Lipids in Health and Disease

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The effects of the gut bacterial product, gassericin A, on obesity in mice

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Abstract

Background Obesity can arise from various physiological disorders. This research examined the impacts of the bacteriocin, gassericin A, which is generated by certain gut bacteria, using an in vivo model of obesity.

Methods Fifty Swiss NIH mice were randomly assigned to five different groups. One group was given a standard diet, while the remaining groups were fed a diet high in fat and sugar. The test groups received gassericin A at doses of 0.75, 1.5, or 3 mIU/kg through intraperitoneal injection, daily for 10 weeks. Body weight, fasting blood sugar, serum lipid profile, and hepatic function indicators were then assessed. Additionally, the blood profile, markers of oxidative stress, and expression levels of specific genes associated with obesity, *Zfp423*, and *Fabp4*, were evaluated in abdominal adipose tissue.

Results A high-calorie diet negatively impacted abdominal fat, serum cholesterol, LDL, and hepatic enzymes. However, gassericin A significantly improved these effects, despite increasing weight gain and abdominal fat. Furthermore, it improved redox status, downregulated the *Zfp423* gene, and enhanced the expression of the *Fabp4* gene. Finally, the bacteriocin caused thrombocytopenia and mild decreases in erythrocytes, hematocrit, and hemoglobin levels.

Conclusions These results suggest that, despite causing weight gain, gassericin A may improve obesity-related complications.

Keywords Obesity, Microbiota, Gassericin A, Obesity complications

Introduction

Obesity is posing as one of the biggest challenges in the 21st century that threats the public health worldwide. According to the World Health Organization, the prevalence of obesity worldwide in adults has more than doubled since 1990. In 2022, an estimated 2.5 billion adults were overweight, while 890 million were classified as obese [1]. This is a disturbing prevalence since obesity has been related to a number of serious health conditions, such as cardiovascular diseases, type 2 diabetes, and certain types of cancer [2].

When the energy consumption in the body equals the energy intake, complex neurohormonal mechanisms regulate energy homeostasis. However, disrupting this balance may increase the number of adipocytes (hyperplasia) or their size (hypertrophy) [3, 4]. Several factors contribute to this disruption, including genetics, environmental influences, medical conditions, and individual behaviors. Recent discoveries of new biological mechanisms have provided innovative therapeutic approaches to prevent obesity [5, 6].

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Recently, numerous studies have investigated the role of intestinal microbiota in the onset of diabetes and obesity. The gut microbiota represents a significant ecological community composed of bacteria, fungi, protozoa, and archaea, all of which directly influence the digestion and absorption of nutrients [7–9]. Amid a complex array of influential factors, the balance between the two predominant phyla in the gastrointestinal microbiota, Bacteroidetes and Firmicutes, along with their specific metabolites, is essential for maintaining health, modulating the immune system, and preventing weight gain by directly affecting the host's physiological processes [10–12]. In a research study carried out by Million et al. [13] to explore the connection between obesity and the gut microbiota, researchers reported the impact of specific *Lactobacillus* species on weight gain and obesity in both animals and humans. In the study by Drissi et al. [14], researchers aimed to identify *Lactobacillus* species and the bacteriocins that are essential for the metabolism of carbohydrates and lipids associated with weight changes. The data obtained from bioinformatics studies revealed that *Lactobacillus* species linked to weight reduction possess mechanisms to increase glycolysis and defend against oxidative stress, whereas those linked to weight gain have a restricted capacity to metabolize glucose or fructose [14]. However, despite the promising potential of microbiota manipulation for obesity treatment and the development of new therapeutic approaches, the underlying molecular mechanisms remain unclear. The microbiota employs various intracellular mechanisms to thrive within the body, including the synthesis of bacteriocins.

Bacteriocins are secondary peptide metabolites produced by a wide range of bacteria that exert antibacterial effects on similar or closely related strains. The recognition of the role of bacteriocins began over two decades ago, and they have significantly impacted the intestinal microbiota [15, 16]. In addition to their important roles in food preservation and antibacterial activity, certain bacteriocins may also activate genes associated with weight gain or loss, depending on the type of bacteria producing them [17, 18]. Bacteriocins may influence weight changes by affecting gut microbial populations in both obese and lean individuals. However, the direct effects of bacteriocins on adipose tissue and the inflammatory diseases linked to obesity are not yet fully understood [19]. A recent study suggested that bacteriocins produced by *Lactobacillus* species, such as *plantarisin*, may contribute to the management of obesity and its complications by affecting the stearoyl-CoA desaturase-1 (SCD-1), a crucial enzyme in adipocyte differentiation [20]. The present research team has previously evaluated the impact of particular bacteriocins on obesity and its associated complications. One of these studies suggested that nisin, a bacteriocin generated by *Lactococcus lactis*, may reduce obesity by inhibiting the expression of specific genes associated with the condition, including *SCD1*, *GLUT4*, *TNFα*, and *Fabp4*, in mice [21]. Another study found that the bacteriocin gassericin A promoted weight gain and downregulated genes associated with adipocyte differentiation [22].

Gassericin A is a cyclic bacteriocin that was first identified in 1991. It is produced by the gram-positive bacterium *Lactobacillus gasseri* LA39 [23]. There are currently no reports on the metabolic effects of this peptide under in vivo conditions. Therefore, the present research assessed the impacts of gassericin A on body weight, certain serum parameters, and the expression of specific genes, with a particular emphasis on obesity and its associated complications.

Materials and methods

The bacteriocin-producing strain, *Lactobacillus gasseri* LA39 was obtained from the Iranian Biological Research Center (IBRC, Tehran, Iran). The purification of the bacteriocin was conducted as previously described [22]. In summary, *L. gasseri* was cultured in DOB-MRS broth (Sigma-Aldrich Co., Steinheim, Germany). Oleic acid was removed from the supernatant using filter paper, and the filtrate underwent dialysis against water (Sigma dialysis tube D7884-1FT, MWCO: 2000, Steinheim, Germany). The dialysate was processed through a hydrophobic chromatography column (TOYOPEARL Butyl-650 S, TOSOH, Tokyo, Japan), where it was eluted with distilled water, 50% (v/v) methanol, and finally 100% methanol. The eluate obtained with water was further subjected to reverse phase chromatography using LiChroprep RP-8 (Merck KGaA, Darmstadt, Germany). The highest dilution of the purified peptide that was capable of inhibiting the growth of the indicator species, *L. delbrueckii bulgaricus* subsp. *bulgaricus* (IBRC, Tehran, Iran), was considered one arbitrary unit [24]. The specified peptide was divided into 0.25 ml aliquots and kept at -80 °C for future studies.

