Research Article

Protective Effects of Almond Oil on Streptozotocin-Induced Diabetic Rats via Regulating Nrf2/HO-1 Pathway and Gut Microbiota

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Received 27 January 2021; Revised 23 April 2021; Accepted 11 May 2021; Published 7 June 2021

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Almond oil has been used as a medicine substitution for its numerous health benefits. This study aimed to evaluate the effect of almond oil on streptozotocin- (STZ-) induced diabetic rats for 4 weeks. The results showed that the administration of almond oil could significantly increase body weight, attenuate abnormally elevated blood glucose, promote insulin secretion, and improve glucose tolerance. Almond oil treatment also suppressed oxidative stress, reduced inflammation reaction, improved liver and kidney function, upregulated the expressions of Nrf2, HO-1, and NQO1, while downregulating the expression of Keap1. Furthermore, almond oil reversed the gut microbiota change by STZ and regulated the gut microbiota associated with glucose metabolism. At the phylum level, the relative abundance of Firmicutes was decreased, while Bacteroidetes was increased by almond oil treatment. More importantly, the ratio of Firmicutes/Bacteroidetes was significantly increased. At the genus level, administration of almond oil increased the abundances of Lactobacillus, Bacteroides, and Lachnospiraceae_NK4A136_group, while decreased the abundances of Ruminococcaceae_UGC-014, Clostridium_sensu_stricto_1, and Fusicatenibacter. These results provided evidence for the regulating effect of almond oil on diabetic rats via the Nrf2/HO-1 pathway and gut microbiota.

1. Introduction

Diabetes is a chronic metabolic disease characterized by hyperglycemia that has been recognized as an increasing global health problem [1]. Genetics and environments are important factors in the pathogenesis of diabetes. In 2017, approximately 451 million adults lived with diabetes, and the number was estimated to rise to 693 million by 2045 throughout the world [2]. Chronic hyperglycemia causes oxidative stress and inflammation in the occurrence of diabetes. Besides, it is frequently associated with dysfunction and severe clinical complications, such as diabetic retinopathy [3], nephropathy [4], neuropathy [5], and peripheral artery disease, owing to the glucotoxicity effects [6, 7], which accelerate the mortality of diabetes. Therefore, controlling a high level of blood glucose and alleviating the generation of reactive oxygen species (ROS) are essential for controlling diabetes or ameliorating diabetic complications.

The nuclear factor erythroid 2-related factor 2 (Nrf2) is an intracellular transcription factor that could maintain cellular redox homeostasis and upregulate cytoprotective proteins, such as heme oxygenase 1 (HO-1) and NAD(P)H quinone oxidoreductase 1 (NQO1). Under normal conditions, the binding of Kelch-like ECH-associated protein 1 (Keap1) to Nrf2 could suppress the Keap1/Nrf2 pathway, which leads to the ubiquitination and degradation of cytoplasmic Nrf2 [8]. The deficiency of expression of Nrf2 could enhance the susceptibility to many oxidative stress-related pathologies [9]. Some agents could protect diabetes-related organs or cells from injuries by upregulating the expression of Nrf2 [10]. Nrf2 regulates the expressions of a series of antioxidant enzymes and is considered an effective target on oxidative stress-
related diseases [11]. Heme oxygenase-1 (HO-1) is a downstream cytokine of Nrf2, which exerts a vital function in maintaining the redox balance of cells [12]. The Nrf2/HO-1 pathway plays a critical role in regulating oxidative stress.

More importantly, the gut microbiota plays a critical role in host homeostasis, and the dysbiosis of the gut microbiota is strongly associated with the onset and progress of metabolic diseases, such as diabetes [13, 14]. A previous study found that commensal bacteria, such as Lactobacillus plantarum, can promote the transfer of insulin vesicles and insulin secretion by ligand binding with NOD1 in islet β cells [15]. Furthermore, a decreased proportion of anaerobes, especially Bacteroides, can lead to hyperglycemia. The aberrant microbiota in STZ-induced diabetic rats and the upsurge of the Gram-negative bacteria could lead to inflammation and the development of a pathological microenvironment [16]. Lipopolysaccharide (LPS) as a gut-derived endotoxin might be crucially involved in chronic inflammation. Release of LPS by pathogenic bacteria in the intestine can enter the bloodstream and trigger low-level inflammation and oxidative damage in rats with high intestinal permeability. Reducing the levels of LPS-producing bacteria in diabetic rats might contribute to reducing systemic inflammation and promoting normal liver insulin signaling [17].

Therefore, regulation of the gutmicroflora may be beneficial for reversing the inflammatory and maintaining the balance of glucose metabolism. Diet is an important factor altering the composition and metabolism of the gut microbiota, especially the main dietary macronutrients whose amount, type, and balance greatly impact the large intestinal microbiota [18].

