rats. (Hematoxylin and eosin staining).
Note: NCG: Negative Control Group; PCG: Positive Control Group; GBG: Green Banana Group

The histological section of the NCG pancreas Figure 4 revealed a normal morphology of the islets of Langerhans with no histopathological abnormalities in either the exocrine or endocrine pancreas, and no associated acute or chronic pancreatitis. In contrast, the sections from the PCG and GBG, while also lacking associated acute or chronic pancreatitis, exhibited a reduction in the volume of the islets of Langerhans due to a decrease in the number of constituent cells. This

reduction was more pronounced in the PCG compared to the GBG. Both groups also presented with adipose tissue deposition within the exocrine pancreas.

According to the renal section, in the NCG, a normal and healthy cellular architecture of the glomeruli, the Bowman's capsule surrounding them, and the renal tubules was observed, with a total absence of interstitial inflammatory infiltrate or tubular necrosis. The renal sections from the PCG displayed a diffuse and uniform thickening of the glomerular basement membrane, with matrix expansion encroaching upon the capillary lumen. However, no interstitial or intra-glomerular inflammatory infiltrate was detected. The renal sections from the GBG demonstrated partial thickening of the Bowman's capsule, without any associated lesions.

The histological examination of the liver in the NCG and GBG revealed no significant abnormalities in either the hepatocytes or the overall architecture. In these two groups, there was no evidence of acute or chronic inflammatory processes, whether lobular or portal. Furthermore, no hepatocellular overload was observed (absence of hepatocellular steatosis). In contrast, in the PCG, minimal microvesicular steatosis was noted, with no associated architectural disturbance or acute or chronic inflammatory process.

Myocardial sections from all three groups showed no histopathological lesions. Indeed, the coronary arteries were free of atheromatous plaques, and the cardiac muscle exhibited no ischemic damage.

The histological examination of testicular sections from the three groups did not reveal any pathological lesions; however, active spermatogenesis was observed, indicating normal testicular function. Nonetheless, the PCG showed slight atrophy of the seminiferous tubules, accompanied by disorganization of intercellular junctions.

6. Discussion

The search for natural solutions to improve diabetes management is a major public health priority. The study of green bananas serves as a prime example: a food rich in resistant starch that has been attracting increasing scientific interest [10]. Indeed, its potential for regulating blood glucose levels and providing other health benefits for diabetics makes it a promising research subject. Our study aimed to evaluate the effects of the regular consumption of green Cavendish banana flour, from a new cultivar grown in Algeria named "The Mediterranean" on Wistar rats rendered diabetic through a HFD and a low dose of STZ.

According to this study, the estimated daily food intake during the first 10 weeks revealed a negative effect of the HFD on PCG and GBG. This negative effect is expressed through a sharp and continuous increase in energy intake, reflected in a substantial progression of weight gain, leading to an overweight state compared to the NCG. The induced overweight mimics the condition commonly observed in most type 2 diabetic patients, as it constitutes a major factor in insulin resistance [14, 17]. Indeed, in parallel, the blood glucose levels significantly increased. This elevation is considered to be the result of a slight dysfunction of the pancreatic β -cells [14, 18]. The HFD also led to a slight increase in daily water consumption. This finding is consistent with the observations of Bhandari, et al. [19] whose study focused on "The effect of high-fat diet-induced obesity on cardiovascular toxicity in wistar albino rats" and aimed to document the development of an animal model and to partially characterize it regarding factors potentially involved in the cardiovascular changes associated with obesity. Conversely, other research has reported a lower consumption with changes in sip size for rats under HFD [20, 21]. This divergency in results may be attributed to the increased daily food intake observed in our rats. Furthermore, after STZ injection, the estimated food intake was significantly reduced, and consequently, the rats' appetite decreased. The same observation was described in the study by Yin, et al. [22] on "The effects of diet and streptozotocin on metabolism and gut microbiota in a mouse model of type 2 diabetes".

