Micronized Dietary Okara Fiber: Characterization, Antioxidant, Antihyperglycemic, Antihyperlipidemic, and Pancreato-Protective Effects in High Fat Diet/Streptozotocin-Induced Diabetes Mellitus

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ABSTRACT: Diabetes mellitus (DM) is a lifelong devastating and debilitating disease with serious chronic complications. Okara is a byproduct generated from soymilk or tofu production and it has been reported to have antioxidant and lipid-lowering effects. However, the antidiabetic effects and pancreatic β-cells’ secretory functions of micronized okara fiber (MOF) have not been reported. Therefore, this study explored the antidiabetic effects and modulatory potentials of MOF on pancreatic β-cells’ secretory functions in a high fat/high sugar/streptozotocin rat model of diabetes mellitus. Fiber-rich okara was prepared by removing fat and proteins from freshly obtained okara, followed by micronization. Fiber-rich okara was prepared, micronized, and characterized for hydrophobicity, thermal stability, structure−function relationship, and antioxidant potentials. We then established a rat model of DM and MOF and two doses (100 and 400 mg kg$^{-1}$) were administered to see its anti-DM effect. Four weeks of MOF supplementation significantly reduced blood glucose, increased serum insulin level, improved hepatorenal functions, glucose tolerance, and regenerated pancreatic β-cells in the treated DM rats. Furthermore, MOF significantly improved the pancreatic antioxidant defense system by significantly elevating glutathione peroxidase, catalase, and superoxide dismutase activities while depleting the malonaldehyde level in the pancreas of the treated diabetic rats. Our results indicated that MOF ameliorated DM by impeding hyperglycemia, hyperlipidemia, and oxidative stress and enhancing the secretory functions of the beta cells, suggesting that MOF might be used as a protective nutrient in DM.

INTRODUCTION

Diabetes mellitus (DM) has grown to become one of the major public health problems in the last 2 decades due to the sporadic surge in the number of people living with the disease as well as the debilitating and life-threatening complications that are associated with the disease. DM is a lifelong incurable disease that arises due to the inability of the body to produce or make use of the insulin it produces leading to persistent hyperglycemia.1,2 The International Diabetes Federation (IDF) reported that 537 million people were diabetic in 2021, as compared to 463 million people reported in 2019 indicating a 9.8% increase within 2 years.3 Prolonged and uncontrolled hyperglycemia causes devastating effects to various organs in the body leading to chronic disorders such as diabetic nephropathy, neuropathy, foot ulcers, and cardiovascular diseases.4−6 Although there are quite a number of approved conventional drugs in use for controlling diabetes, however, their effectiveness in slowing down hyperglycemia-induced diabetic complications as well as the associated side effects of these drugs have raised some concerns.7 As such, there is an urgent need to explore potential novel and safe treatment strategies for treating DM.

In recent years, researchers have devoted great importance to discover alternative therapeutic avenues for preventing and treating diabetes and diabetic complications. Rigorous attention has been directed to find antidiabetic entities from nature. Several studies have suggested the huge therapeutic potential embedded in agricultural-food waste, which is produced in enormous quantities annually across the world. Particularly, agricultural-food wastes are inexpensive sources of bioactive constituents including polyphenols, fibers, phenolic compounds, polysaccharides, and carbohydrates, which have been proven to show various pharmacological effects such as antioxidant, anti-inflammatory, antidiabetic, and antimicrobial properties.8,9 Okara is a byproduct generated from soymilk or tofu production and it is rich in proteins that are similar to soy protein isolate and insoluble polysaccharides such as cellulose,
hemicellulose, and lignin.\textsuperscript{10} In fact, dried okara consists of 5.5% fiber, 21% proteins, 13–14% fats and oils, 1.5% ash, and about 10% moisture.\textsuperscript{11} The use of okara is rapidly translating from serving as animal feeds to the formulation of food products with health values. Okara rich dietary fiber has been confirmed to possess antioxidant, hypolipidemic, and hypoglycemic effects.\textsuperscript{12,13} To utilize the functional compounds in okara, pre-treatment is required.\textsuperscript{14} Processing and structural modification of okara for improved biological activities, nutritional benefits, and overall valorization have included high-pressure homogenization and ultrasonication,\textsuperscript{15} thermal and hydrothermal processing,\textsuperscript{16} solid-state fermentation,\textsuperscript{17} high energy wet media milling,\textsuperscript{18} enzymatic processing,\textsuperscript{19} and steam explosion.\textsuperscript{20} Previous studies have reported the antihyperlipidemic effects of micronized okara fiber (MOF) in mice.\textsuperscript{21} However, no information is available on the micronization of okara for hypoglycemic or antidiabetic potentials, yet the knowledge of particle size plays an important role in determining okara properties and applications, as found in tofu’s fortification with okara particles within the micrometer range.\textsuperscript{19} Therefore, the aim of this study was to explore the antidiabetic effects and modulatory potentials of MOF on pancreatic β-cells’ secretory functions in high fat/high sugar and streptozotocin rat model of DM. Fiber-rich okara was prepared by removing fat and proteins from freshly obtained okara, followed by micronization. The MOF particles were investigated for antidiabetic effects.