Some natural products isolated from plant sources had therapeutic and antioxidant properties and could be used as a form of supplement or replacement with antioxidant activity and fewer side effects, which should be searched for attenuating the risk of diabetes. Almond (Prunus amygdalus) is the most popular nuts and widely used as ingredients in some processed foods such as bakery and confectionery products. A previous study showed that the consumption of almonds could reduce the risk of chronic diseases and prevent colon cancer [19]. Besides, almond contains proteins, certain minerals, vitamins E and D, and about 50% oil. Almond oil has high amounts of monounsaturated fatty acids (MUFA), especially a rich concentration of oleic fatty acids [20], which could alleviate cellular apoptosis, oxidative stress, mitochondrial dysfunction, and inflammation in hepatocytes [21]. Almond oil has long been used as a medicine substitution [22] and has significant potential in biomedical and pharmaceutical studies. Previous studies illustrated that the consumption of almond oil could inhibit lipid peroxidation processes in CCl4-induced hepatic damage rats and boost immunity [23, 24] due to its anti-inflammatory and antioxidant activity and free radical scavenging capacity. However, the gut microbiota changes in STZ-induced diabetes by the dietary supplement of almond oil were hardly reported.

Due to the excellent antioxidant activity of almond oil, we speculated the beneficial effect of almond oil on diabetes. Therefore, our present study aimed to investigate the effects of almond oil on diabetic rats induced by STZ via the regulation of oxidative stress and inflammatory response, Nrf2/HO-1 signaling pathway, and gut microbiota changes. The study would provide a beneficial suggestion for choosing a diet supplement.

2. Materials and Methods

2.1. Chemicals. The almond oil was kindly provided by Laiyuan Almond Processing Co., Ltd., in Hebei, China, in November 2019. The sunflower seed oil was purchased from a local grocery store in Baoding, Hebei, China, in November 2019, as the positive control. They were both stored at 4°C before use.

Streptozotocin (STZ) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

The biochemical kits of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), alanine transaminase (ALT), aspartate transaminase (AST), creatinine (CRE), and blood urea nitrogen (BUN) were obtained from Jiancheng Bioengineering Research Institute (Nanjing, China). The enzyme-linked immunosorbent assay (ELISA) kits of inflammation indicators of tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β), insulin (INS), and advanced glycation end products (AGEs) were purchased from Boster Biological Technology Co., Ltd. (Wuhan, China).

2.2. Composition Analysis of Almond Oil. The composition analysis of almond oil was detailed in Table S1.

2.3. Animals and Experimental Treatment. Male Sprague Dawley rats (200–220 g) were purchased from Beijing HFK Bioscience Co., Ltd. The rats were kept under standardized conditions at a constant temperature of 23–25°C, and 50 ± 5% humidity with a 12 h light/dark cycle, and they had free access to standard diet and water for one week adjustment period. All experimental procedures and animal welfare were performed under a protocol that was approved by the Research Ethics Committee of the College of Food Science and Technology in Hebei Agricultural University.

After the adjustment period, the rats were weighed and randomly divided into six groups (n = 10): the control group (CON), the model control group (MC), the low dose of almond oil group (LAO), medium dose of almond oil group (MAO), high dose of almond oil group (HAO), and sunflower seed oil group (SSO). All rats were fasted for 12 h, and rats in MC, LAO, MAO, HAO, and SSO were intraperitoneally injected STZ with 60 mg/kg, which was dissolved in 0.1 M citrate buffer (pH 4.2–4.5), while those in CON were intraperitoneally injected with the same dose of citrate buffer solution. Fasting blood glucose (FBG) was determined three days after the injection. The rats with levels of FBG >16.7 mmol/L were considered as diabetic rats for experiments.

Rats in LAO, MAO, and HAO were intragastrically administrated with almond oil at doses of 2, 4, and 8 g/kg b.w. and SSO administrated with sunflower seed oil at a dose of 4 g/kg b.w. for 4 continuous weeks, respectively, while rats in CON and MC were given at an equal volume of sterile water.

Body weights and FBG of all the rats were monitored weekly throughout the whole experimental period. At the
The last week of the experiment, oral glucose tolerance test (OGTT) was determined. At the end of the experiment, the rats were deprived of food overnight and 10% chloral hydrate (6 ml/kg) was intraperitoneally injected for anesthesia. The blood samples were collected from the abdominal aorta of rats and separated by centrifugation at 3000 rpm for 15 min to obtain serum and then stored at −80°C for further assay. The liver samples were rinsed with saline and dried on filter paper, then quickly snap-frozen, and preserved at −80°C for Western blotting analysis.

The colon contents sample of each rat was collected at a sterile condition and preserved at −80°C for further microbiota analysis.

2.4. Measurement of Fasting Glucose and OGTT. FBG levels were measured from the tail vein of rats after overnight (12h) fasting using a glucose meter. The OGTT was estimated at the last week after administration of AO or SSO. The blood glucose levels were estimated at 0, 30, 60, and 120 minutes after administration with glucose solution (2 g/kg BW). The area under the curve (AUC) during the OGTT was calculated by the trapezoidal rule.