According to our experimental data, blood glucose levels exceeding 3.5 g/L (19.4 mmol/L) were observed, thereby confirming the establishment of T2DM. Rats with hyperglycemia exhibited a sharp increase in estimated daily water intake and considerable polyuria. In accordance with good practice guidelines [3], T2DM is defined by the presence of symptoms such as polyuria and polydipsia, associated with a venous plasma glucose level greater than or equal to 2 g/L (11.1 mmol/L). As reported West, et al. [23], STZ induces significant changes in blood insulin and glucose concentrations. These changes reflect abnormalities in pancreatic β -cell function and may ultimately lead to their damage. Its intracellular action results in DNA modifications, notably fragmentation [24, 25]. Numerous prior studies have suggested that the induced cell death is primarily due to DNA alkylation [26, 27].

By the second week, the rats had begun to recover their appetite, with the PCG persisting in its increased food consumption, while the GBG group began to adjust its intake. This is more likely attributable to the presence of fiber and resistant starch found in the green banana flour. Bananas are considered an exceptional source of fiber, vitamins, minerals, and resistant starch, all of which may have beneficial effects on health, particularly when the bananas are still green [11, 28]. They reduce energy intake and significantly enhance the sensation of satiety and fullness [29, 30] while reducing postprandial hunger [31, 32].

During the first two weeks of experimentation, a weight loss associated with the injection was observed, which was immediately followed by a return to baseline status, after which the weight increased again. In the GBG, this weight gain was mitigated compared to the PCG, which continued to show intensive weight gain. This mitigation altered the impact of fibers and resistant starch on weight loss. In the literature, research Sumbe and Salve [29]; Rosado, et al. [33] has confirmed that a HFD enriched with green banana flour can reduce body weight gain.

Simultaneously, the blood glucose levels of rats in the GBG began to decrease, reaching a value of 1.69 g/L by the fourth week, end of the experiment, demonstrating that green banana possesses a hypoglycemic effect. Increasing evidence has also revealed the capability to reduce postprandial blood glucose levels in healthy subjects [34, 35] as well as hyperglycemia in diabetic patients [36, 37].

Regarding the biochemical analysis results, a deleterious effect was observed from RHL and the low dose of STZ used to induce T2DM. This effect was manifested in the PCG by a significant increase in serum concentrations of TC, LDL-C, TTG, AST, and creatinine, compared to the NCG. In contrast, no difference was observed in plasma levels of HDL-C and ALT. According to Noordin, et al. [38] all these indicated biochemical parameters reveal a significant increase, which may support and reinforce our findings. This disorder affects not only the lipid profile, renal and hepatic parameters but also reduces serum testosterone levels. Similar results were reported in Turkey by Ellidag, et al. [39] in the study titled "Serum Testosterone Levels and Oxidative Stress in Type 1 Diabetes, Type 2 Diabetes, and Obesity."

Under the same conditions, the GBG demonstrated a significant similarity in serum levels of CT, HDL-C, AST, and ALT compared to the NCG. Additionally, there was a significant decrease in serum levels of LDL-C and TTG when compared to the PCG. This improvement is likely attributable to the enrichment of its HFD with green banana flour. The effect of green bananas on biochemical parameters related to lipids, liver function, and renal health in the context of T2DM and HFD is poorly documented. We were compelled to refer to related studies, while taking into account differences in experimental protocols. The effect of green bananas on lipid, hepatic, and renal biochemical parameters in the context of T2DM and HFD has been poorly documented. This forced us to refer to related studies, taking into account differences in protocols. According to the Brazilian study of Costa, et al. [40] and the Egyptian studies of Zakaria, et al. [41] and Rabeih, et al. [42] it has been observed that supplementation with banana fruit significantly improved the lipid profile, as well as hepatic and renal functions, in diabetic rats. These results may be attributed to the constituents of banana fruit, such as phenolic compounds, vitamins, minerals, and dietary fibers, which exhibit antioxidant, hepatoprotective, antilipidemic, and antidiabetic activities. Reddy, et al. [43] and Bai, et al. [44] have demonstrated that banana-derived resistant starch significantly improved metabolic health in diabetic and hyperlipidemic rat models. Resistant starch regulates blood glucose and lipid profiles, attenuates oxidative stress, and positively modulates hepatic and renal function, suggesting its potential as a therapeutic ingredient for managing diabetes and related metabolic disorders. Furthermore, previous studies Rosado, et al. [45] and Fu, et al. [46] have demonstrated that supplementation with green bananas, due to their high resistant starch content, exerts significant protective effects against metabolic disturbances induced by HFD. It contributes to improving serum levels of lipid, liver, and kidney biomarkers. These findings suggest that incorporating green banana into the diet could represent a promising therapeutic strategy for preventing and managing metabolic disorders associated with a high-fat diet. However, Lousek, et al. [47] reported in their study that there were no significant changes in the observed parameters were attributable to green banana biomass over the 8-week intervention period, suggesting an absence of interference with the hyperlipidemic model during the analysis period.

