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Characterization and potential health benefits of millet flour and banana peel mixtures on rats fed with a high-fat diet

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ABSTRACT

Millet (M) and banana peel (Bp) possess significant nutritional qualities and have been shown to reduce obesity resulting from a high-fat diet (HFD). The present research assessed the effect of millet flour and banana peel mixtures on lipid profiles, liver and kidney functions, and characterized food products derived from these mixtures. Thirty-five male albino rats were allocated into five groups for a biochemical analysis. The control group $(n = 7)$ received a basal diet, while the remaining 28 rats were fed a high-fat diet (HFD) for 8 weeks to induce obesity. These rats were then separated into four sub-groups ($n = 7$ each): sub-group 1 as the positive control (+ve) receiving only HFD, while sub-groups 2, 3, and 4 were administered HFD supplemented with millet flour and banana peel mixtures $(M90+Bp10 \%)$, $M80+Bp20 \%$, and $M70+Bp30 \%$), respectively for an additional 8 weeks. The chemical composition analysis showed that banana peel (Bp) has higher levels of fat, ash, fiber, magnesium, and potassium, while millet flour is richer in carbohydrates. Bp also had superior antioxidant activity and total phenol content (13.32 % and 10.54 mg/100g) compared to millet flour (3.75 % and 4.55 mg/100g). Biochemical tests on the HFD plus (M70+Bp30 %) group revealed improved lipid profiles, leptin, antioxidant enzymes, and kidney and liver functions. Glucose levels were higher in the HFD group (137.33 mg/ dl) than in the control (85.70 mg/dl), but these levels were reduced with millet and banana peel treatment. The histology of liver tissues confirmed the biochemical results. Sensory evaluation of pancakes and toast from the (M70+Bp30 %) mixture by forty panelists showed high acceptability, aligning with the biochemical outcomes. This study suggests that a banana peel and millet flour mixture could help reduce obesity.

1. Introduction

The prevalence of obesity has emerged as a significant health concern in industrialized nations, impacting an estimated 1.9 billion persons who are either overweight or obese [[1](#page-11-0)]. In Egypt, about 19 million adults, representing roughly 35 % of the total adult population, are overweight [[2](#page-11-0)]. In 2017, the World Health Organisation (WHO) reported that just over 39 % of individuals globally were categorized as overweight, while 13 % were classified as obese [[3](#page-11-0)]. WHO [\[3\]](#page-11-0) also discusses similar global trends in obesity in their study. The chronic condition of obesity heightens the susceptibility to metabolic syndrome, including hypertension, increased

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triglyceride levels, reduced HDL cholesterol, and hypercholesterolemia. These conditions collectively contribute to an increase in heart disease, type 2 diabetes, renal impairment, and fatty liver disease [\[4\]](#page-11-0). The incidence of obesity is influenced by both hereditary and environmental variables, with nutrition being a key environmental determinant [[5](#page-11-0)].

Anti-obesity drugs have been demonstrated to have side effects. For example, orlistat has been associated with gastrointestinal disturbances, and sibutramine has been linked to increased cardiovascular risks [\[6\]](#page-11-0)**.** Semaglutide, a GLP-1 analog, is cleared for treating type 2 diabetes and reducing cardiovascular risk in adults at doses up to 1 mg weekly. It has also been proven to cause decreased body mass in people with type 2 diabetes and adults with obesity, as demonstrated in phase 2 trials by Sodhi et al. [\[7\]](#page-11-0) and Wilding et al. [\[8\]](#page-11-0). Therefore, it is vital to investigate alternative approaches, such as a diet full of whole grain foods and plant-based dietary compounds like polyphenols, dietary fibers, probiotics, and prebiotics, which have been shown to offer potential anti-obesity benefits [9–[11\]](#page-11-0).

Millet (*Eleusine coracana* L.) is a tropical cereal grain widely cultivated in several African countries, including Egypt [\[12](#page-12-0)]. Millet is notable for its use as a cereal because of its high protein, lipid, and carbohydrate content, which is similar to wheat but superior to rice [\[13](#page-12-0)]. It also boasts a rich nutritional profile, contributing significantly to dietary needs with its high quantities of iron, calcium, zinc, phosphorus, and potassium [[14\]](#page-12-0). Furthermore, Sharma and Niranjan [[15\]](#page-12-0) found that millet contains a substantial amount of phenolic chemicals recognized for their antioxidant and metal-chelating consequences. The effects of millet on metabolic disorders, and metabolic illnesses like diabetes and obesity have attracted considerable attention in academic literature [\[16](#page-12-0)]. Therefore, it is evident that consuming millet-based foods may help mitigate overweight and obesity. Millet eating has been shown by Wang et al. [\[17](#page-12-0)] to mitigate the severity of overweight and obesity, namely those with a BMI above 30. Millet reduces the absorption of cholesterol and fat, prolongs the emptying of the stomach, and enhances the volume of alimentary contents.

Bananas are taxonomically classified within the Musaceae family and the Musa genus. They are widely recognized as a highly significant and commonly consumed fruit, both in Egypt and globally [[18\]](#page-12-0). Banana peels, a byproduct of banana processing, have been identified to possess considerable bioactive chemicals [[19\]](#page-12-0). They also contain a high concentration of polyphenols [[20\]](#page-12-0). Additionally, banana peels have a significant amount of phytochemicals, including anthocyanin, delphinidin, cyanidin, and vitamins C, E, and $β$ -carotene [[21\]](#page-12-0). The presence of flavonoids and phenols in banana peels has been observed to potentially reduce cholesterol, free fatty acids, and triglycerides in both serum and tissue [[22\]](#page-12-0). Furthermore, Njapndounke et al. [\[23](#page-12-0)] demonstrated that biscuits made from a combination flour of *Musa sapientum* and *Vigna unguiculata* were rich in various nutrients and had a low glycemic index.