2.5. Biochemical Parameters Analysis. The enzyme activities of SOD, CAT, MDA, ALT, AST, CRE, and BUN in the serum were determined by biochemical kits; INS, TNF-α, IL-1β, and AGEs in the serum were quantified by ELISA kits strictly according to the instructions of instruments and reagents.

2.6. Western Blot Analysis. The expressions of Keap1, Nrf2, HO-1, and NQO1 protein in liver were determined by Western blotting. Western blotting analysis of liver homogenates (10% SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane. The membranes were blocked with 5% BSA for 1 h and incubated in primary antibodies against Keap1, Nrf2 (1:1000), HO-1, NQO1 (1:10000), and β-actin (1:4000) at 4°C overnight. The membranes were washed 5 times in TBST and incubated with HRP-conjugated anti-rabbit or anti-mouse secondary antibodies at room temperature for 1 h. After washing 7 times with TBST, the protein bands were analyzed using a Bio-Rad Chemidoc XRS Imaging System (Bio-Rad, CA, USA). The expression of Nrf2, NQO1, and HO-1 were determined by image analysis system (Bio-Rad, CA, USA).

2.7. Fecal DNA Extraction and Sequencing. According to the manufacturer’s instructions, total DNA was extracted from colon contents using the TIANamp Stool DNA kit (Tiangen, Beijing, China). The bacterial 16S rRNA gene V3-V4 region was amplified with primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAAT-3′). The PCR amplification program was performed as follows: initial denaturation at 95°C for 3 min, followed by 27 cycles PCR (95°C for 30 s, 55°C for 30 s, and 72°C for 30 s), finally 72°C for 10 min. The PCR product was extracted from 2% agarose gel, purified using AxyPrep DNA (Axygen Biosciences, Union City, CA, USA), quantified using QuantiFluor Meter (Promega, USA), and sequenced using Illumina MiSeq platform (Illumina, San Diego, CA, USA) and finally sequenced using Illumina MiSeq platform (Illumina, San Diego, CA, USA) at Majorbio Bio-Pharm Technology company (Shanghai, China).

Sequences with ≥97% similarity were assigned to the same operational taxonomic units (OTUs). The sequences were trimmed with UCHIME and annotated using RDP Classifier (http://rdp.cme.msu.edu/) based on the SILVA database (SSU123) at the threshold of 70%.

2.8. Statistical Analysis. Data were expressed as mean ± SD. Statistical analysis was performed using SPSS 17.0. Statistical significance between groups was analyzed using one-way analysis of variance (ANOVA) followed by Duncan’s new multiple range test. P values <0.05 or P values <0.01 were considered statistically significant or statistically highly significant.

3. Results

3.1. Effect of Almond Oil on Body Weight in Diabetic Rats. The changes in body weight were presented in Figure 1. Body weight of the rats decreased significantly one week after the injection of STZ (P < 0.01). The body weight among diabetic groups had no significant differences during the first two weeks. However, treatment with almond oil could obviously reverse the reduction during 4-week administration. Compared with the MC group, the body weight among MAO group was significantly increased at week 2 (P < 0.05); the three doses of almond oil and the SSO group significantly increased body weight (P < 0.01) at weeks 3 and 4. The results showed that different doses of almond oil could reduce the weight loss of diabetic rats.

3.2. Effect of Almond Oil on FBG, OGTT, and Insulin Levels in Diabetic Rats. Elevated FBG is one of the main characteristics of diabetes. The FBG was estimated every week. As shown in Figure 2(a), FBG of the MC group was significantly higher than the CON group after STZ induction (P < 0.01), which proved that the diabetes model had been established. Compared with the MC group, there was no significant difference in FBG in rats during the first two weeks’ intervention. However, FBG of the LAO and HAO groups exhibited a significant descent tendency (P < 0.05) compared with the MC group at week 3, and FBG was decreased by 20.9% in the LAO group at the last week. The results suggested that AO could suppress the increase in FBG.

OGTT aims to measure the ability to tolerate glucose in rats. Results of OGTT among all groups are shown in Figure 2(b). The maximum blood glucose level occurred 30 minutes after the oral administration of glucose, and the MC group had a significantly lower level of glucose tolerance compared with the CON group (P < 0.01). The LAO group
Figure 1: Effect of almond oil on body weight in STZ-induced diabetic rats. CON, control group; MC, model control group; LAO, low dose of almond oil-treated group; MAO, middle dose of almond oil-treated group; HAO, high dose of almond oil-treated group; SSO, sunflower seed oil-treated group. Data were expressed as means ± SD. The MC group compared with the CON group, #P < 0.05, ##P < 0.01; the LAO, MAO, HAO, and SSO groups compared with the MC group, *P < 0.05, **P < 0.01.

Figure 2: Continued.
significantly suppressed the blood glucose elevation after gavaging glucose 30 minutes. At 60 minutes, significantly lower glucose levels were observed among almond oil groups and SSO group (P < 0.05). The glucose levels of LAO and MAO were significantly reduced at 120 minutes compared with the MC group (P < 0.05).