### MATERIALS AND METHODS

**Okara Specimen.** Okara derived from soymilk production was obtained from a local factory and was immediately dried to avoid spoilage. The fat and protein contents were determined to be 10–12% and 12% (nitrogen content (N) × 6.25) by the Kjeldahl method,\textsuperscript{22} respectively.

**Preparation and Micronization of Okara Fiber.** Dried okara was powdered with a high-speed rotary grinder and defatted using petroleum ether at 45 °C in a Soxhlet extractor for 8 h. The method of Ashaolu and Zhao\textsuperscript{22} was followed for protein removal. The defatted okara was dispersed in deionized water using a high-speed rotary grinder and was then centrifuged at 4000 × g for 15 min. The supernatant was removed, while the residue was dried and defined as okara fiber (OF). Determination of insoluble protein content in the OF yielded insignificant amounts (less than 1%) using the Kjeldahl method. OF was micronized with ILaser light scattering Mastersizer 3000 (Malvern Instruments Ltd., Worcestershire, UK) using ultrapure water as the disperse medium and a circulation pump operating at 3000 rpm. The micronized OF was defined as MOF.

**Fourier Transform Infrared Spectroscopy.** Fourier transform infrared spectroscopy (FT-IR) analysis was carried out with a Spectrum 100 FT-IR detector (PerkinElmer, USA) using a potassium bromide (KBr) disc consisting of 3.3% finely ground samples made in an agate mortar. Thirty-two spectra scans were taken per sample from 400 to 4000 cm\textsuperscript{-1} at a resolution of 4 cm\textsuperscript{-1}.

**Hydrophobicity Measurement.** The hydrophobicity of MOF and OF (control) was measured using a contact angle apparatus goniometer (JC200D, China). Each sample (0.2 g) was pressed into a standard tablet under 30 MPa by a pressure machine. Water droplet (10 µL) was released onto the tablet surface, and the contact angle was computed using the drop image recorded after 5s according to the Laplace–Young equation. The contact angle was defined as the angle between the baseline and the tangent to the drop boundary.\textsuperscript{23}

**Thermogravimetric Analysis.** Thermograms of MOF and OF were obtained using a thermogravimetric analyzer (TA, USA). The analysis was carried out under the protection of nitrogen using 8 mg of each sample. The temperatures of the samples were increased from 35 to 600 °C at a heating rate of 10 °C/min. The weight loss of each sample was measured as a function of temperature.

**Antioxidant Capacity of MOF.** The antioxidant potentials of MOF were investigated by ferric reduction antioxidant power (FRAP), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays following previously reported protocols.\textsuperscript{24–27} Briefly, for the FRAP assay, stock solutions were prepared using 300 mM acetate buffer (3.1 g C\textsubscript{6}H\textsubscript{5}NaO\textsubscript{2}·3H\textsubscript{2}O and 16 mL C\textsubscript{6}H\textsubscript{12}O\textsubscript{6}), pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl\textsubscript{3}·6H\textsubscript{2}O solution. A mixture consisting of 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of FeCl\textsubscript{3}·6H\textsubscript{2}O solution was freshly prepared as the working solution, which was warmed at 37 °C before use. MOF and OF solutions were allowed to react with the FRAP solution (1:20 v/v) for 30 min in the dark. The absorbance of the colored product [ferrous tripyridyltriazine complex] was taken at 593 nm with a spectrophotometer.