In terms of absolute and relative weight, the pancreas exhibited an atrophy in both the PCG and GBG. This alteration is likely due to the onset of diabetes induced by STZ and HFD. Histological examination of the pancreas provided illustrated evidence of a reduction in the number of cells in the islets of Langerhans, consequently leading to a decrease in their volume. Additionally, it was observed that this atrophy was less pronounced in the GBG, indicating the potential of green banana flour to mitigate atrophy and regenerate β -cells. Both groups also displayed early signs of fat deposition in the exocrine pancreas, suggesting a disturbance in lipid metabolism. According to Noordin, et al. [38] pancreatic histology in the T2DM rat group reveals atrophied and irregular islets of Langerhans containing vacuolated cells, and acini presenting swollen cells with fat accumulations. The study by Bai, et al. [44] on "Banana starch intervention ameliorates diabetes-induced mood disorders via modulation of the gut microbiota-brain axis in diabetic rats" demonstrated that while diabetes has damaged the ultrastructure of the pancreas in rats, the intervention of resistant starch extracted from green bananas mitigates these damages.

The weight of the kidneys in the PCG was significantly elevated, suggesting possible hypertrophy. Indeed, renal sections from this group demonstrated a diffuse and uniform thickening of the glomerular basement membrane, with matrix expansion encroaching upon the capillary lumen. Despite the absence of inflammatory infiltrate, it is highly probable that these alterations may manifest as complications of diabetes. Wu, et al. [48] have similarly demonstrated comparable results, revealing a significant increase in the mesangial matrix fraction in diabetic rats. According to Fu, et al. [49] the induction of diabetes leads to significant renal alterations characterized by glomerular hypertrophy, associated with inflammation and lipid degeneration of tubular epithelial cells. In line with our study, renal sections from the GBG showed partial thickening of Bowman's capsule, without other associated lesions. This result suggests a strong potential of green banana flour against diabetes-associated renal alterations. Fu, et al. [49] has also reported analogous findings, indicating that the administration of green plantain flour attenuated renal damage due to diabetes by inhibiting glomerular cell proliferation and reducing tubular steatosis. The non-significant changes in the weight of the liver, heart, and both testes of rats across the three studied groups may be attributed to a lack of sensitivity to STZ. Furthermore, it is likely that no damage occurred due to HFD or diabetes.

Histological examination of the liver revealed minimal microvesicular steatosis in the PCG, indicating the onset of triglyceride deposition. This triglyceride may act as an alternative precursor for glucose, since hepatic glycogen is typically depleted in STZ-induced diabetic rats, or it could simply be the result of the liver's susceptibility to STZ provocation [50]. Over time, the liver is likely to develop more significant diabetic hepatic steatosis, manifested by hypertrophy of the organ. It is worth noting that, according to Wickramasinghe, et al. [51] an absence of hepatic steatosis was observed across all experimental groups, whether healthy or diabetic. However, histopathological alterations, including hepatic steatosis with lipid accumulation and lobular inflammation, have been reported in other studies [14, 52]. In our study, the GBG exhibited a healthy histological structure analogous to that of the NCG, thereby clearly demonstrating the beneficial effect of green banana flour in mitigating hepatic steatosis. The histological results from Rosado, et al. [45] corroborate our findings and indicate that green banana, due to its high resistant starch content, has prevented the onset of hepatic steatosis, characterized by a significant reduction in lipid accumulation.