The AUC levels were presented in Figure 2(c). The MC group had a significantly higher level of glucose AUC compared with the CON group (P < 0.01). Significantly lower AUC levels were observed in LAO and MAO groups (P < 0.01, P < 0.05) compared with the MC group.

Based on the results of blood glucose, changes in insulin levels in serum were also detected. As shown in Figure 2(d), the insulin level was significantly reduced in STZ-induced rats compared with the CON group (P < 0.01); however, the amount of insulin released was significantly increased in the LAO and MAO groups (P < 0.05, P < 0.05). These results showed that the AO treatment could effectively maintain glucose homeostasis in STZ-induced rats.

3.3. Effect of Almond Oil on the Oxidative and Inflammation-Related Factors in Diabetic Rats. The results of oxidative stress in the serum are shown in Figure 3. The STZ-induced rats had significantly lower SOD and CAT antioxidant enzymes activity and higher MDA level (P < 0.01), while almond oil administration could reverse the changes. Treatments with almond oil for four weeks and the activity levels of SOD in LAO, SSO (P < 0.01, P < 0.01), and MAO, HAO groups (P < 0.05, P < 0.05) were increased compared with MC group; levels of CAT in LAO and MAO groups were increased (P < 0.05, P < 0.05). MDA level in LAO and MAO groups (P < 0.01, P < 0.01) and HAO and SSO groups (P < 0.05, P < 0.05) were decreased compared with MC group. The results suggested that almond oil had the ability to suppress oxidative stress via increasing the activity of antioxidant enzymes and inhibiting lipid peroxidation in diabetic rats.

As shown in Figure 4, the levels of inflammatory factors were significantly elevated in the MC group compared with the CON group. TNF-α and IL-1β levels were significantly reduced in LAO, MAO, and HAO groups (P < 0.01,
3.4. Effect of Almond Oil on Biochemical Parameters in Diabetic Rats. As shown in Figure 5, the levels of ALT, AST, CRE, BUN, and AGEs were significantly increased ($P < 0.01$) after STZ-induced diabetes in rats compared with the CON group. The levels of serum ALT and AST are important indicators of liver damage. The three doses of AO and SSO treatment groups significantly decreased ALT levels compared with the MC group ($P < 0.01$, $P < 0.05$, $P < 0.05$), and AST level was significantly reduced in LAO and MAO groups ($P < 0.05$, $P < 0.05$). BUN, CRE, and AGEs are also indicators of kidney damage. As shown in Figure 6, the levels of BUN, CRE, and AGEs in STZ-induced diabetic rats were significantly upregulated compared with the CON group ($P < 0.01$). Conversely, lower levels of BUN and CRE were observed in LAO and MAO groups ($P < 0.05$, $P < 0.05$) and LAO group ($P < 0.05$) compared to those of the MC group, respectively. AGEs level was significantly enhanced in all AO and SSO treatment groups ($P < 0.01$, $P < 0.05$, $P < 0.05$, $P < 0.05$). The results demonstrated that almond oil treatment could alleviate liver and kidney dysfunctions.

3.5. Effects of Almond Oil on Nrf2/HO-1 Pathway in Diabetic Rats. To determine whether almond oil affects the antioxidant ability in diabetes through the Nrf2/HO-1 pathway, the expressions of Keap1, Nrf2, HO-1, and NQO1 were measured by Western blot. As shown in Figure 7, compared with the CON group, the expressions of Nrf2 and HO-1 ($P < 0.01$, $P < 0.01$) were significantly downregulated while the expression of Keap1 ($P < 0.01$) was upregulated in STZ-induced rats, which showed that the Nrf2 antioxidant signaling

Figure 4: Effects of almond oil on serum TNF-α and IL-1β in STZ-induced rats. TNF-α, tumor necrosis factor-α; IL-1β, interleukin 1β. Data were expressed as means ± SD. The MC group was compared with the CON group, $\# P < 0.05$, $\#\# P < 0.01$; the LAO, MAO, HAO, and SSO groups were compared with the MC group, $^* P < 0.05$, $^*^* P < 0.01$.

Figure 5: Effects of almond oil on serum ALT and AST in STZ-induced rats. ALT, alanine transaminase; AST, aspartate transaminase. Data were expressed as means ± SD. The MC group compared with the CON group, $\# P < 0.05$, $\#\# P < 0.01$; the LAO, MAO, HAO, and SSO groups compared with the MC group, $^* P < 0.05$, $^*^* P < 0.01$.

Figure 6: Effects of almond oil on serum CRE, BUN, and AGEs in STZ-induced rats. CRE, creatinine; BUN, blood urea nitrogen; AGEs, advanced glycation end products. Data were expressed as means ± SD. The MC group compared with the CON group, $\# P < 0.05$, $\#\# P < 0.01$; the LAO, MAO, HAO, and SSO groups compared with the MC group, $^* P < 0.05$, $^*^* P < 0.01$. 