Animals and treatment

Healthy male NIH Swiss mice, six weeks old and weighing 32±0.4 g, were selected for the study. The mice were purchased from the Animal Unit at Mashhad University of Medical Sciences. The animals were kept in the animal facility of the School of Veterinary Medicine at Ferdowsi University of Mashhad and allowed to acclimatize for one week before the initiation of the experiments. Temperature was maintained at 25 °C with 12-hour cycles of light and darkness. The animals had *ad libitum* access to both water and food. The animals were weighed, then placed into individual cages.

Forty-six male mice were randomly divided into five groups, with each group consisting of 8 to 10 mice. The

animals were weighed and then placed in individual cages. The two control groups were given either a standard diet (the N group) or a high-fat and high-sugar (HFS) diet (the C group), along with a placebo (saline). The three test groups were fed an HFS diet and received gassericin A at 0.75, 1.5, or 3 mIU/kg intraperitoneally (designated as the L, M, or H group, respectively) daily for 10 weeks. The composition of the standard diet (Javaneh Khorasan Co., Mashhad, Iran) is detailed in Table 1. The HFS diet was prepared by uniformly incorporating 25% highly saturated fat, vegetable shortening, and 32.5% sugar into the standard diet [25]. The final diet contained 5061 kcal/kg which is an increase of 84% over the standard diet (2750 kcal/kg).

Sampling

At the end of each week, body weight and food intake were recorded. Feeding was halted 16 h prior to blood sampling at the end of 10 weeks. The animals were anesthetized by an intraperitoneal injection of sodium thiopental (50 mg/kg), and the abdominal wall was excised. Blood samples were collected from the hearts of the mice for a complete blood count (CBC) and serum analysis. The animals were then euthanized via removal of the heat. The mesenteric fat was removed from the abdominal cavity, weighed, and preserved in liquid nitrogen for subsequent molecular and cellular studies. Approximately 300 mg of liver tissue was extracted and stored at -80 °C for the assessment of redox status.

The biochemical parameters of the collected samples, including fasting blood sugar (FBS), cholesterol, triglycerides, creatinine, blood urea nitrogen (BUN), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and liver enzymes such as alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) were measured using a BT1500 autoanalyzer (Biotecnica Instruments, Rome, Italy). All parameters were assessed using commercial kits (Pars Azmoon Inc., Tehran, Iran).

A complete blood count, which included measurements of red and white blood cells, platelets, hematocrit, and hemoglobin levels, was performed using an automatic hematology analyzer (Nihon Kohden, Tokyo, Japan).

Table 1 The nutritional content of the standard diet

Nutrients	Quantity
Energy (kcal/kg)	2750
Protein (%)	20–21
Fat (%)	2–3
Fiber (%)	5–6
Lysine (%)	0.05
Methionine (%)	0.05
P/Ca ratio	1.5–2.5
Salt (%)	0.5
Ash (%)	4

Markers of redox status

Commercial kits (KushanZist Co., Tehran, Iran) were used to measure malondialdehyde (MDA), catalase (CAT) activity, and total antioxidant capacity (TAC). The method employed to assess CAT activity takes advantage of the peroxidative properties of CAT. This technique involves the interaction between methanol and CAT in the presence of H_2O_2 to generate formaldehyde. The presence of formaldehyde is quantified using a chromogen, 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole, that transforms the aldehyde into a purple color, allowing for photometric analysis. The procedure involved mixing 20 µL of tissue homogenate with 30 µL of potassium phosphate buffer, 100 µL of formaldehyde, and 20 µL of methanol. After adding hydrogen peroxide and incubating for 20 min on a shaker, 30 µL each of potassium hydroxide and the chromogen were added for another 10 min of incubation. Finally, the absorbance was measured at 50 nm five minutes after adding potassium periodate. The thiobarbituric acid (TBA) technique was employed to assess the concentration of MDA, a biomarker for lipid peroxidation, in liver tissue. Thiobarbituric acid reacts with MDA in the presence of butylated hydroxytoluene and ferric chloride. The optical absorption is subsequently measured at 532 nm [26]. A peroxidase chromogenic substrate was employed to assess the TAC of serum. Following oxidation by ferryl myoglobin radicals, this substrate generates a blue-green chromogen. The presence of antioxidants limits the formation rate of colored chromogens, which can be assessed using photometric methods at a wavelength of 412 nm [26].

Extraction of RNA and gene expression

To extract RNA from mesenteric fat, 300 mg of the tissue was homogenized in 500 µL of TRIzol (Parstous Co., Mashhad, Iran). The RNA was separated by adding 500 µL of chloroform, followed by 500 µL of isopropanol for precipitation. The RNA pellet was then purified by washing it with 75% ethanol and allowed to air-dry. The pellet was then resuspended in sterile distilled water. The concentration and purity of the RNA were assessed using a NanoDrop spectrophotometer (BioTek Epoch 2, Watertown, USA) at 230 nm and by performing electrophoresis on a 1% agarose gel. The RNA samples were stored at -80 °C for further analysis.

Two genes including *Zfp423* and *Fabp4*, also known as *422/ap2*, were analyzed via RT-qPCR (Ampliqon Onestep RT qPCR Kit, Odense, Denmark) to investigate the impact of gasserin A on the expression levels of adipocyte-specific genes.

Table 2 The sequences of the primers used in RT-qPCR

Gene	Forward primer	Reverse primer
422ap2	TGAAATCACCGCAGACGACA	ACACATTCCACCACCAGCTT
TBP	CCTATCACTCCTGCCACACC	ATGACTGCAGCAAATCGCTTG
Zfp-423	CCGCGATCGGTGAAAGTTGA	ACGCTGTTCCTGTCTTCCAG

The primers (Table 2) were crafted to target exon-exon junctions to minimize the risk of DNA contamination on the RT-qPCR results. Regarding its relatively high stability in adipocytes, TATA-binding protein (TBP) was selected as the reference gene [16].