While many studies have examined dietary interventions for obesity, few have explored the combined effects of millet flour and banana peels—a blend high in dietary fibers, antioxidants, and bioactive substances. This study fills that gap by assessing the impacts of this mixture on lipid profiles, liver and kidney function, and antioxidant enzymes in rats fed a diet high in fat. Furthermore, the sensory qualities of bakery products made from the optimal mixture were evaluated to determine their potential as functional foods for obesity management. Unlike prior research focusing on individual components, our study uniquely investigates this combination, offering a comprehensive analysis of its biochemical and histological benefits in obesity management.

2. Materials and methods

2.1. Materials

2.1.1. Millet and banana peels

Ripe yellow bananas (*Musa* sp.), finger millet grains (*Eleusine coracana* L.), along with dry ingredients such as salt, sugar, baking powder, butter, eggs, and vanilla essence were sourced from a local store in Alexandria, Egypt.

2.1.2. Chemicals and kits

Chemicals of analytical and pure grade were acquired from vendors for Merck and Sigma-Aldrich Co. located in St. Louis, MO, USA. The kits were procured from Biosystems GmbH in Spain, Diamond GmbH in Germany, and Randox GmbH in the United Kingdom.

2.1.3. Animals

Thirty-five male albino rats of the Wistar strain were obtained from Alexandria University's Institute of Graduate Studies and Research. The rats had an average body weight of 150 ± 10 g.

2.2. Preparation of millet flour

The millet grains were ground for 1 min utilizing a Moulinex LM2428EG (400 Watt, Germany). The ground millet was then sifted through a sieve with 180 mm openings to obtain fine, powdery flour, which was stored individually in polyethylene bags in the refrigerator until use.

2.3. Preparation of banana peel powder

The fully ripe banana peels (ripe yellow) were first rinsed with tap water to remove impurities. They were then cleaned, sliced into smaller pieces, and immersed in a citric acid solution. The peel powder was obtained by drying the mixture using a lyophilization apparatus (Alph 1–2 LD plus, Martin Christ Gefriertrocknungsanlagen GmbH, Untere Söse 50, 37520 Osterode Harz, Germany) for 48 h. The resulting powder was stored in a refrigerator until use [\[24](#page-12-0)].

2.4. Chemical composition and some minerals

The chemical composition of millet flour and banana peels was analyzed at the Central Laboratory, Faculty of Agriculture, University of Alexandria. Protein, ash, fiber, fat, and moisture levels were measured according to the procedures outlined by the Association of Official Analytical Chemists [\[25](#page-12-0)]. Total carbohydrates were calculated by difference, according to the AOAC [\[25](#page-12-0)] method, as indicated in equation [1].

$$
Carboblydrates (%) = 100 - (protein % + ash% + fat% + fiber %)
$$
\n[1]

Mineral content (K and Mg) was determined by atomic absorption spectrometry (NC.9423-400-30042, England) following the procedure outlined by AOAC [\[25](#page-12-0)].

2.5. Determination of antioxidant activities, phenolic and total carotenoid content

The antioxidant activities of millet flour and banana peels were estimated by assessing their ability to neutralize free radicals using the widely accepted 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay. The total phenolic of the methanolic extracts from millet flour and banana peels was assessed using the Folin-Ciocalteu reagent [\[26](#page-12-0)]. The total carotenoid content in the 80 % acetone extract using the approach established by Yang et al. [\[27](#page-12-0)].

2.6. Biological study

2.6.1. Experimental design

The study was conducted at the Graduate Studies and Research Department of Alexandria University. The rats lived in wire boxes maintained at 25 ℃. The elements of the basal diet are detailed in Table 1. The rodents were accommodated in wire enclosures keeping a temperature of 25 ◦C. All the components of the baseline diet are enumerated in Table 1. Following two weeks of acclimation, the rats were separated into two primary groups. The initial cohort of seven rats was provided with the baseline diet and functioned as the negative control group. The second cohort, consisting of 28 rats, was administered a high-fat diet (HFD) by incorporating animal fat into the basal diet (refer to Table 1). To promote obesity, the rats were fed a high-fat diet for eight weeks. Following the specified time frame, blood samples were obtained from the rats following the High-Fat Diet (HFD). Next, serum analysis was conducted to evaluate the lipid profile by quantifying triglyceride and cholesterol levels to confirm the occurrence of obesity. Therefore, the rats were separated into four subgroups, each consisting of seven rats:

Sub-Group 1: Rats fed the HFD, serving as the positive control group $(+ve)$.

Sub-Group 2: Rats fed the HFD augmented with 90 % millet flour (M) and 10 % banana peel (Bp).

Sub-Group 3: Rats fed the HFD augmented with 80 % millet flour (M) and 20 % banana peel (Bp).

Sub-Group 4: Rats fed the HFD augmented with 70 % millet flour (M) and 30 % banana peel (Bp).

After the experimental duration, which lasted an additional eight weeks, the following biological parameter, body weight gain (BWG), was estimated. Dietary intake and body weight were documented weekly during the trial period. A daily record was kept of the quantities of food ingested and/or wasted, and the total feed intake (FI) was computed. A weekly assessment was conducted to determine the individual body weight (BW) of rats in each group. Calculations of the body weight gain percentage (BWG%) and feed efficiency ratio (FER) were performed in formulas (2, 3) outlined by Stachiewicz and Quastel [[28\]](#page-12-0).

BWG (
$$
\%
$$
) = $\frac{\text{Final weight}(g) - \text{Initial weight}(g)}{\text{Initial weight}(g)} \times 100$ [2]

$$
FER = \frac{\text{Body weight gain percentage } (g/day)}{\text{Total feed intake } (g/day)}
$$
 [3]

Table (1)

Ingredients of basal diet and high-fat diet with different mixtures of millet and banana peel flour (mg/g).

Source [[103,104\]](#page-14-0)

The body mass index (BMI) is determined by multiplying the body weight (g) by the square of the body length (cm). Body length (measured from nose to anus) was recorded every four weeks using a measuring tape under light anesthesia [\[29](#page-12-0)].