Conversely, histological sections confirmed a healthy tissue architecture of the cardiac muscle, which refutes their sensitivity to both STZ and HFD. Previous studies support and confirm cardiac insensitivity to STZ [53]. However, numerous others contradict our result regarding the influence of HFD Karam, et al. [54] highlighting myocardial steatosis and intermuscular lipid infiltration, while Naderi, et al. [55] shows alterations in cardiac tissues, including interstitial edema, inflammatory infiltration, myocardial necrosis, and a loss of the normal architecture of cardiomyocytes in diabetic rats. The short duration of the experiment provides a plausible explanation for our results obtained in streptozotocin-induced diabetic rats.

The histological examination of testicular tissue from the PCG revealed slight atrophy of the seminiferous tubules, accompanied by disorganization of intercellular junctions, without significantly affecting spermatogenesis or testicular weight. This alteration is likely elicited by STZ and, consequently, the induced diabetic state. In contrast, the GBG exhibited testicular histology homologous to the NCG, indicating the protective capacity of green banana flour's anti-diabetic effects against the previously documented structural changes. According to Budak [56] histological examination of the diabetic group's testes revealed severe atrophy in most seminiferous tubules, a reduction in the number and layers of spermatogenic cells, and a separation of spermatogenic arrests from intercellular junctions. Indeed, studies conducted on the green banana *Musa paradisiaca* [57-59] have shown that animal models with diabetes-induced testicular disorders can experience a reversal of testicular lesions upon its administration. The precise mechanism through which green banana exerts its effects remains uncertain. Green bananas have hypoglycemic effects in both normal and diabetic animal models, which may account for their ability to enhance male sexual function in diabetic animals. This is further corroborated when the ripe green plantain is consumed at a moderate dose, as revealed in the study by Alabi, et al. [59].

The significant increase in the absolute and relative weight of abdominal, visceral, and epididymal adipose tissues in the PCG reflects the effect of the HFD consumed by the rats throughout the experimental period. The GBG was subjected to the same diet; however, it was supplemented with green banana flour after diabetes induction. Consequently, the absolute and relative weights of its adipose tissues exhibited levels significantly lower than those observed in the LTP group. This improvement is likely attributable to the incorporation of green banana flour, owing to its high content of resistant starch and dietary fiber. According to Santiago, et al. [60] the incorporation of green banana flour (*Musa cavendish*) promoted a reduction in caloric intake and abdominal fat accumulation in rats subjected to an HFD.

7. Conclusion

The implementation of a high-fat diet is capable of inducing insulin resistance. In parallel, the administration of streptozotocin (STZ) has been shown to cause alterations indicative of functional abnormalities or even damage to pancreatic β -cells. Consequently, the combination of a low-dose STZ treatment following a period of high-fat diet in rats can generate a model of type 2 diabetes that mimics the pathophysiology of the human disease, including the development of its associated complications.

The incorporation of green banana flour into the diet, even one high in fat, emerges as a promising nutritional strategy for managing diabetes in rats. The collected data reveal a beneficial modulation of feeding behaviors, suggesting an improvement in satiety signaling and potentially better overall energy intake control. Furthermore, its association with body weight regulation and a significant reduction in blood glucose levels underscores a substantial hypoglycemic effect, opening perspectives for the management of the hyperglycemia characteristic of diabetes. Notably, the concurrent improvement in the lipid profile, liver and kidney function markers, as well as the restoration of serum testosterone levels, indicates that green banana flour may exert pleiotropic effects, targeting several metabolic and endocrine disturbances frequently observed in diabetes and its complications. These preclinical observations suggest the importance for further exploration of the therapeutic potential of green banana flour in modulating diabetes and its comorbidities.

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