$P < 0.05$, $P < 0.05$ and LAO, MAO, and SSO groups ($P < 0.01$, $P < 0.05$, $P < 0.05$) compared with MC group, respectively.
pathway was impaired. Conversely, treatment with almond oil could reverse the changes compared with the MC group. Nrf2, HO-1, and NQO1 expressions were significantly upregulated in LAO, MAO, and HAO groups \( (P < 0.05, \# P < 0.01) \), LAO, MAO, HAO, and SSO groups \( (P < 0.01, \# P < 0.05) \), and LAO and MAO groups \( (P < 0.05, \# P < 0.05) \). Keap1 expression was significantly downregulated in LAO, MAO, HAO, and SSO groups \( (P < 0.01, \# P < 0.01) \). However, no significant changes in Nrf2, HO-1, or NQO1 expressions were observed between the SSO and MC groups. The results demonstrated that almond oil treatment could protect against oxidative-mediated injury by activating the Nrf2/HO-1 signaling pathway, which was correlated to its antioxidant effects.

3.6. Almond Oil Modified the Gut Microbiota Structure.

The imbalance of interactions between host and gut microbiota could cause many important metabolic disorders, such as diabetes. In order to assess the effects of AO on the modulation of gut microbiota composition in diabetic rats, colon digesta samples were analyzed by 16S rDNA
The effects of AO treatments on the changes of gut microbiota are shown in Figures 8–10.

The different microbial composition with overlaps was observed in the Venn diagram (Figure 8). Specifically, the CON group had 542 OTUs, MC group had 673 OTUs, SSO group had 584 OTUs, and the AO groups (LAO, MAO, and HAO) had 689, 648, and 518 OTUs, respectively. 331 OTUs were shared by these groups.

As shown in 1, compared with the CON group, Chao 1, Ace, and Shannon indices ($P < 0.01$, $P < 0.01$) were significantly increased, while Simpson index ($P < 0.01$) was decreased in STZ-induced rats.

In the beta diversity analysis, the principal coordinate analysis (PCoA) was used to reflect the compositional differences among groups. As shown in Figure 10, there was a clear separation of gut microbiota composition between the CON and MC groups, and the microbial composition in the almond oil treatment groups was more close to that in the CON group.

We analyzed the phylum and genus level characteristics further to explore the gut microbiota structure differences among groups. The relative abundance in the phylum level was shown in Figure 10(a). In the CON group, the dominant phyla were Firmicutes (60.74%) and Bacteroidetes (26.79%). Compared with the CON group, the abundance of Firmicutes increased to 72.57%, while the Bacteroidetes significantly decreased to 16.85% in the MC group ($P < 0.05$). However, the consumption of almond oil modified the gut microbiota. Specifically, the middle dose of almond oil decreased the abundance of Firmicutes to 62.87% and significantly increased Bacteroidetes to 21.89% ($P < 0.05$).

At the genus level, the microbiota of the MC group changed greatly compared to that of the normal rats (Figure 10(b)). The abundances of Ruminococaceae_UCG-014, Clostridium_sensu_stricto_1, and Fusicatenibacter were increased, while Lactobacillus, Bacteroides, and Lachnospiraceae_NK4A136_group were decreased in the MC group compared with the CON group. Different doses of AO treatment can partially restore the microbiota change. The high dose of AO decreased Ruminococaceae_UCG-014 ($P < 0.05$), the low dose of AO decreased Clostridium_sensu_stricto_1 and Fusicatenibacter ($P < 0.05$, $P < 0.05$), the high dose of AO increased Lactobacillus and Lachnospiraceae_NK4A136_group ($P < 0.05$, $P < 0.05$), and the middle dose of AO increased Bacteroides ($P < 0.05$).

3.7. Spearman’s Correlation Analysis. Spearman’s correlation analysis was used to further explore the relationship between gut microbiota and metabolic parameters (Figure 11). We analyzed the correlation between bacteria genus, FBG, insulin (INS), body weight (BW), SOD, CAT, MDA, TNF-α, IL-1β, Keap1, Nrf2, HO-1, and NQO1. The abundances of Clostridium_sensu_stricto_1 and Fusicatenibacter were positively correlated with FBG, MDA, TNF-α, IL-1β, and Keap1, while being negatively correlated with INS, BW, SOD, CAT, Nrf2, and HO-1. The abundance of Ruminococaceae_UCG-014 was negatively correlated with BW, SOD, Nrf2, and HO-1. The abundance of Lachnospiraceae_NK4A136_group was positively correlated with SOD and Nrf2, while being negatively correlated with BW, SOD, CAT, Nrf2, and HO-1. The abundance of glucose metabolism, oxidative stress, and inflammation.

4. Discussion

As a chronic metabolic disease, diabetes has proven to be a major threat to health risks. In recent years, the diet has been considered a crucial tool in the prevention and protection from disease and its complications, and dietary lipids have been regarded as one of the most necessary macronutrients for human health. Searching for dietary lipids with antidiabetic activity and few side effects has been essential to control the development of diabetes. In the present study, we exhibited that oral administration of almond oil for 4 weeks alleviated the symptoms of diabetes induced by STZ.