2.6.2. Assessment of biochemical parameters in serum

Following the conclusion of the experimental stage, the rats underwent an overnight fasting period before being sedated with ether and further dissected. Blood samples were obtained and subjected to centrifugation at 3000 revolutions per minute (rpm) to isolate the serum, which was subsequently used for multifaceted biochemical investigations. Total cholesterol was determined using the method of Richmond [[30\]](#page-12-0), triglycerides were measured according to Fossati and Prencipe [\[31](#page-12-0)], and high-density lipoprotein **(**HDL) was assessed [\[32](#page-12-0)]. Low-density lipoprotein (LDL-C), and very low-density lipoprotein (VLDL-C) were determined as described by Ferri et al. [\[33](#page-12-0)]. The activity of antioxidant enzymes, including glutathione peroxidase (GPX), was measured according to Wendel [[34\]](#page-12-0). Serum malondialdehyde (MDA) levels and catalase (CAT) activity were evaluated based on the methods of Hadwan [\[35](#page-12-0)] and Tsikas [[36\]](#page-12-0), respectively, while superoxide dismutase (SOD) activity was assessed using the procedure of Rigo et al. [[37\]](#page-12-0). Alkaline phosphatase (ALP) was measured following Lavie et al. [\[38](#page-12-0)], aspartate transaminase AST according to Yagi et al. [\[39](#page-12-0)], alanine transaminase (ALT) activity following Williamson [[40\]](#page-12-0), lactate dehydrogenase (LDH) activity according to Bergmeyer and Bernt [\[41\]](#page-12-0), urea and creatinine levels based on Perez-Gomez et al. [[42\]](#page-12-0), and glucose levels according to Lott and Turner [\[43](#page-12-0)].

2.6.3. Histological of liver tissues

The histology of liver tissues was examined in detail. Specimens were obtained from each experimental group and preserved in a 10 % phosphate-buffered formalin solution for 10 h. The specimens were next meticulously rinsed in 70 % alcohol for additional analysis. After fixation, the specimens were dried in graded ethanol, encased in wax, sectioned into 5-micron-thick pieces, and dyed with Hematoxylin and Eosin using the procedure of Carleton et al. [\[44](#page-12-0)]. The stained slices were further analyzed using an Olympus light microscope.

2.7. Bakery products

2.7.1. Preparing millet flour and banana peels bakery products

The ingredients used in the formulation of bakery products (pancakes and toast bread) derived from a blend of millet flour and banana peel (M70 + Bp 30 %), which yielded the best results in biochemical analyses, are listed in Tables 2 and 3.

2.7.2. Method of preparing

Sugar and yeast were dissolved in warm water. The flour was blended with the yeast mixture for 2 min and then left to ferment for 1 h. After fermentation, egg, vanilla essence, butter, skimmed milk, and salt were added and blended. Oil was heated in a frying pan, and portions of the batter were placed in the pan. The pancakes were cooked on low heat until the bottoms were browned, then flipped to cook the other side. This process was repeated until all the batter was used. The prepared pancakes were then evaluated for sensory characteristics [\[45](#page-12-0)].

2.7.3. Sensory evaluation of millet flour and banana peels mixture food products

Some millet flour and banana peel bakery products (pancakes and toast bread) were formulated and evaluated for overall acceptability by forty people (staff members and students) from Alexandria University, following the method described by Lorenz and Coulter [[46\]](#page-12-0). The sensory parameters evaluated were color, taste, odor, and flavor, using a 9-point hedonic scale.

2.8. Statistical analysis

Analysis of the data was conducted using IBM SPSS software package version 20.0, developed by IBM Corp; Armonk, NY. The numerical data were displayed as the mean and standard error parameters. An evaluation of the normality of the data distribution was conducted using the Shapiro-Wilk test, which verified that the data adhered to a normal distribution. Additionally, homogeneity of

A: control **B:** Millet and banana peel flour mixture.

Table (3) Composition of ingredients in toast bread.

A: Control **B**: Millet flour and banana peels.

variance was tested using Levene's test, and the results indicated that this assumption was met, making a log transformation of the data unnecessary. A Student's t-test was used to compare two categories, and an F-test (ANOVA) was used to compare multiple groups. For pairwise comparisons, a Post Hoc test was applied [[47\]](#page-12-0)**.** This analysis approach is consistent with the study by Njapndounke et al. [[23\]](#page-12-0).

3. Results and discussions

3.1. Chemical composition and some minerals

The chemical composition of millet flour and banana peel is shown in Table 4. The findings align with those presented by Audu et al. [[48](#page-12-0)], who demonstrated that the protein, ash, fiber, and total carbohydrate contents were 8.42 %, 2.51 %, 2.51 %, and 73.32 %, respectively. Additionally, finger millet was shown to be high in potassium (K) and magnesium (Mg), with concentrations of 9.95 mg/100g and 16.42 mg/100g, respectively. Finger millet could, therefore, be utilized as an alternative source of protein substitute in diets and protein supplements and as a good source of energy. In contrast, Ibrahem and Sayed [[12\]](#page-12-0) reported millet's fat, protein, ash, dietary fiber, and carbohydrate contents to be 7.3 %, 1.3 %, 3 %, 19.1 %, and 72.6 %, respectively. According to Ahmed et al. [[49\]](#page-12-0), millet was less in protein but greater in fiber and ash content, with potassium content at 345.9 mg/100g and magnesium at 115.4 mg/100g. Potassium is essential for the synthesis of proteins and amino acids.

Regarding banana peels, Ragab et al. [\[50](#page-12-0)], found that the ash and protein concentrations were 11.19 % and 5.62 %, respectively, with potassium and magnesium content estimated at 3812.50 mg/100g and 138.50 mg/100g, respectively. In another investigation by Salem et al. [\[51](#page-12-0)], the nutritional composition of banana peel powder was analyzed, revealing average values for protein, fat, and ash content of 14.50 %, 4.04 %, and 14.43 %, respectively. The high fiber level of banana peels suggests that they have the potential to enhance general health and well-being. A study by Amini Khoozani et al. [\[52](#page-12-0)] also demonstrated that banana peel powder has a considerable mineral composition, particularly potassium and magnesium contents, at 1485.0 mg/100g and 179.00 mg/100g, respectively. The composition of banana peel powder includes 14.38 % fiber, 57.13 % carbohydrates, and 10.22 % fat. Incorporating this powder into food products could enhance their nutritional value. The observed disparities in the chemical analysis results may be attributed to variations in plant species and environmental factors, as noted by several researchers [\[53](#page-12-0)].