For the ABTS assay, the stock solutions of 7.4 mM ABTS** and 2.6 mM potassium persulfate were freshly prepared. The two stock solutions were mixed in equal quantities and allowed to react for 12 h at room temperature in the dark. This working solution was diluted by mixing 1 mL of ABTS** solution with 60 mL of methanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm. MOF and OF solutions were allowed to react with the ABTS** solution (1:20 v/v), respectively, for 2 h in the dark and the absorbance was measured at 734 nm.

For the DPPH assay, the stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol and then stored at 20 °C until further use. To make the working solution, a 10 mL stock solution was mixed with 45 mL of methanol to obtain an absorbance of 1.1 ± 0.02 units at 515 nm. MOF and OF solutions were allowed to react with the DPPH solution (1:20 v/v), respectively for 24 h in the dark, followed by absorbance readings at 515 nm. All assays were performed in triplicates, and the results were expressed in μmoles of Trolox equivalent (TE)/g of solids using calibration curves of Trolox.

**Animals.** Healthy 7 weeks old male Wistar rats weighing 170 ± 20 g were used for the study. The rats were housed in clean stainless-steel cages in an animal house facility under controlled conditions (temperature: 22 ± 2 °C, light/dark cycle: 12:12 h and relative humidity: 55 ± 10%). The rats were acclimatized for 1 week prior to the experiment and were allowed unrestricted access to a standard rodent diet and water. The handling, care, and animal use were in accordance with the specification of the National Institute of Health Guide to the Care and Use of Laboratory Animals, while approval was obtained from the Institutional Ethics Committee of Anhui Medical College (approval number: Anhuiyxygdkx-2020-09018).

**Induction of Insulin Resistance and Type 2 Diabetes in Experimental Animals.** The rats were divided into two groups of diet regimens. Six rats in the first group were fed with...
a normal rodent diet and normal water, while 24 rats in the second group were fed with high-fat diet (HFD), in addition to 30% fructose solution as their drinking water for 4 weeks. Upon completion of the dietary manipulation, the rats fed with HFD were fasted overnight and a single intraperitoneal injection of freshly prepared streptozotocin (STZ; 40 mg/kg) in 0.1 M cold citrate buffer (pH 4.5) was administered to the rats. The rats in the normal diet group were also administered with the same volume of cold citrate buffer. After 72 h, rats with fasting blood glucose (FBG) level ≥ 13.9 mmol/L were considered diabetic and included in further experiments.

**Experimental Protocol.** Diabetic rats were randomly divided into four groups (n = 6) as follows: diabetic control group (group 2); diabetic rats administered with 100 mg/kg body weight of MOF (group 3); diabetic rats administered with 400 mg/kg body weight of MOF (group 4); and diabetic rats administered with 250 mg/kg body weight of metformin (group 5). The non-diabetic rats fed with normal diet were designated as normal control (group 1). MOF was dissolved in 5% tween 80 and administered to the rats by oral gavage for 28 days. The choice of the dose of MOF was based on our preliminary investigation as well as previous reports. The body weight of the rats was measured on day 0 before the commencement of treatment and on day 28 after the last treatment. The food and water consumption was measured daily, while the initial and final FBG levels were determined before and after the final treatment using a glucometer (Roche Diagnostics, Germany).