Histology

A portion of the mesenteric adipose tissue was immersed in 10% buffered formaldehyde for one day. The specimens were subsequently dehydrated and encased in paraffin blocks, which were then sectioned into 5-micrometer slices and stained with hematoxylin and eosin. The cytometry of adipocytes was then conducted under the supervision of an expert histologist. For each animal, three replicates were considered, and four fields were randomly chosen from each replicate. The diameter of adipocytes was measured using an Olympus BX51 fluorescence microscope (Takachiho Manufacturing, Tokyo, Japan) with DP2-BSW software.

Statistical analysis

The statistical calculations and graphs were generated using GraphPad Prism v9.5.1 software (GraphPad Software Inc, Boston, USA). The nonparametric Mann-Whitney test was used to analyze the RT-PCR results. All other statistical comparisons were conducted using one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test. The data are presented as medians and quartiles for the gene expressions and as means ± standard error of the mean (SEM) for the other parameters studied. In all cases, a *P*-value of less than 0.05 was considered statistically significant.

Results

Body weight, abdominal fat, and food intake

As shown in Fig. 1A, the average dose of gassericin A caused a significant increase in the percentage of weight gain relative to the control group. A diet high in fat and sugar resulted in an increase in abdominal fat relative to the standard diet (Fig. 1B). Conversely, the weight of abdominal fat was significantly greater in the animals receiving the highest dose of gassericin A compared to the control group. Additionally, as depicted in Fig. 1C, food intake was not influenced by either the diet or treatment with gassericin A.

Fasting blood sugar, lipid profile, and BUN

The impacts of gassericin A on serum levels of FBS, HDL, LDL, total cholesterol, and triglycerides are illustrated in Fig. 2. The high-calorie diet caused significant increases in the levels of FBS (Fig. 2A), cholesterol (Fig. 2B), HDL (Fig. 2C), and LDL (Fig. 2D). Gassericin A appears to counteract all of these effects. Conversely, neither the HFS diet nor gassericin A exhibited any significant impact on serum triglyceride levels (Fig. 2E). Additionally, while the HFS diet reduced the BUN level (Fig. 2F), the bacteriocin did not significantly influence this biomarker.

Serum biomarkers, redox status, and gene expression

The serum markers, including AST, ALP, and ALT, along with the levels of MDA, CAT, and TAC, were measured. The high-calorie diet significantly increased the serum levels of the liver enzymes (Fig. 3) and MDA (Fig. 4A); however, it did not have a significant impact on catalase (Fig. 4B) or TAC (Fig. 4C). Treatment with gassericin A

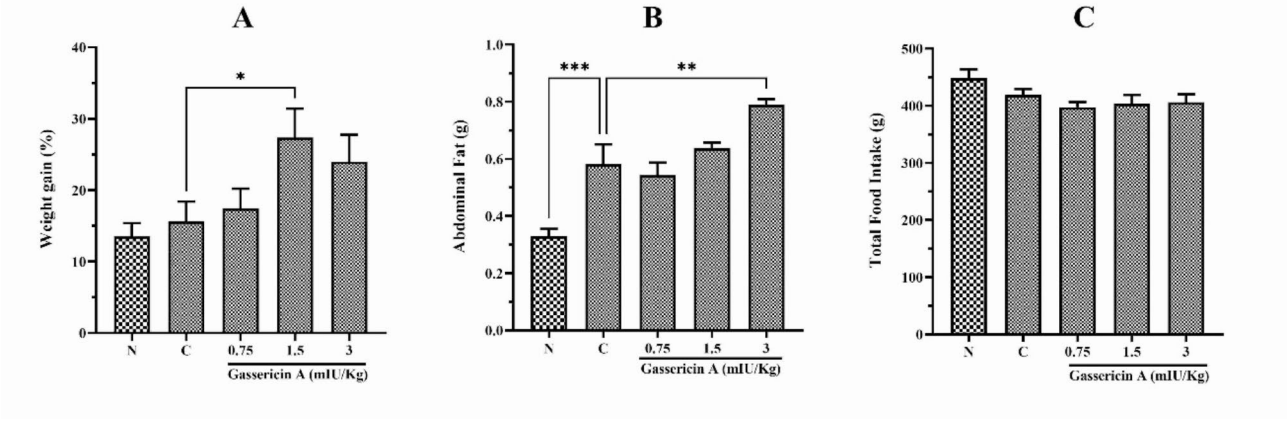

Fig. 1 Impact of gassericin A on body weight and related markers. A standard diet was fed to the N group, whereas the other groups received a high-calorie diet ($n = 10$, each). The mice were administered a placebo or different doses of gassericin A (i.p.) daily for 10 weeks. The data are presented as the mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