3.2. Antioxidant activity, total phenol, and total carotene

The antioxidant activity and total phenolic level of millet and banana peel flour are displayed in [Table 5](#page-5-0). The findings indicated that banana peel is rich in antioxidant activity, total phenol, and total carotenoids (13.32 % and 10.54 mg/100g), compared to millet flour (3.75 % and 4.55 mg/100g). Additionally, the banana peel was determined to have a high concentration of total carotenoids, with a concentration of 113.03 mg/100g.

An analysis of millet's flavonoids and phenolic compounds demonstrated that millet contains a substantial reservoir of flavonoids, antioxidants, and phenolic acids, which possess free radical scavenging properties. These properties contribute to the treatment of oxidative stress-related disorders, including obesity and cardiovascular diseases [\[54](#page-12-0)]. The research of Amini Khoozani et al. [\[52](#page-12-0)], the use of banana peel and millet flour as dietary sources of natural antioxidants has the potential to be highly beneficial. These ingredients

Table (4)

Data are presented as mean \pm SEM (n = 3). Statistical analysis was performed using Student's t-test. Significant differences (P \leq 0.05) were observed between banana peel powder and millet flour for fat, ash, fiber, carbohydrates, Mg, and K content. No significant difference was found in protein content (P *>* 0.05).

Table (5)

Antioxidant activity, total phenolic contents, and total Carotenoids of millet and banana peel flour.

Data are presented as mean \pm SEM (n = 3). Statistical analysis was performed using Student's t-test. Significant differences (P \leq 0.05) were observed between banana peel powder and millet flour for antioxidant activity and total phenol content.

can be regarded as functional, facilitating the creation of functional food items with added value.

Salama et al. [\[55](#page-12-0)] reported that antioxidant activity and total phenolic content in banana peel were 12.65 % and 11.59 mg/g, respectively. However, Islam et al. [[56\]](#page-12-0) discovered that the total phenolic content of banana peels ranges from 17.89 to 26.96 GAE/g. Furthermore, Tirfesa Olana [\[57](#page-12-0)] observed that the phenol content increased with a higher percentage of millet added to wheat flour, reaching 5.60 mg/100g. Nassarawa and Sulaiman [\[58](#page-13-0)] reported that millet exhibited high total phenolic content (2.62 mg GAE/g), which showed significant antioxidant activity against DPPH radicals (1.68 μg/ml). The phenolic compounds in millet have been noted for their anti-inflammatory and antioxidant activities [[59\]](#page-13-0).

Moreover, the current study found that banana peel powder contained 113.03 mg/100g of total carotenoids. Following the findings of Elhassaneen et al. [\[60](#page-13-0)], who reported that banana peel powder contained 186.25 mg/100g of total carotenoids. However, Ahmed et al. [\[61](#page-13-0)], noted that the highest stage of ripe yellow banana peel contained total carotenoids of 846.58 mg/100g. This variation in total carotenoids may be attributed to differences in banana variety and ripening stage. Carotenoids have numerous beneficial effects on human health, including improving fat metabolism, reducing body size, regulating blood sugar levels associated with weight gain, and serving as precursors of vitamin A. They also have antioxidant, anti-cancer, anti-obesity, and bone anabolic impacts [[62\]](#page-13-0). Overall, the study of banana peels as food by-products suggests they can be effectively utilized in culinary applications owing to their substantial bioactive chemical content.

3.3. Biological parameters

3.3.1. Body weight gain

The impact of millet and banana peel flour mixtures on feed efficiency ratio (FER), feed intake (FI), body weight gain (BWG), and body mass index (BMI) in obese rats is presented in Table (6). The estimated FI for rats fed a basal diet (control -) was 31.74 g/day/rat, while those on a high-fat diet (HFD, control +) consumed 32.90 g /day/rat. The estimated FER for the basal diet control group was 3.72, compared to 4.89 for the HFD control group. Among the treatment groups, FER values varied, with the lowest being 1.86 for rats fed HFD plus M70+Bp30 %. These results suggest that the M70+Bp30 % treatment effectively reduced FER, indicating an improvement in health status, potentially due to the interference with lipid absorption caused by the presence of soluble fibers.

Additionally, the findings indicated that the positive control group, which was only fed on a high-fat diet (HFD), had a statistically significant (p *<* 0.05) rise in body weight gain (BWG) (161 %) relative to the control group (-ve) (118 %). In contrast, BWG was 94.22 %, 81.94 %, and 60.90 % in rats fed on HFD plus different levels of millet and banana peel flour mixtures (M90+Bp10 %, M80+Bp20 %, and M70+Bp30 %), respectively. The most significant reduction in BWG was indicated in the M70+Bp30 % group compared to other treatments and controls. Furthermore, BMI values supported these findings, with the basal diet control group having a BMI of 1.04 g/cm², the HFD alone group showing an elevated BMI of 1.52 g/cm², and the treatment groups exhibiting reduced BMIs. These results highlight the effectiveness of the millet and banana peel flour mixtures, especially the M70+Bp30 % combination, in reducing BMI and supporting their potential role as a natural weight loss supplement.

According to Gomez-Hernandez et al. [\[63](#page-13-0)], high-fat diets have been widely utilized as a prominent model for developing obesity in animals, primarily due to their ability to closely replicate the typical progression of obesity in humans. The current results align with research undertaken by Cabanillas et al. [\[64](#page-13-0)], which revealed a notable rise in body weight among rats fed a high-fat diet (HFD) related to those provided with a standard diet. The correlation between millet consumption and the reduction in body weight becomes more evident as millet intake levels increase, potentially serving as a preventive measure against weight gain. A study by Sarma et al. [[65\]](#page-13-0), found that the polysaccharide in millet can mitigate obesity in rats induced with a high-fat diet. Similarly, Konda et al. [\[66](#page-13-0)] indicated that administering oral augmention of banana juice at a dosage of 5 mL/kg bw/day found a substantial reduction in body weight

Table (6)

Effect of millet and banana peel flour mixtures on body weight parameters in rats fed with a high-fat diet.