STZ is commonly used to induce experimental diabetes in rodents. STZ enters pancreatic β-cells through the GlUT2 glucose transporter [25] and induces the production of ROS, which rapidly and irreversibly destroys pancreatic β cells [26, 27]. As the characteristics of diabetes, polyphagia, polydipsia, hyperglycemia, and reduced BW were observed after STZ induction, which were demonstrated in previous research [28].

The main bioactive compound of almond oil is the oleic (n-9) fatty acids. Previous studies found that n-9 fatty acids could reduce the local inflammatory response [29]. Oleic
Figure 9: Principal coordinate analysis (PCoA) between different groups.

Figure 10: Modulation effect of almond oil on the gut microbiota in rats. (a) The effect of almond oil on microbial community structures at the phylum level. (b) The effect of almond oil on relative abundances at the genus level.
acids also have a cytoprotective effect for β-cells, and dietary MUFAs have the potential to act as regulators of glucose homeostasis in either the inner-digestive or postabsorptive period [30]. Previous research demonstrated that oleic acid could suppress the deleterious effects of palmitic acid on the homeostasis in either the inner-digestive or postabsorptive period [30]. Previous research demonstrated that oleic acid could suppress the deleterious effects of palmitic acid on the homeostasis in either the inner-digestive or postabsorptive period [30]. Previous research demonstrated that oleic acid could suppress the deleterious effects of palmitic acid on the homeostasis in either the inner-digestive or postabsorptive period [30]. Previous research demonstrated that oleic acid could suppress the deleterious effects of palmitic acid on the homeostasis in either the inner-digestive or postabsorptive period [30]. Previous research demonstrated that oleic acid could suppress the deleterious effects of palmitic acid on the homeostasis in either the inner-digestive or postabsorptive period [30]. Previous research demonstrated that oleic acid could suppress the deleterious effects of palmitic acid on the homeostasis in either the inner-digestive or postabsorptive period [30]. Previous research demonstrated that oleic acid could suppress the deleterious effects of palmitic acid on the homeostasis in either the inner-digestive or postabsorptive period [30].

Chronic hyperglycemia leads to the excessive generation of ROS, such as hydrogen peroxide (H₂O₂), superoxide (O₂⁻), hydroxyl (OH), or peroxyl (OOH) radicals through glucose autoxidation [32]. Oxidative stress occurs as the excessive accumulation of ROS exceeding local antioxidant capacity, which may damage cellular organelles and causes an increase in lipid peroxidation [33]. More importantly, the oxidative status level, which plays a crucial role in the antioxidant system, was evaluated by measuring MDA, SOD, and CAT activities in our study. SOD is an essential endogenous antioxidant enzyme used to eliminate superoxide radicals and protect cells against the toxic byproducts of aerobics metabolism [34]. SOD could convert superoxide radicals to hydrogen peroxide (H₂O₂), which is decomposed into oxygen and water by catalase, and maintain the oxidation balance. Polyunsaturated fatty acids in the cell membrane were attacked by free radicals and formed MDA. MDA was formed by the attacking free radicals of polyunsaturated fatty acids in the cell membrane. As an oxidation end product and a biomarker of lipid peroxidation, MDA could indirectly reflect the extent of oxidative damage in the body [35]. The present study found that SOD and CAT activity were significantly decreased, and MDA content was increased in the MC group rats treated by STZ. In contrast, the results were reversed after almond oil treatment. Consistent results have proved that almond oil had an antioxidant modulating effect on rats with hepatic injury [24].

Oxidative stress has a crucial effect on the inflammatory reaction, which increases the excretion of inflammation-related cytokines [36]. TNF-α and IL-β are considered the central proinflammatory mediators for the inflammatory reaction, which are the major parts of the inflammatory process leading to β-cell destruction and closely associated with the progression of diabetes [37]. TNF-α is a cytokotoxin mainly produced by monocytes and macrophages and could induce the activation and accumulation of IL-1β, stimulating the immune disorder [38]. IL-1β affects the activation of nuclear factor kappa B (NF-kB), promoting the expression of several β-cells genes [39]. The overproduction of IL-1β leads to the pathogenesis of inflammation and autoimmune diseases and correlates with the recruitment of immune cells and β-cell damage in islets [40]. In our study, almond oil treatment significantly reduced the levels of TNF-α and IL-1β induced by STZ, suggesting that it had the potential effects of suppressing the expression of proinflammatory cytokines. However, no significant change of TNF-α was observed between the sunflower seed oil treatment group and the STZ-induced group. The sunflower seed oil contained about 67% linoleic acid (LA). LA is a direct precursor of the proinflammatory arachidonic acid (AA). AA can participate in cell signaling and trigger inflammation, which is linked to oxygen-free radical rise and results in more severe reactions in some tissues [41]. In contrast, the inflammatory markers of people with high MUFAs dietary habits have been considerably reduced, and oleic acid can reverse the inhibitory effect of TNF-α on insulin production, revealing that oleic acid has the potential therapeutilic effect in an inflammatory context [42]. In summary, the anti-inflammatory effect of almond oil was better than sunflower seed oil according to the results of inflammatory factors due to its high content of oleic acid probably.