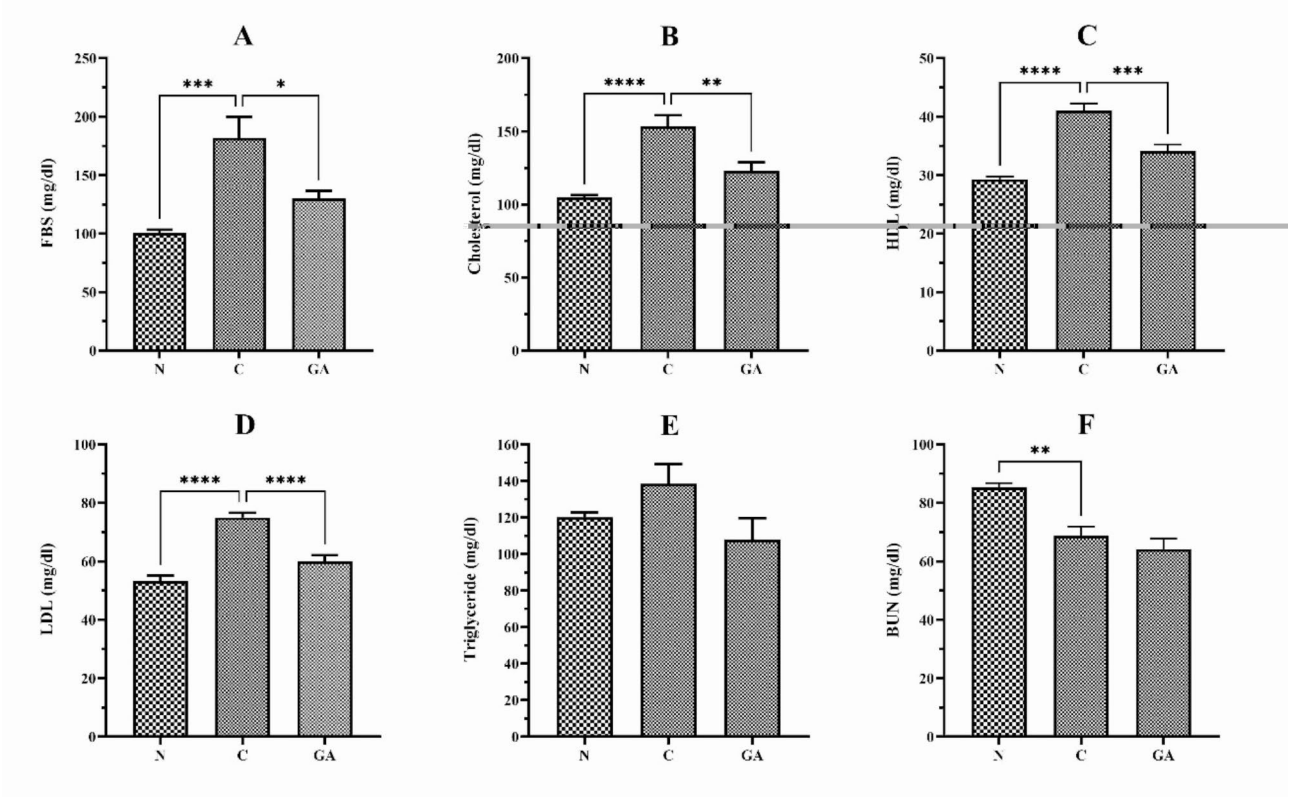

Fig. 2 The effects of gassericin A on specific serum biomarkers. The N group was fed standard a diet, whereas the other groups were given a high-fat and high sugar diet ($n = 10$, each). Over a period of 10 weeks, the mice received either a placebo or gassericin A (3 mIU/kg, i.p.) daily. The data are presented as the mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, BUN: blood urea nitrogen, C: control, GA: gassericin A

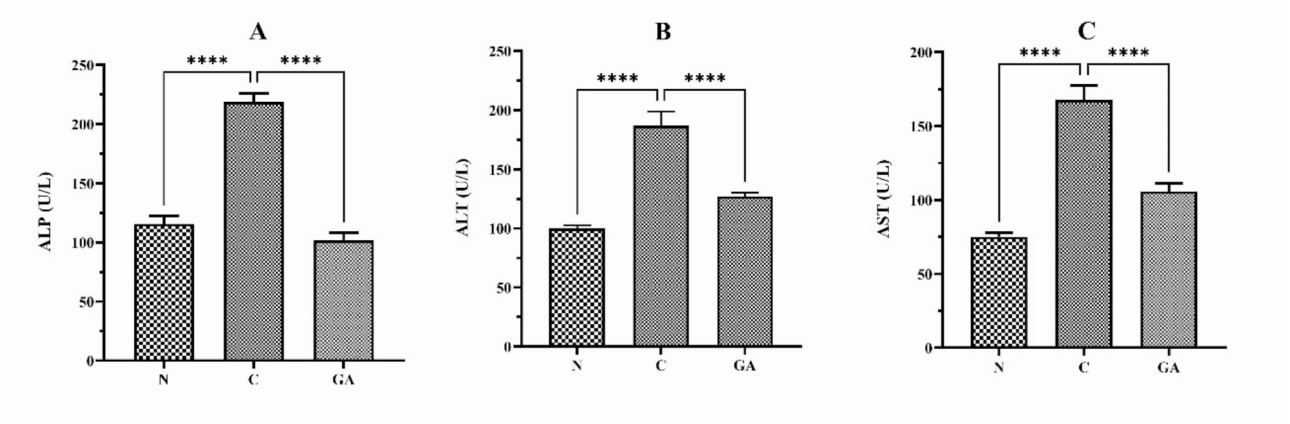

Fig. 3 The impact of gassericin A on particular liver enzymes. The N group was given a standard diet, while the remaining groups were subjected to a high-fat and high-sugar diet ($n = 10$, each). Over a duration of ten weeks, the mice were administered either a placebo or different dosages of gassericin A daily. The data are expressed as the mean ± SEM. ****$P < 0.0001$, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GA: gassericin A

at a dosage of 3 mIU/kg significantly reduced the levels of hepatic enzymes. Furthermore, the treatment increased hepatic CAT activity; however, it did not affect the levels of MDA or TAC.

The RT-qPCR data indicated that the expression level of the *Zfp423* gene significantly decreased in response to gassericin A (Fig. 5A), while the expression of the *Fabp4* gene significantly increased (Fig. 5B).

Blood profile

The effects of the HFS diet and gassericin A on CBC are shown in Fig. 6. Accordingly, the diet did not affect any of the studied CBC parameters (Fig. 6A-I). However,