Data are presented as mean \pm SEM (n = 7). Statistical significance was determined using ANOVA followed by LSD post hoc test for pairwise comparisons. Significant differences (P ≤ 0.05) were observed for BWG%, FER, and BMI among the treatment groups, but not for FI (P *>* 0.05).

among obese rats on a high-fat diet (HFD), as compared to the control group receiving only the HFD. Additionally, rats consuming a mixture of licorice and banana peel lost significantly more body weight than rats fed a high-fat diet.

The current findings revealed that rats who were given a high-fat diet for 8 weeks had a notable rise in body weight % and feed efficiency ratio (FER), coupled with a non-significant increase in food consumption, in comparison to rats that were given a standard baseline diet. Additionally, rats fed an HFD plus M70+Bp30 % showed decreases in FI, FER, BWG, and BMI. These findings are consistent with [REF], who reported that supplementing a high-fat diet with millet at levels of 5 %, 10 %, and 15 % resulted in a decrease in body weight percentage (BWG%), fat index (FI), and fat equivalent ratio (FER) than the high-fat diet alone. Higher quantities of millet led to a more significant decrease in both food intake and body weight.

Sarma et al. [[65\]](#page-13-0) found that arabinoxylan, a polysaccharide present in millet, can prevent adiposity in rats induced with a high-fat diet. Additionally, Ortega et al. [\[67](#page-13-0)] noted that millets are abundant in micronutrients, devoid of gluten, have a low glycemic index, and are abundant in fiber, boasting antioxidant characteristics that are crucial in managing obesity rates. Epidemiological studies provide consistent evidence that consuming dietary fiber effectively reduces the risk of obesity and is negatively correlated with body fat and body mass index, regardless of the amount of fat consumed.

Ibrahem and Sayed [\[12](#page-12-0)] reported that substituting 70 % wheat flour with fermented millet flour in a high-carbohydrate diet led to reductions in body weight gain (BWG) from 64.20 to 30.11, the feed efficiency ratio (FER) from 0.035 to 0.016, and body mass index (BMI) from 0.59 to 0.49, indicating strong anti-obesity effects. Leidy et al. [[68\]](#page-13-0) found that high-protein diets reduce energy intake and enhance physique characteristics in humans. Additionally, oral supplementation of PPBJ at 5 mL/kg bw/day dramatically reduced body weight in HFD-treated obese rats from 700 ± 0.04 g to 380 ± 0.34 g, with corresponding decreases in total fat, fat-free mass, BMI, and fat percentage.

Konda et al. [\[66](#page-13-0)] found that a diet containing 20 percent unripe banana flour (B20) had a notably reduced impact on weight growth in animals when in comparison to the usual diet (p *<* 0.05). The aforementioned discovery underscores the capacity of dehydrated banana flour as a nutritional element for regulating weight increase [[69\]](#page-13-0).

3.3.2. Liver function

The effect of millet and banana peel flour mixtures on liver function, specifically aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), in rats fed a high-fat diet (HFD) is shown in Table 7. Administering a high-fat diet increased the levels of serum AST, ALT, and ALP in comparison to the negative control. The HFD group displayed the most pronounced activities of these enzymes in comparison to the other treatments and the control group. However, the serum activities of AST, ALT, and ALP were reduced in the HFD groups supplemented with millet and banana peel mixtures (M90+Bp10 %, M80+Bp20 %, and M70+Bp30 %). Notably, the highest reduction in liver enzyme levels was observed in the M70+Bp30 % mixture. These results are consistent with those reported by Konda et al. [[66\]](#page-13-0), where the experimental group consuming the HFD showed a statistically significant increase (P *<* 0.05) in liver enzyme levels (AST, ALT, and ALP) related to the negative control group.

Nevertheless, the HFD supplemented with varying doses of millet and banana peels resulted in a reduction of liver enzymes than the positive control group. Similarly, Shi et al. [[70\]](#page-13-0) observed that rats fed germinated millet flour showed decreased ALT and AST levels relative to the HFD group. Additionally, Barroso et al. [\[71](#page-13-0)] found that adding 100 mg of banana peel extract prevented the increase in AST and ALT in the HFD group. Konda et al. [\[66](#page-13-0)] reported elevations in liver enzymes—AST, ALT, and ALP—in the HFD group compared to the negative control group fed a basal diet. Furthermore, it was demonstrated that HFD groups supplemented with 6 % banana peel experienced a significant drop in AST, ALT, and ALP levels than the HFD group alone. In a study by Wang et al. [\[72](#page-13-0)], the application of banana peel significantly reduced AST and ALT levels in a rat model of carbon tetrachloride (CCl4)-induced liver injury. These results indicate improved liver function in the treatments involving the HFD plus millet and banana peel mixtures.

3.3.3. Kidney function and glucose

The effects of millet and banana peel flour mixtures on kidney function (urea, creatinine) and glucose in rats fed an HFD are presented in [Table 8.](#page-7-0) The levels of urea and creatinine were significantly higher in HFD rats compared to the negative control group, with urea and creatinine levels at 51.0 mg/dl and 1.85 mg/dl, respectively, compared to 17.33 mg/dl and 0.48 mg/dl in the control (-ve) group. However, treatments with HFD supplemented with millet and banana peel powder mixtures resulted in a reduction of urea and creatinine levels, approaching those observed in the negative control group. Similarly, the glucose concentration in HFD rats was elevated, with a level of 137.33 mg/dl compared to 85.70 mg/dl in the control (-ve) group. Supplementation with millet and banana

Table (7)

Effect of millet and banana peels flour mixtures on Liver function in rats fed with a high-fat diet.