The chronic accumulation of circulating glucose might cause damage to the whole body blood vessels, which affected the functionality of some vital organs and increased the risk of various life-threatening health complications, such as chronic liver and kidney disease. ALT and AST activities may reflect the damage of hepatocytes and the markers of hepatocyte integrity. The contents of ALT and AST are low in the serum of normal rats but released into the blood due to the damage of hepatocytes or cell membrane permeability. In the present study, ALT and AST levels were increased in diabetes groups induced by STZ. Simultaneously, the administration of almond oil significantly reduced those levels, which was consistent with the result that treatment with almond oil suppressed the acute hepatic damage [23].

### Table 1: Effects of almond oil on Alpha diversity.

<table>
<thead>
<tr>
<th>Group</th>
<th>Chao 1</th>
<th>Community richness</th>
<th>Ace</th>
<th>Community diversity</th>
<th>Simpson</th>
<th>Shannon</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>416.97 ± 44.77</td>
<td></td>
<td>419.59 ± 46.40</td>
<td>0.3175 ± 0.1282</td>
<td>2.6319 ± 0.3733</td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>541.84 ± 21.36#</td>
<td></td>
<td>533.70 ± 24.61#</td>
<td>0.0713 ± 0.0253#</td>
<td>3.7212 ± 0.2476#</td>
<td></td>
</tr>
<tr>
<td>LAO</td>
<td>475.53 ± 69.38</td>
<td></td>
<td>480.19 ± 77.50</td>
<td>0.0802 ± 0.0335</td>
<td>3.6362 ± 0.4064</td>
<td></td>
</tr>
<tr>
<td>MAO</td>
<td>428.57 ± 51.25**</td>
<td></td>
<td>424.33 ± 45.05**</td>
<td>0.1277 ± 0.0902</td>
<td>3.3834 ± 0.5983</td>
<td></td>
</tr>
<tr>
<td>HAO</td>
<td>395.07 ± 33.71**</td>
<td></td>
<td>394.07 ± 39.80**</td>
<td>0.1804 ± 0.1007</td>
<td>2.8502 ± 0.6498</td>
<td></td>
</tr>
<tr>
<td>SSO</td>
<td>480.08 ± 58.78</td>
<td></td>
<td>481.47 ± 55.62</td>
<td>0.1325 ± 0.1546</td>
<td>3.5032 ± 0.9386</td>
<td></td>
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</table>

*Figure 11: Significant correlation between the abundance of bacteria genus and metabolic parameters was selected using Spearman correlation analysis (*P < 0.05, **P < 0.01).*
Hyperglycemia is characterized by the high content of glycated proteins and binding of monosaccharides to amino groups of proteins, leading to the formation of AGEs and changes in the structure and functions of proteins. Besides, glycated proteins can activate membrane receptors by glycation end products and induce intracellular oxidative stress and a proinflammatory status [43], consequently affecting kidney functions. CRE and BUN are also the biochemical markers of kidney functions. In the present study, the increased CRE, BUN, and AGEs levels in diabetic rats were reversed by almond oil treatment, which were consistent with the result that administration with almond oil changed the levels of CRE and BUN in rats exposed to a sublethal concentration of lead [44].

Nrf2/HO-1 is the main signaling pathway for antioxidative stress [12]. Under normal conditions, Nrf2 is bound by Keap1 in the cytoplasm and then degraded by the ubiquitination system. Under oxidative stress or inflammatory conditions, Nrf2 is released from Keap1 and translocated into the nucleus, where it combines with antioxidant response elements [10], upregulates the transcription of HO-1 and NQO1, and then participates in the antioxidation processes. HO-1 is a redox-sensitive inducible stress protein activated by Nrf2. HO-1 degrades heme into biliverdin, free iron, and carbon monoxide, which exerts a protective role by reducing oxidative injury and attenuating inflammatory response [45]. Our results found that the antioxidant mechanism of almond oil appeared to activate Nrf2 signaling pathway and upregulate the downstream of Nrf2. The consistent result also found that taurine could reduce the severity of oxidative stress by activating the antioxidative defense signaling pathway in diabetic rats [46]. The results also showed that the antioxidant ability of almond oil was better than sunflower seed oil.

Previous studies suggested that the blood glucose levels were also regulated in the intestine, which was a major organ involved in the glucose homeostasis, carbohydrate digestion, and secretion of glucose into the circulation, and the energy substrates can be used by intestinal colonized microbiota [4]. It indicated that gut microbiota has a protective effect on metabolism regulation and has a positive effect on glucose metabolism [47].

The Chao 1 and Ace indices were used to reflect the richness of the communities; the Simpson and Shannon indices were used to reflect the diversity of the communities. The richness and diversity of gut microbiota in STZ-induced rats were increased compared with the CON group, which were consistent with the previous report. The increase of gut microbiota diversity and richness could be associated with the development of disease [48].