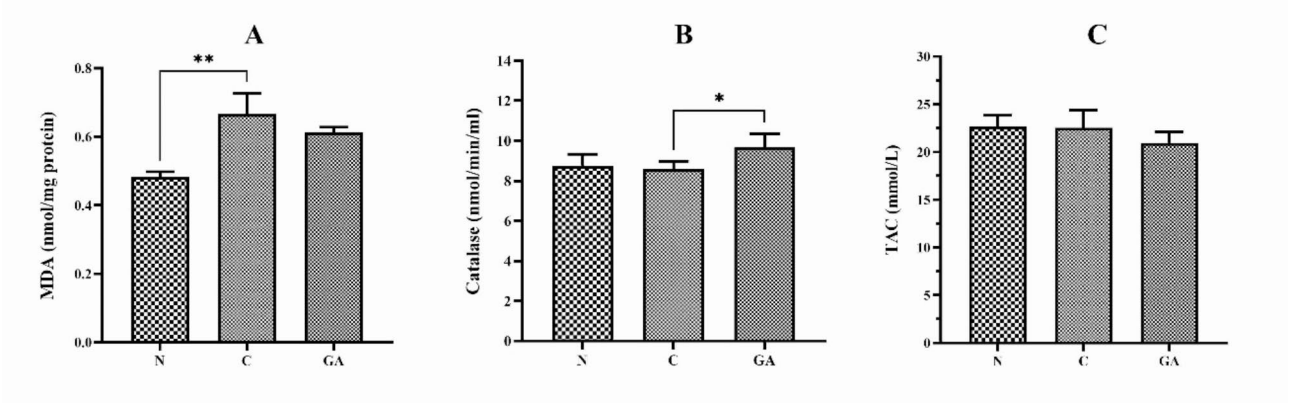

Fig. 4 The effect of gassericin A on redox status. The N group was fed a standard diet and the other two groups were given a high-calorie diet ($n = 10$, each). The mice received either a placebo or gassericin A (3 mIU/kg, i.p.) daily for ten weeks. The data are presented as the mean ± SEM. *$P < 0.05$, **$P < 0.01$, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GA: gassericin A, TAC: total antioxidant capacity

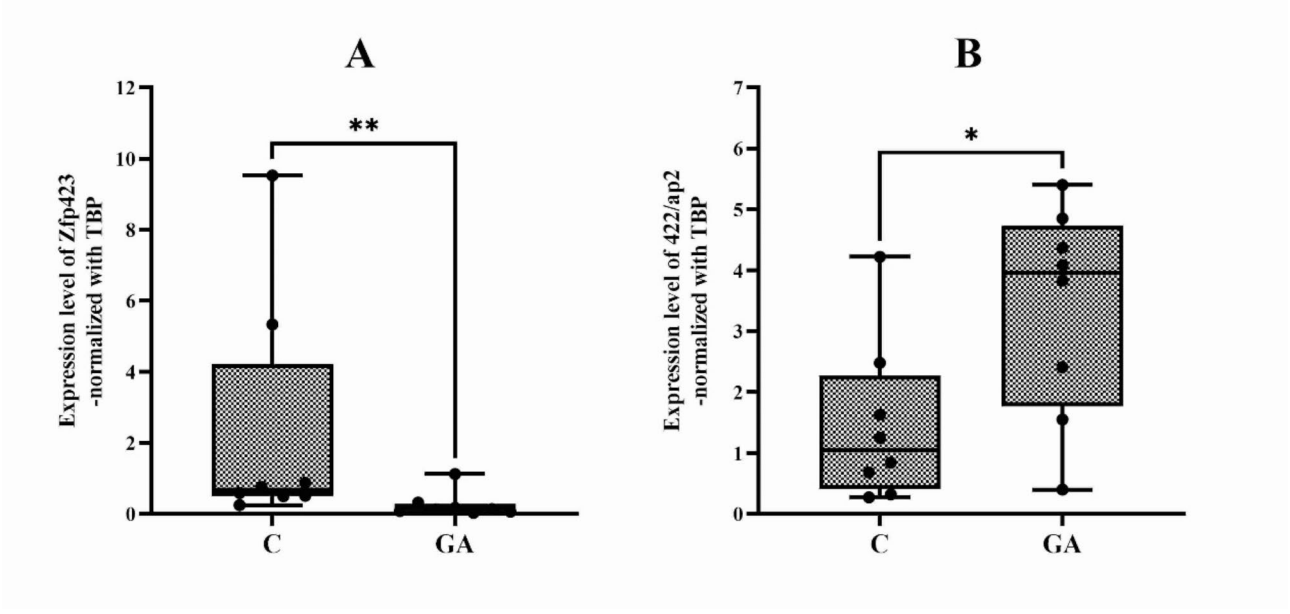

Fig. 5 Impacts of gassericin A on the genes associated with obesity. The control (C) group received placebo while the GA group ($n = 10$, each) received gassericin A (3 mIU/kg, i.p.) daily for 10 weeks. The gene expression levels in mesenteric fat are reported as medians and quartiles. *$P < 0.05$, **$P < 0.01$

the bacteriocin caused significant decreases in hematocrit (Fig. 6A), RBCs (Fig. 6B), hemoglobin (Fig. 6C), and platelets (Fig. 6I). On the other hand, the average volume of RBC (MCV, Fig. 6D), average hemoglobin content in RBC (MCH, Fig. 6E), average concentration of hemoglobin in RBC (MCHC, Fig. 6F), variation in RBC size (RDW, Fig. 6G), and the count of white blood cells (Fig. 6H) were not affected by the treatment.

Histological evaluation

As shown in Fig. 7, the HFS diet significantly increased the diameter of the fat cells. The cytometry results revealed a significant shrinkage in the size of abdominal adipocytes in the H group relative to the control group (23.3 ± 0.6 vs. 46.0 ± 1.5 µm, respectively; $p < 0.001$).

Discussion

An expanding body of research implies that intestinal microbiota are essential in the onset and advancement of obesity [27]. *Lactobacillus spp*, such as *L. gasseri*, have been effectively utilized in in vivo studies on obesity [28]. However, there are limited studies on the biological impacts of bacteriocins produced by *Lactobacillus gasseri*, such as gassericin A. A bioinformatics study revealed that this bacteriocin may contribute to weight gain [14]. The present research team employed an in vivo model to evaluate the effects of gassericin A on the culture medium of 3T3-L1 preadipocyte cell line, both before and after they differentiated into mature fat cells. They assessed the morphology and viability of the cells, as well as the expression levels of specific genes related