Data are presented as mean \pm SEM (n = 7). Statistical analysis was performed using ANOVA followed by LSD post hoc test for pairwise comparisons. Significant differences ($P \le 0.05$) were observed among the treatment groups for AST, ALT, and ALP levels.

peel flour mixtures reduced glucose levels, bringing them closer to those of the negative control group.

Moreover, HFD supplemented with varying levels of millet and banana peel showed improvements in kidney function and glucose levels compared to the positive control group. Serum creatinine and urea are well-established biomarkers for predicting renal dysfunction, as they show significant elevation in the presence of kidney failure [[73\]](#page-13-0). A study by Jungert et al. [[74\]](#page-13-0) demonstrated that rats fed an HFD experienced significant elevations in serum creatinine, urea, and glucose levels compared to negative control rats. In contrast, rats fed a diet supplemented with millet at three various concentrations (5 %, 10 %, and 15 %) exhibited notable reductions in blood creatinine, urea, and glucose levels than the HFD group, with the 15 % millet group indicating the most significant improvement in these serum variables.

According to Narayanan et al. [[75](#page-13-0)], millet consumption has the potential to lower both fasting and postprandial blood glucose amounts. These results are consistent with prior research that has shown millet can improve antioxidant status and regulate blood sugar concentrations [[76\]](#page-13-0). Millet has also been revealed to be helpful for individuals with type 2 diabetes [\[77](#page-13-0)].

Furthermore, Almaski et al. [[78\]](#page-13-0) concluded that millet can lower fasting and postprandial glucose levels in both healthy and type 2 diabetic people. Additionally, Zakaria et al. [[79\]](#page-13-0) performed an investigation in which diabetic rats were fed a diet enriched with banana peel at two different levels (5 % and 10 %), revealing a considerable reduction in serum creatinine, urea, and glucose levels than the positive control group. Zaini et al. [[80\]](#page-13-0) further found that the dietary fibers present in banana peel contributed to reducing postprandial blood sugar levels.

3.3.4. Antioxidant enzymes

The impact of millet and banana peel flour mixtures on antioxidant enzymes— catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA),—and glutathione peroxidase (GPx) levels, indicating oxidative stress in rats fed a high-fat diet (HFD), is shown in [Table 9](#page-8-0). The results reveal that the HFD induced a significant increase in serum MDA levels and a decrease in the activities of SOD, CAT, and GPx compared to the control (-ve) and other treatments. The inclusion of millet and banana peel mixtures (M90 + Bp10 %, M80 + Bp20 %, and M70 + Bp30 %) in the diet led to increased antioxidant enzyme activities and a reduction in MDA levels. Notably, the highest increase in antioxidant enzyme activities was observed in the M70 $+$ Bp30 % mixture. These findings are consistent with those reported by Wise et al. [\[81](#page-13-0)], where male rats on an HFD had significantly lower SOD levels compared to those on a control diet. Similarly, Kovačević et al. [\[82](#page-13-0)] demonstrated that rats fed an HFD exhibited decreased activity of blood antioxidant enzymes and increased lipid peroxidation, as indicated by elevated MDA levels.

Furthermore, a study by Bai et al. [[83\]](#page-13-0) showed that the consumption of a high-fat diet (HFD) alone resulted in a notable elevation in blood malondialdehyde (MDA) levels. Additionally, the activities of glutathione (GSH) and glutathione peroxidase (GPx) enzymes were reduced compared to the control group (-ve). Incorporating various levels of millet into the HFD led to a considerable decrease in serum MDA levels and an increase in antioxidant enzyme activities compared to the consumption of HFD alone. Rezq et al. [[84\]](#page-13-0) demonstrated that millet consumption had a normalizing effect on the increased lipid peroxidation and reduced susceptibility to oxidative stress in obese-hyperlipidemic rats, often associated with a depletion of antioxidants. The potential antioxidant effects of millet can be attributed to its phenolic and flavonoid components. Konda et al. [[66\]](#page-13-0) showed that hepatic MDA levels were significantly elevated in rats with HFD-induced obesity compared to those in the control group. Similarly, Liu et al. [[85\]](#page-13-0) found that a high-fat diet impairs antioxidant enzymes and increases lipid peroxidation; however, supplementing this diet with millet improved antioxidant status in rats, reducing MDA levels, increasing GSH levels, and boosting antioxidant enzyme activity. Millet's antioxidant benefits are likely due to its phenolic and flavonoid compounds. Additionally, oral supplementation of banana peel juice led to a noteworthy decrease in MDA levels. The study found that banana peel juice possesses significant antioxidative activity, though HFD caused a decline in the antioxidant defense mechanism, with a drop in catalase and glutathione enzyme activities in the hepatic tissue of rats relative to the control group. Oral administration of banana peel juice increased these enzyme activities. The current study suggests that banana peel and millet flour may possess antioxidant capacities, potentially offering protection against obesity. These bioactive compounds, including fibers, polyphenols, and flavonoids, hold promise for future dietary applications in obesity treatment.

3.3.5. Lipid profile

[Table 10](#page-8-0) displays the impact of combinations of millet and banana peel flour on the lipid profile measurements, including total cholesterol (T.C.), triacylglycerol (T.G.), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and leptin, in rats that were fed a high-fat diet (HFD). The results demonstrated that a high-fat diet (HFD) had a substantial (P *<*

The effect of millet and banana peels flour mixtures on antioxidant enzymes and oxidative stress in rats fed with high-fat.

Data are presented as mean \pm SEM (n = 7). Statistical analysis was conducted using ANOVA followed by LSD post hoc test for pairwise comparisons. Significant differences ($P \le 0.05$) were found among the treatment groups for all variables.

Table (9)

Data are presented as mean \pm SEM (n = 7). Statistical analysis was performed using ANOVA followed by LSD post hoc test for pairwise comparisons. Significant differences ($P \le 0.05$) were observed among the treatment groups for all variables.