**Fasting Blood Glucose and Intraperitoneal Glucose Tolerance Test.** Before the commencement of treatment, the rats were fasted overnight and FBG levels were determined from the blood obtained from the tail vein using an Accu-Chek guide glucometer. Likewise, the FBG levels of rats were determined after 28 days of administration. After the last day of treatment, the IPGTT was performed in 12 h fasted rats by

| Table 1. Particle Size, Contact Angle, and Antioxidant Activity of MOF<sup>4d</sup> |
|---------------------------------|-----------------|-----------------|-----------------|
| particle size (μm) | hydrophobicity (θ) | ABTS (μmol TE/g solid) | FRAP (μmol TE/g solid) | DPPH (μmol TE/g solid) |
| OF | 580 | 105.0° | 42.3 ± 1.1 | 8.2 ± 0.4 | 2.1 ± 0.2 |
| MOF | 88 | 95.0° | 52.2 ± 1.21<sup>*</sup> | 7.2 ± 0.44 | 2.4 ± 0.24 |

Antioxidant activity data are presented as means (n = 3) ± standard deviation. Values with significant different at p < 0.05 within the same column are represented with *. TE = Trolox equivalents. Antioxidant capacity were determined by the DPPH assay (DPPH), the ABTS* assay (ABTS) and ferric reducing antioxidant power (FRAP). MOF = micronized okara fiber.
administering 2 g/kg of glucose solution intraperitoneally, and their blood glucose levels were measured using blood collected from the tail vein at 0, 30, 60, 90, and 120 min.

Sacrifice and Biochemical Estimations. After the treatment, the rats were anesthetized and blood samples were withdrawn by cardiac puncture. The blood samples were centrifuged and the sera collected were used for various biochemical analyses. The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glycated haemoglobin (HbA1c), serum creatinine (SCr), albumin, blood urea nitrogen (BUN), and insulin were determined using an automatic biochemical analyzer (Dirui CS 600B auto chemistry analyzer, Japan) and assay kits obtained from Nanjing Jiancheng Bioengineering Institute (China). Homeostatic model assessment index (HOMA-IR) was calculated using the formula stated below.

Histopathological Studies. After animal sacrifice, the pancreas tissues were quickly excised, washed with normal saline to remove any residual blood, and a small portion of the tissues were fixed in 10% neutral buffered formalin solution. The fixed samples were processed by dehydration in graded series of alcohol, embedded in paraffin and sectioned into 5 μm thickness. The tissue sections were further stained with hematoxylin and eosin and examined under a light microscope (Olympus DP73, Japan).

Assessment of Oxidative Stress Biomarkers in the Pancreatic Tissue Homogenate. Another portion of the excised pancreatic tissues was homogenized in phosphate buffer and centrifuged at 3000 rmp for 15 min at 4 °C. The supernatant obtained was used for the evaluation of pancreatic catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and lipid peroxidation product malonaldehyde (MDA) content using assay kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturer’s instructions.

Data Analysis. Data were expressed as mean ± SD (n = 6) and statistical differences at p < 0.05 among the groups were analyzed by one-way ANOVA, followed by Bonferroni’s multiple comparison tests using GraphPad Prism 5.0 software (GraphPad Software, San Diego, California, USA).

RESULTS

Physicochemical Properties of MOF. The average diameter of the MOF particle size is presented in Table 1. MOF is 88 μm in size, much smaller than its native OF (580 μm). The water contact angle or hydrophobicity measurement literally provides useful information on the wetting behavior of a solid particle (in this case, MOF) by a liquid (in this case, water). Water droplet contact angles for hydrophilic and highly hydrophilic particles are below 90 and 50°, respectively, while the angles for hydrophobic and highly hydrophobic particles are larger than 90 and 130°, respectively. As observed in Table 1, MOF improved its hydrophilicity (95.0°) and solubility when compared to its native form, OF (105.0°). MOF was also thermally stable as shown in Figure 1B. Thermal decomposition of MOF (Figure 1B) started from 68.29 to 326.96 °C. The first endothermic peak indicated the removal of water from MOF, while the second/third endothermic peaks might imply that the particles contained some matter, which were undergoing further decomposition of the polysaccharides into flammable gases such as carbon monoxide and carbon dioxide. General observations showed no major differences between MOF and OF stability (both had high decomposing temperatures) and therefore implied strong molecularity and high stability.
FT-IR analysis (Figure 1C) indicated that the spectra of MOF and OF were absorbed at 1015, 1650, 2925, and 3424 cm⁻¹ regions, respectively. In comparison with the control (OF) and based on consistency, increasing minor shifts in peaks of MOF could be related to the impact of downsizing/milling, which also affected the covalent interactions of hydrogen bonds.