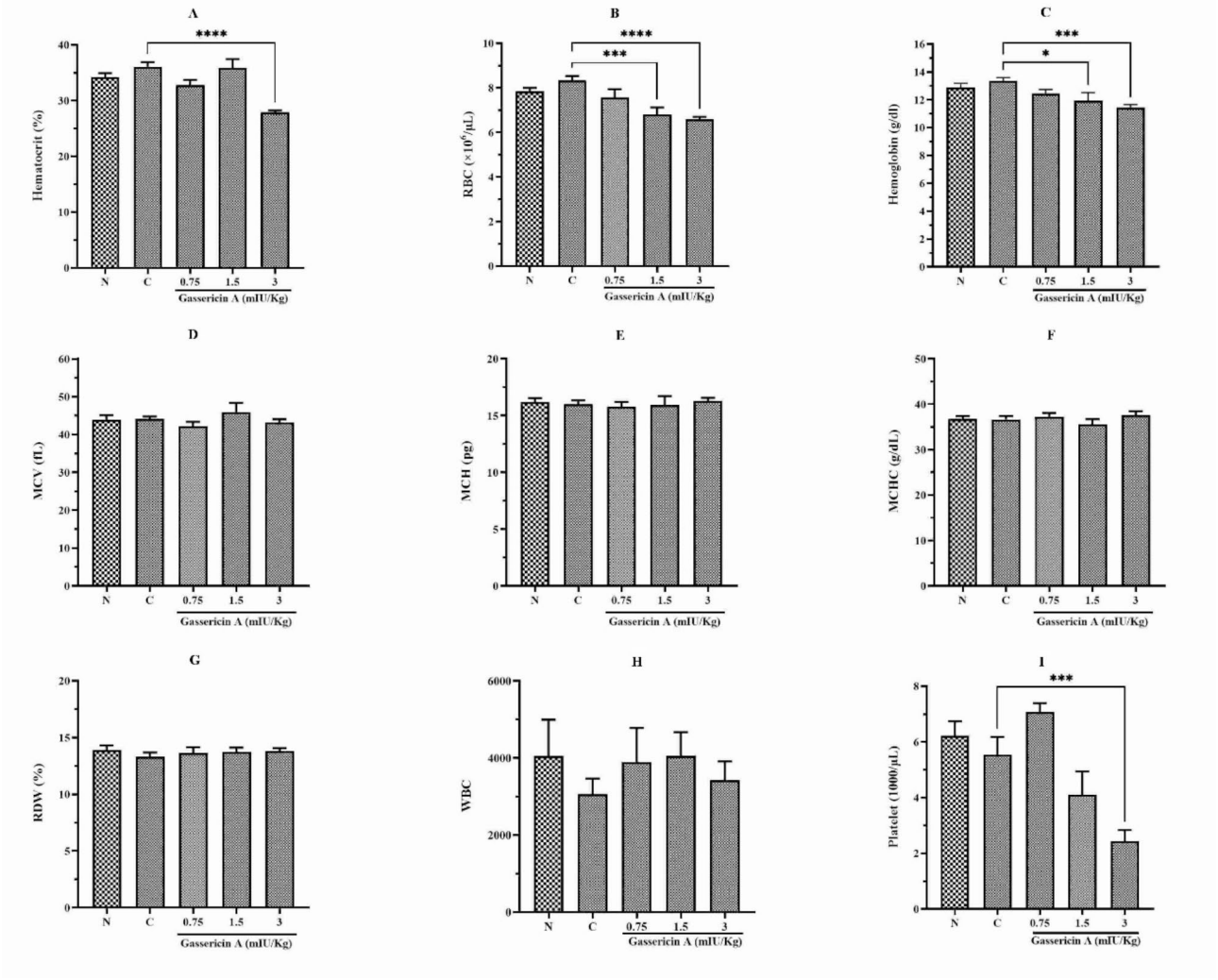

Fig. 6 Impact of gassericin A on the blood profile. A standard diet was fed to the N group ($n = 10$), whereas the remaining groups ($n = 10$, each) received a high-calorie diet for a duration of 10 weeks. The test groups were treated with gassericin A (i.p.) daily for 10 weeks. The results are presented as the means ± SEMs. $^{*}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$, RBC: red blood cell, RDW: red cell distribution width, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cell

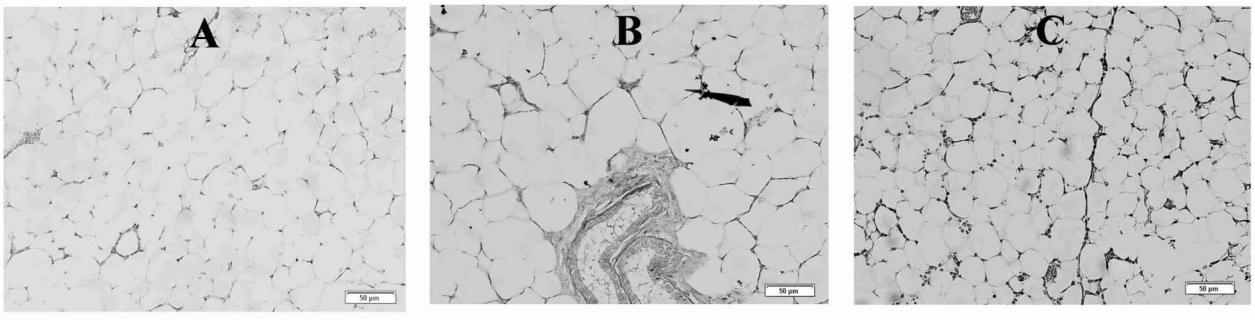

Fig. 7 Light microscope images of mesenteric adipose tissue. **A**: The N group, which received a standard diet; **B**: The C group, which received a high-calorie diet; **C**: The H group, which was treated with gassericin A (3 mIU/kg, i.p.) daily for 10 weeks. Magnification: 200x

to obesity, including *SCD-1*, *UCP-1*, *GLUT4*, *Zfp423*, *TNF-α*, and *Fabp4*. The results indicated that gassericin A may increase the number of adipocytes and contribute to hyperplastic obesity, while also offering protection against obesity-related complications [22]. However, there are currently no reports utilizing an in vivo model in this context.

The current research investigated the effects of gassericin A on obesity and its associated biomarkers in mice fed an HFS diet. The results indicated that specific doses of the peptide may increase weight gain and abdominal fat content when administered through intraperitoneal injection. Bioinformatics studies on *Lactobacilli*, as opposed to experimental evaluations of the peptide, indicated that gassericin A may have weight-protective effects [14]. Similar outcomes have been observed in meta-analyses of clinical trials or experimental models involving the impacts of *Lactobacillus gasseri* on both animals and humans [13]. However, these findings contradict the results of the present report. It is important to note that the previous studies cannot be directly compared to the current one, as none of them evaluated the effects of the bacteriocin itself under in vivo conditions.