0.05) impact on elevating the levels of blood lipid profile markers and leptin. However, treatment with HFD plus millet and banana peel mixtures (M90 + Bp10 %, M80 + Bp20 %, and M70 + Bp30 %) decreased all serum lipid profiles and leptin levels while increasing HDL-C compared to the HFD and control (-ve) groups. These findings suggest that the lipid-lowering effects in the serum are likely related to the influence of banana peels and millet flour.

Silvester et al. [[86\]](#page-13-0) reported that the HFD group had a notable rise in blood levels of T.G., T.C., and LDL-C while experiencing a decline in serum HDL-C, as opposed to the control group. The present findings align with the results reported by Lordan et al. [[87\]](#page-13-0), where HFD supplemented with millet at varying concentrations (5 %, 10 %, and 15 %) resulted in a notable decrease in serum levels of T.G., T.C., and LDL-C while simultaneously increasing serum levels of HDL-C. Kumari and Thayumanavan [[88\]](#page-13-0) observed that rats who were given a meal containing processed starch from barnyard millet exhibited reductions in blood glucose levels, as well as reductions in serum cholesterol and triglyceride levels. Similarly, Park et al. [\[89](#page-13-0)] observed that millet supplementation improved high-density lipoprotein (HDL) cholesterol levels in genetically obese type-2 diabetic mice under high-fat conditions. Additionally, Antonowski et al. [\[90](#page-13-0)] revealed a notable decrease in serum triglyceride levels caused by millet. This finding implies that millet has the potential to mitigate cardiovascular disease by lowering plasma triglycerides in hyperlipidemic rats. Moreover, Elhassaneen et al. [[60\]](#page-13-0) showed that adding 1 % w/w banana peel powder to a rat's diet significantly altered the blood lipid profile by decreasing T.C., T.G., LDL, and VLDL levels, while increasing HDL levels.

Leptin, a ubiquitous protein synthesized by adipose tissue, exhibits a strong association with body fat, indicating that those who are obese typically lack sensitivity to the creation of leptin from inside their own body. Serum levels of this hormone are often positively associated with the amount of triglyceride reserves in adipocytes, making it a crucial controller of dietary intake and fat-derived energy expenditure produced from fat [\[91](#page-13-0)]. In comparison to the normal control group, the high-fat diet control group had a substantial increase in blood leptin levels. Studies have demonstrated that replacing fat with dietary carbohydrates leads to an elevation in plasma leptin levels [\[92](#page-13-0)]. Handjieva-Darlenska and Boyadjieva [\[93](#page-13-0)] reported that an experimental diet high in fat might elevate leptin levels. Administering millet to obese-hyperlipidemic rats led to a notable reduction in blood leptin concentrations in comparison to their untreated counterparts. These findings indicate that the reduction in blood concentrations of leptin following millet supplementation may be due to its impact on lowering food consumption and body weight, thereby subsequently reducing the buildup of fat in adipocytes. This highlights the role of millet and banana peels not only in managing weight but also in modulating key metabolic hormones like leptin, thereby offering a comprehensive approach to obesity management [[94\]](#page-13-0). A notable decrease in average leptin quantities was observed in obese groups that were fed 3 % and 6 % unripe banana or banana peels, as compared to both the positive control group and the high-fat diet (HFD) groups. The experiment found that green banana flour supplementation reduced leptin levels in mice fed a high-fat diet, suggesting an improved response to leptin, potentially linked to better weight regulation.

3.3.6. Histology of liver tissue

A photomicrograph of liver tissue from the control (-ve) group ([Fig. 1A](#page-9-0)) shows slices of untreated control rats exhibiting the typical histological architecture of hepatic lobules, with liver tissue (hepatocytes) radiating from a central vein. Blood sinusoids are welldeveloped between the cords of hepatocytes, converging toward the central vein, and the liver cells display normal basal nuclei and eosinophilic cytoplasm. In contrast, the liver sections of the HFD group [\(Fig. 1B](#page-9-0)) revealed blood expansion, fibrosis, infiltrations, and edema in the central veins, blood sinusoids, and portal veins. Additionally, new bile ductules were identified, and some

Effect of millet and banana peel flour mixtures on Lipid Profile and Leptin in rats fed with a high-fat diet.

Data are presented as mean \pm SEM (n = 7). Statistical analysis was performed using ANOVA followed by LSD post hoc test for pairwise comparisons. Significant differences ($P \le 0.05$) were observed among the treatment groups for all lipid profile parameters and leptin levels.

 $A:$ Light micrograph of liver from control (-ve) rats group showing the normal structure of hepatocytes (H). hepatic cord (Hc), blood sinusoid (S), and central vein (CV) (H&E X400).

C: Light micrograph of liver from HFD plus M90+Bp10 % showing mild dilatation and congestion of central vein (CV) and blood sinusoids (S). Severe congestion of portal vein (P.V.) with mild cellular infiltrations (arrow) around components of a portal tract (H&E X100).

 \mathbf{B} : Light micrograph of liver from HFD-treated rats group showing dilatations and congestion of central vein (CV), sinusoidal areas (S), and portal vein (P.V.), which is surrounded by fibrotic area. The portal tract edema (*) is associated with newly formed bile ductules (arrow) (H&E X100).

 ${\bf D}$: Light micrograph of liver from HFD plus M80+Bp20 % showing almost normal structure with regular arrangement of hepatocyte cords (Hc), mild congestion of central vein (CV) and portal vein (P.V.) with mild cellular infiltrations (arrow) around components of a portal tract (H&E X100).

 $E:$ Light micrograph of liver from HFD plus M70+ Bp 30% showing slight congestion of blood sinusoids (S) and few inflammatory cells (arrow) around the central veins (CV). Most hepatocytes (H) appear normal, and few cells contain some cytoplasmic vacuoles (arrowhead) (H&E X400)

Fig. 1. A–E Photomicrograph of liver tissue from untreated control (-ve) group and four treated sub-groups showing slices of histological architecture of hepatic lobules with liver tissue (hepatocytes) forrats

hepatocytes were observed to lack nuclei, accompanied by fatty degeneration (accumulation of fat) in liver cells due to obesity.