Firmicutes and Bacteroidetes were the most abundant phyla in normal rats. The ratio of Firmicutes/Bacteroidetes was widely regarded as an indicator of gut microbiota dysbiosis in many metabolic diseases [49]. A decrease in Bacteroidetes has been associated with pathologies such as inflammatory bowel disease and diabetes [50]. A reduced relative abundance of Bacteroidetes and an increase in Firmicutes were observed in STZ-induced rats, resulting in an increased ratio of Firmicutes/Bacteroidetes, which were consistent with the previous study [4]. However, the ratio was downregulated upon treatment with almond oil.

Lactobacillus can promote glucose metabolism through directly consuming glucose in the host intestines [51]. Lactobacillus can inhibit the growth of acid-sensitive pathogenic bacteria by lowering the pH of the intestinal environment. It also competes with pathogens to protect the gut barrier and immune response of the host and improves intestinal oxidative stress in rats with liver damage [52]. In our study, the abundance of Lactobacillus was decreased in the MC group, while the change was reversed by oral administration of almond oil, which was in line with the previous report that dietary administration with pistachio nuts increased the abundance of Lactobacillus in diabetic rats [53]. Some bacteria in Ruminococcaceae are proinflammatory bacteria and associated with inflammatory bowel diseases [54]. The previous research analyzed the correlation between gut microbiota and blood glucose, and results showed that Ruminococcaceae_UCG-014 had a positive correlation with the occurrence and development of diabetes [55]. In this study, the abundance of Ruminococcaceae_UCG-014 was increased in the MC group, while the level of Ruminococcaceae_UCG-014 was decreased by almond oil. Clostridium_sensu_stricto_1 is considered as an LPS-producing pathogenic bacteria associated with intestinal inflammation and infection [56, 57]. High level of LPS accumulates in the intestine and participates in the inflammation of diabetics. Reducing the level of LPS-producing bacteria could reduce harmful substances from the intestines into the blood and inhibit the incidence of oxidative stress and inflammation [58]. Clostridium_sensu_stricto_1 and Fusicatenibacter were positively correlated with FBG levels. Our study also found that the abundance of Clostridium_sensu_stricto_1 and Fusicatenibacter was decreased by almond oil treatment compared to the MC group, which was in accordance with the report that propolis decreased the abundance of Clostridium_sensu_stricto_1 and Fusicatenibacter in diabetic rats [59]. Lachnospiraceae_NK4A136_group and Bacteroides are both short-chain fatty acids- (SCFA-) producing intestinal bacteria, which enhance the gut barrier function [60]. Lachnospiraceae_NK4A136_group inhabits the human gut and produces butyric acid that is considered as an intestinal barrier protector, which is associated with maintaining intestinal health [61]. Bacteroides is beneficial bacteria that plays an important role in glucose metabolism in the host [62]. The present study found that almond oil increased the abundances of Lachnospiraceae_NK4A136_group and Bacteroides compared with the MC group induced by STZ, which were consistent with the report that Lycium barbarum alleviated gut microbiota dysbiosis [63]. The results indicated that almond oil could reduce the relative abundance of proinflammatory bacteria and enhance the butyrate-producing bacteria, which alleviated abnormal glucose metabolism. Our results showed that the intake of low dose almond oil presented the best protective effect; however, the high dose almond oil presented a better impact on the antidysbiosis of gut microbiota. According to the correlation analysis, the abundance of Clostridium_sensu_stricto_1 and
**5. Conclusions**

In conclusion, almond oil alleviated the development of STZ-induced diabetes in rats.

The administration of almond oil significantly decreased the levels of FBG, promoted insulin secretion, increased BW and the ability to glucose tolerance, suppressed oxidative stress and inflammatory reaction, and improved liver and kidney function. The beneficial effect of almond oil appeared to be associated with activating Nrf2/HO-1 pathway against oxidative stress and regulating gut microbiota on glucose metabolism.

These results showed that the effect of almond oil on ameliorating diabetes is associated with its antioxidative and anti-inflammatory properties in STZ-induced diabetic rats, which indicated that replacing the common oil in the diet with almond oil may have a potential beneficial effect on diabetes, suggesting that almond oil could be used as an adjunct treatment strategy for diabetes.

**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Ethical Approval**

All the experimental procedures and animal welfare were in accordance with the related ethical regulation guidelines and approved by the Research Ethics Committee of the College of Food Science and Technology in Hebei Agricultural University (2017-006).

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Acknowledgments**

This work was supported by the Hebei Province Modern Agricultural Industrial Technology System Sheep Industry Innovation Team Special (No. HBCT2018140203); the Food Processing Discipline Group of Hebei Agricultural University (No. 2020-04); and the Research and Demonstration of the Key Technology of Using Nanocellulose Stabilized Pickering Emulsion Substitute Fat In Low-Fat Meat Products (No. 20327116D).

**Supplementary Materials**

The fatty acid composition analysis of almond oil was shown in Table S1 in the Supplementary Material. (Supplementary Materials)

**References**