Adipose tissue has two influential metabolic processes related to fat storage in its cells: hypertrophy, in which the cell volume is increased by storing fat particles, and hyperplasia, in which the fat cells multiply through cell division. In general, cell hyperplasia is not linked to metabolic or morphological disorders. In contrast, an increase in cell size may be associated with conditions such as local hypoxia in adipose tissue and various cellular disorders [29]. In the present research study, gassericin A decreased the size of fat cells, which is consistent with the previous findings in 3T3-L1 cells [22]. Smaller adipocytes are associated with a greater resistance to metabolic disorders, such as diabetes. Conversely, larger adipocytes are linked to a reduced production of anti-inflammatory adipokines like adiponectin, increased lipolysis, and heightened release of inflammatory cytokines [29]. These results indicate that gassericin A may offer protection against obesity-related complications by promoting hyperplastic fat cells. However, further research is necessary to monitor the levels of adipokines and inflammatory cytokines in this context.

In this study, gassericin A increased the expression of the *Fabp4* gene in fat tissue. This gene, also known as *422/ap2*, encodes the adipocyte fatty acid-binding protein 4. This finding is consistent with the increased abdominal fat and body weight in the mice treated with gassericin A. This result is also consistent with previous findings on cultured 3T3-L1 cells, both before and after their differentiation into adipocytes [22]. Adipocyte fatty acid-binding protein 4 is expressed in adipocytes following the activation of genes specific to adipose tissue, such as peroxisome proliferator-activated receptor (*PPAR*) *γ*, *C/EBPα*, and *C/EBPβ* [30]. The expression of the *Fabp4* gene rises during the transformation of preadipocytes into adipocytes [31]. In fact, genetic deletions of *Fabp4* and *Fabp5* provide greater protection against fatty liver disease, type 2 diabetes, insulin resistance, and obesity. Furthermore, these deletions are associated with decreased hepatic expression of *SCD-1* gene [32]. However, the overexpression of the *Fabp4* gene in the present study did not coincide with obesity-related disorders. The discrepancy observed in these findings necessitates further studies using additional distinct markers of fully developed adipocytes.

The additional gene of focus, *Zfp423*, encodes a zinc finger protein which is crucial for preserving the characteristics of white adipose tissue (WAT). Disruption of this gene has led to a significant reduction in adipose tissue [33]. White adipocytes are responsible for energy storage and resistance to thermogenesis. The *Zfp423* gene is a critical regulatory factor in white adipocytes. It modulates the levels of *PPARγ* in preadipocyte mural cells and guides them during the process of adipogenesis. It is also found in fully developed white adipocytes. Genetic deletion of *Zfp423* in white fat cells results in the development of beige adipocytes. *Zfp423* is crucial for preserving the characteristics of white adipocytes by inhibiting the expression of thermogenic genes. White adipocytes show a higher expression of *Zfp423* compared to brown adipocytes. This gene is downregulated upon exposure to cold [34]. Therefore, by reducing the expression of this gene, gassericin A may disrupt the process of fat whitening, leading to the formation of brown or beige adipocytes. Furthermore, gassericin A may reduce the overall size of white adipocyte tissues by decreasing the expression of this gene in the abdominal fat cells of mice. However, more studies are needed in this area. A previous in vitro study from the present team suggested that gassericin A may be capable of inducing changes in the characteristics of white or beige adipocytes by decreasing the expression of the *Zfp423* gene in the culture media of both preadipocytes and mature fat cells [22].

As there are no existing studies on the biological effects of gassericin A under in vivo conditions, the present study conducted a preliminary investigation into its biological impacts, including those related to obesity. Accordingly, gassericin A decreased RBC, hematocrit, hemoglobin, and platelet counts, whereas the leukocyte count remained unaffected. The underlying mechanisms and their potential physiological and therapeutic significance remain unclear. Although the decreases in RBC, hematocrit, and hemoglobin levels were mild, prolonged exposure to or higher doses of gassericin A may result in normocytic-normochromic anemia, primarily due to impaired RBC production. The concurrent decline in

platelet levels may be attributed to bone marrow suppression, a condition that can be induced by certain medications and toxins [35]. The current research was preliminary; therefore, further studies are necessary to conduct a more comprehensive investigation in this field.

Healthy nutrition is one of the primary measures for managing diabetes. An HFS diet leads to the buildup of fat in the body, the onset of metabolic syndrome, and impaired glucose tolerance, which is a precursor to diabetes [36]. Diabetes is among the most prevalent endocrine disorders and is defined by an increase in blood glucose levels resulting from a disorder in glucose metabolism [37]. *Lactobacillus fermentum* strain RS-2 has demonstrated the ability to prevent the onset of diabetes under certain conditions. This probiotic reduces oxidative stress by minimizing inflammation and enhancing the performance of antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase in diabetic rats [38].

Oxidative stress and the damage it causes contribute to various pathological processes in numerous diseases, including diabetes. Given the beneficial effects of probiotics and their capacity to lower glucose levels, their therapeutic potential in the management of diabetes is not surprising [37]. Feeding an HFS diet resulted in a remarkable rise in fasting glucose levels in the present study. However, the administration of gassericin A effectively mitigated this increase. Mosallami et al. (2020) investigated the effects of *Lactobacillus plantarum* on various biochemical parameters in diabetic rats. The administration of *Lactobacillus plantarum* suspension to diabetic rats led to a notable decline in their fasting blood glucose levels [39]. In a study investigating the impact of intestinal microbiota modulation on metabolic syndrome in mice, *Lactobacillus reuteri* significantly reduced blood sugar levels [40]. Despite the observed similarities, these findings cannot be directly compared to the current results due to the differences between the studies.