Antioxidant Properties of MOF. Moreover, different and high-molecular-weight profiles observed in OF and MOF could have contributed to their antioxidant activity, as represented in Table 1. They both showed high ABTS, FRAP, and DPPH scavenging capacities (42.3 ± 1.1, 52.2 ± 1.21; 8.2 ± 0.4, 7.2 ± 0.44; and 2.1 ± 0.2, 2.4 ± 0.24 μmol TE/g solid, respectively), with MOF demonstrating much better potential in the ABTS" and DPPH scavenging activities (52.2 ± 1.21 and 2.4 ± 0.24 μmol TE/g solid, respectively). It was also observed that both had higher ABTS" radical scavenging values than FRAP. Usually, the reducing power is denoted by the reaction of electron donors or antioxidant compounds with free radicals.

Effects of MOF on Body Weight, Food, and Water Consumption in Diabetic Rats. As shown in Figure 2A, the initial body weight of the normal control, non-treated diabetic rats and MOF-treated diabetic rats showed no significant differences. In contrast, there was a drastic reduction in the final body weight of the untreated diabetic rats compared to the normal control. Interestingly, the diabetic rats administered with either 100 or 400 mg/kg of MOF showed significant body weight gain when compared to non-treated diabetic rats. Likewise, the food and water consumption of the non-treated diabetic rats were substantially increased compared to the normal control (Figure 2B,C). Nonetheless, the food and water consumption of diabetic rats treated with either 100 or 400 mg/kg MOF were significantly decreased compared to the non-treated diabetic rats (Figure 2B,C).

Effects of MOF on the FBG and IPGTT in Diabetic Rats. Figure 2D showed the effect of MOF on the FBG level of rats after 28 days of treatment. The initial and final FBG levels of the non-treated diabetic rats were significantly increased compared to the normal control group. In the MOF-treated groups, the initial FBG levels were not significantly different from the non-treated diabetic group. Whereas after 28 days of treatment, the final FBG levels of diabetic rats treated with either 100 or 400 mg/kg MOF were markedly suppressed compared to the non-treated diabetic control group (Figure 2D). Moreover, the results from the IPGTT indicated that diabetic rats showed significantly increased blood glucose levels at 0, 0.5, 1, 1.5, and 2 h after the administration of glucose when compared to the normal control group. Contrastingly, the administration of MOF markedly reduced blood glucose levels after glucose loading compared with rats in the non-treated diabetic group (Figure 2E). Effects of MOF on insulin and HOMA-IR in diabetic rats. The diabetic rats showed a significant decrease in serum insulin levels (Figure 2F), as well as a marked increase in HOMA-IR (Figure 2G) compared to that of normal control rats. Furthermore, HOMA-IR was significantly elevated from 4 to 13.7% in the non-treated diabetic group (Figure 2G). Conversely, the diabetic rats treated with 100 and 400 mg/kg of MOF showed significantly increased serum insulin levels, while HOMA-IR values were remarkably reduced compared to the untreated diabetic rats (Figure 2F,G). In addition, MOF at 100 and 400 mg/kg markedly decreased HbA1c in comparison to the DC group (Figure 2H).

Effects of MOF on Serum Biomarkers of Hepatorenal Function in Diabetic Rats. As shown in Figure 3, significant elevation in SCr, BUN, ALT, and AST was observed in the non-treated diabetic rats compared to normal control, whereas MOF (100 and 400 mg/kg) induced significant reduction in these parameters compared to non-treated diabetic control (Figure 3A–D).
Effects of MOF on serum lipid profile in diabetic rats.

The effect of MOF on serum lipid profiles is presented in Figure 3E–H. Diabetic rats displayed significantly increased TC, TG, and LDL-C levels compared to normal control rats. In addition, HDL-C was also observed to be significantly reduced in the non-treated diabetic rats. However, oral administration of MOF to diabetic rats prominently reduced the level of TC, TG, and LDL-C while concomitantly increasing the HDL-C level in a dose-dependent manner compared to the untreated diabetic rats (Figure 3E–H).