Liver sections from the HFD plus millet and banana peel mixtures (M90 + Bp10 % and M80 + Bp20 %) (Fig. 1C–D) exhibited cellular degeneration, activation of Kupffer cells, mild dilatation, and some congestion of the central veins and hepatic portal blood vessels, infiltrated by leukocyte inflammatory cells. In contrast, liver sections from the HFD plus millet and banana peel mixture (M70 + Bp30 %) (Fig. 1E) displayed only slight congestion in the sinusoidal blood, central veins, and portal veins, along with mild dilatation and minimal cellular infiltration around the central vein and portal tract components. Most hepatocytes appeared normal.

The current study indicates that rats in the control (-ve) group had normal liver structures, while those in the HFD group exhibited blood-engorged pelvic veins, inflammation, and necrosis. In contrast, the liver from the HFD plus millet and banana peel mixture (M70

+ Bp30 %) showed normal architecture and blood sinusoids. The improved structure of the hepatic lobules may be due to the antioxidative effects of the mixtures, reducing liver damage.

Yousef et al. [[95\]](#page-13-0) reported that liver slices from normal rats showed no pathological alterations, while those fed HFD displayed hepatic vacuolar degeneration, necrosis, inflammatory cell infiltration, and minor vacuolization. However, liver sections obtained from rats that were given a 15 % millet showed no significant changes. Similarly, Bucalen [[96\]](#page-13-0) reported that banana peel extract had a protective effect on the liver and attenuated lipid peroxidation in rats. Barroso et al. [[71\]](#page-13-0) demonstrated that the HFD group exhibited hepatic steatosis, lobular inflammation, and hepatocellular ballooning in their histological investigation, while banana peel doses reduced hepatic steatosis and had pronounced effects on hepatocyte lipids. The study observed that adding banana peel powder to the rat diet did not result in any histological abnormalities in the liver. The potential protective mechanism of banana peel powder could be attributed to the presence of vitamin E, which possesses antioxidant properties [\[71](#page-13-0)]. The current results revealed that the histological findings of the liver were corroborated by our biochemical results.

3.4. Sensory evaluation

The sensory evaluation of pancakes and toast bread made from a millet flour and banana peel mixture (M70 $+$ Bp30 %), which yielded the best results in biochemical analyses, is presented in [Table 11](#page-11-0) and [Fig. 2.](#page-11-0) The data showed that while there were color differences, the products were still deemed acceptable. There were no notable disparities detected in terms of flavor, smell, consistency, and overall satisfaction when compared to the control. These findings align with the results reported by Rajiv et al. [[97\]](#page-14-0)**,** where adding finger millet flour (FMF) to snacks as a substitute for wheat flour at ratios of 70 % and 100 % FMF improved all sensory attributes and overall acceptability, with a more pronounced improvement in the taste of the resultant snacks. Similarly, Florence et al. [\[98](#page-14-0)] revealed that cakes and cookies prepared with a 50:50 wheat + millet blend had better sensory profiles than other combinations. According to Dias-Martins et al. [\[99](#page-14-0)]**,** pearl millet exhibits suitability for various food applications, including the production of infant foods, snack foods, and bread items, due to its nutritional content and health-enhancing attributes. Zidan [\[100\]](#page-14-0) also examined the potential use of millet flour in toast bread preparation, focusing on improving its nutritional properties and sensory characteristics.

Mahmoud et al. [[24\]](#page-12-0) conducted a study where banana peel powder was used to enrich crackers, leading to noticeable improvements in texture and aroma, although the color and taste remained comparable between samples containing 10 % and 15 % banana peel powder and the control group. Similarly, Ho et al. [\[101\]](#page-14-0) found that bread formulated containing 10 % banana pseudo-stem flour (BPF) was just as well-received as the control bread in terms of overall acceptance. Amini Khoozani [\[102\]](#page-14-0) highlighted that using banana peel flour is a viable opportunity to enhance the nutritional composition of cereal-based goods, with no significant impact on the acceptability of the products as perceived by panelists. Therefore, there is potential to increase the quantities of BPF in cereal-based products such as biscuits, bread, and pasta.

4. Conclusion

Millet and banana peels exhibited strong antioxidant activity, high total phenol content, and valuable nutrients and minerals such as magnesium (Mg) and potassium (K). The combination of a high-fat diet (HFD) with a millet and banana peel mixture (M70 + Bp30 %) led to reduced body weight gain (BWG), improved lipid profiles, and enhanced levels of leptin, antioxidant enzymes, as well as improved liver and kidney function. The histological analysis of the liver supported the biochemical findings. Additionally, the sensory evaluation of pancakes and toast bread made from the $(M70 + Bp30%)$ mixture was considered acceptable by panelists, with this mixture producing the best results in the biochemical analyses. This study highlights the potential benefits of incorporating millet flour and banana peel into the human diet to promote better health in individuals with obesity.

CRediT authorship contribution statement

Azizah A. Alshehri: Writing – review & editing, Supervision, Software, Resources, Funding acquisition. **Nashwa M. Younes:** Visualization, Supervision, Project administration, Formal analysis, Conceptualization. **Reham Kamel:** Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Investigation, Formal analysis, Data curation. **Samar M. Shawir:** Writing – review & editing, Validation, Resources, Investigation, Funding acquisition, Formal analysis.

Ethical approval

All study experiments were ethically approved by the Scientific Research Ethics Committee, Faculty of Specific Education, Alexandria University (Approval no27-8-2023 SREC 09).

Consent for publication

All authors agree to publication.

Availability of data and materials

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Table (11)

Sensory evaluations of prepared pancake and toast bread.

Data are presented as mean \pm SEM (n = 7). Statistical analysis was performed using the Student's t-test. Significant differences (P < 0.05) were observed in the color and acceptance parameters for both pancake and toast bread between the control (A) and the treatment (B) groups (M70 + Bp30). Other sensory parameters did not show significant differences.

Fig. 2. Image depicting sensory evaluations of prepared pancake and toast bread.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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