The present study revealed that gassericin A significantly decreases serum levels of LDL, HDL, and cholesterol. Recent research suggests that probiotics can lower cholesterol levels and help prevent atherosclerosis [41]. The administration of *Lactobacillus gasseri* MG4524 to obese rats led to a decline in serum cholesterol levels [42]. These observations align with the findings of the current study, despite the differences between the two treatment protocols. In the previous study conducted by the present team on 3T3-L1 cells, gassericin A resulted in a decrease in the expression of the *SCD-1* gene during the differentiation of adipocyte precursor cells [22]. Consequently, the reduction in the lipid profile induced by gassericin A may be linked to its effects on cholesterol esterification pathways, lipoprotein transporters, and *SCD-1* gene expression. Reducing the expression of this gene may improve various metabolic disorders and is also associated with a reduction in obesity-related complications, such as an improved lipid profile [43]. *SCD-1* reduces the production of single-chain monounsaturated fatty acids, leading to a decrease in the synthesis of triglycerides and essential blood lipids. Therefore, the decrease in *SCD-1* expression caused by gassericin A may be the reason for the improvement in the lipid profile in mice [44].

In the present study, the HFS diet resulted in a decrease in BUN levels. However, this parameter was not influenced by gassericin A. Urea and creatinine are well-established biomarkers of kidney damage, and elevated serum levels of these substances are frequently associated with renal dysfunction [45]. Certain species of *Lactobacillus* have been demonstrated to lower serum BUN levels [46, 47]. Nevertheless, the current results obtained through parenteral injection of gassericin A cannot be directly compared to those achieved with oral probiotics.

Abnormalities in peripheral metabolism caused by obesity and weight gain can result in hepatic dysfunction. Liver enzymes are crucial biomarkers for detecting liver injury [48]. Liver diseases are linked to significant rises in ALP, AST, and ALT enzyme levels in the bloodstream. A diet rich in fat and the buildup of fat in liver cells increase the sensitivity and vulnerability of this tissue to other damaging factors. Consequently, this may cause fatty liver disease to advance to hepatic fibrosis and cirrhosis [49]. Given that the liver is essential for the metabolism of fatty acids, glucose, and energy [50], abnormal peripheral metabolism caused by obesity and weight gain can lead to the buildup of lipids in this tissue. In this study, the HFS diet resulted in substantial rises in ASP, ALP, and ALT enzymes. In contrast, treatment with gassericin A significantly inhibited the elevation of these enzymes. In a 2021 study examining the impacts of *Lactobacillus acidophilus* on hepatic tissue, serum levels of ALT and AST enzymes decreased in rats treated with this probiotic, resulting in improved liver function [51]. Probiotics, including *Lactobacillus* species, have shown similar beneficial effects in nonalcoholic fatty liver disease [52]. Additionally, SCD-1 activity has been reported to increase in obese mice with hepatic steatosis. The level of hepatic saturated fatty acids exhibits an inverse relationship with liver *SCD-1*. This indicates that a reduction in *SCD-1* leads to the buildup of saturated fatty acids in the cytosol of cells. Consequently, the transfer of cytosolic fatty acids to the mitochondria is impeded, resulting in increased fatty acid oxidation [53]. Therefore, the decrease in liver enzyme levels and the improvement in liver steatosis may be linked to the suppression of *SCD-1* gene expression by gassericin A [22]. Gassericin A appears to offer protective effects against hepatic damage induced by an HFS diet.

Comparisons with other studies: what does the current work add to the existing knowledge

Despite numerous studies examining the effects of gut microbiota on metabolism and fat accumulation, only limited number of reports have addressed the impact of bacteriocins synthetized by the same bacteria on obesity and its associated complications. To the authors' knowledge, this team is the first to provide experimental evidence regarding the potential therapeutic effects of gassericin A on obesity. In a previous study, this research team demonstrated the beneficial effects of gassericin A in 3T3-L1 cells [22]. By employing an in vivo model of obesity, the present study confirms the former in vitro results.

Study strengths and limitations

The most important advantage of this study is its novelty, as there are very few similar studies, even concerning other bacteriocins. Consequently, this study is preliminary, and one of its main limitations is the lack of prior research in this field. It remains unclear whether gassericin A is absorbed from the intestine under physiological conditions. Additionally, the pharmacokinetics and pharmacodynamics of the peptide are not well understood. Although three doses of the peptide were examined, the optimal effective dose has yet to be determined. Western blotting should also be conducted to assess the effects of gassericin A on protein levels. If the absorption of this bacteriocin from the intestine is feasible, potential physiological effects can be anticipated. However, the correlation between the doses administered in this study and the physiological concentration of this peptide is a subject that requires further investigation. Finally, gassericin A is not commercially available and must be produced in the laboratory in small quantities, which is both time-consuming and costly.

Conclusion

The objective of this research was to examine the impacts of i.p. administration of gassericin A on obesity and related parameters. Despite the increased weight gain and abdominal fat content, the bacteriocin improved the FBS, serum lipid profile, liver enzymes, and markers of redox status. Therefore, it appears to alleviate complications related to obesity. Consistently, the bacteriocin reduced the size of adipocytes, indicating hyperplastic obesity, which is consistent with the aforementioned beneficial effects. Finally, gassericin A downregulated the *Zfp423* gene, suggesting a potential stimulatory effect on the browning of WAT. However, these findings are preliminary, and further studies are necessary before any clinical recommendations can be made. Future research must investigate the reproducibility of these findings in humans, especially with long-term administration. Finally, the extensive physiological effects of gut bacterial products, as indicated by these results, warrant further investigation from both physiological and pharmacological perspectives.

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
CBC	Complete blood count
C/EBP	CCAAT/enhancer binding protein
CAT	Catalase
FABP	Fatty acid binding protein
FBS	Fasting blood sugar
HDL	High density lipoprotein
HFS	High-fat, high-sugar
ip	Intra peritoneal
LDL	Low density lipoprotein
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MDA	Malondialdehyde
RBC	Red blood cell
RDW	Red cell distribution width
rpm	Revolutions per minute
RT-qPCR	Reverse transcriptase quantitative polymerase chain reaction
SCD-1	Stearoyl-CoA desaturase- 1
SEM	Standard error of the mean
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TBP	TATA-binding protein
WAT	White adipose tissue
WBC	White blood cell
Zfp423	Zinc finger protein 423

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Author contributions

VM, HB, and FT conducted the research and wrote the initial draft. HRK designed the research, performed the statistical analysis and provided the revised manuscript. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All procedures followed the protocols established by the Animal Ethics Committee at Ferdowsi University of Mashhad (IR.UM.REC.1400.250).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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