Effects of MOF on the Histology of the Pancreas in Diabetic Rats. The photomicrographs showing the effects of MOF on the histology of the pancreas are depicted in Figure 4. The histopathological sections of the normal control pancreas revealed normal and healthy architecture of the pancreas with large and distinct islets of Langerhans (Figure 4A), whereas the non-treated diabetic rats showed a significant reduction in the mass of the islets of Langerhans (Figure 4B). These changes were obviously attenuated to a varying extent by MOF (100 and 400 mg/kg) treatment with an increase in the size of the islets of Langerhans (Figure 4C–E).

Effects of MOF on Pancreatic Oxidative Stress Markers in Diabetic Rats. As depicted in Figure 5A–D, the diabetic control rats showed significant declines in pancreatic antioxidant enzyme activities including SOD, CAT, and GPx, with an associated increase in the MDA level compared to the normal control. Interestingly, the oral administration of MOF (100 and 400 mg/kg) to diabetic rats significantly restored the activities of pancreatic SOD, CAT, and GPx, while pancreatic MDA content was markedly reduced compared to non-treated diabetic rats (Figure 5A–D).

**DISCUSSION**

Diabetes is a chronic metabolic disorder having hyperglycemia and hyperlipidemia as the major hallmark which arises due to abnormalities in insulin metabolism. Regarding the epidemiology of the disease, low- and middle-income countries are greatly lagging behind in terms of management of the disease.
as well as the fatality ratio due to lack or insufficient basic and necessary healthcare and socio-economic requirements needed for the management of the disease. With the increase in the yearly incidence of the disease as well as the International Diabetes Federation projection of 645 million diabetic patients by 2045, the discovery of new, cost-effective, and safer antidiabetic therapies has become a necessity. The present study demonstrated that the administration of MOF could effectively attenuate hyperglycemia, dyslipidemia, hepatorenal dysfunction, and oxidative stress in HFD/STZ-induced diabetic rats.

First, an overview of the characterized MOF is hereby documented. The particle size changed when okara fiber was micronized. The reduction in the particle size as a result of the mechanical process could be ascribed to the breakage of well-ordered crystalline regions and amorphous domains of OF cellulose content. In other studies where a laser particle size analyzer was used to obtain nano-sized particulates from starch and chitosan, 245 and 308 nm were obtained, respectively. This was not without many difficulties as the grinding beads mechanical friction, machine type, and the studied product affected the particle size that could be attained. Indeed, it took Ullah et al. 6 h to reduce the particle size of okara insoluble dietary fiber from 66.7 μm to 544.3 nm.

For the wettability study, the water contact angles positively correlated with the MOF particle size, as down-sizing OF made the particles to possess a slightly improved hydrophilic characteristic from 105.0° down to 95.0°. This value indicated an interference with the composition of MOF due to micronization, which then exposed the hydrophilic groups. Furthermore, the results indicated that there were no major differences between MOF and OF thermal stability, as both samples had high decomposing temperatures, and therefore implies strong molecularity and high stability. Thus, MOF intermolecular and intramolecular hydrogen and electrostatic bonds became broken as the temperature increased, leading to a drastic reduction in the hydrophobic interaction. This phenomenon interestingly relates to the particle size and spectra of MOF obtained, which then affects the overall antioxidative capacity of the sample.

FT-IR involves the use of infrared light to scan test samples, identify molecular chemical bonds, detect functional groups, and/or characterize covalent bonds. The samples (OF and MOF) exhibited similar spectral profiles but with varying intensities, indicating that OF milling resulted in different degrees of structural changes, yet with similar functional groups. Sharp and broad bands in the regions of 611, 896, and 1450 cm$^{-1}$ were also considered as peaks produced by conjugated oxy-hydroxyl groups of OF. The minor shifts of intensities in some MOF peaks could be attributed to the splitting of intermolecular hydrogen bonds of cellulose and hemicellulose by redistributing to new structural features of the considered amorphous and soluble polysaccharides. Moreover, when compared with the control (OF), minor shifts in MOF peaks could be related to the impact of downsizing, which might have exposed the hydrogen and carbon moieties to manifest reorientation and conformational changes that would also affect MOF thermal stability and hydrophilic and antioxidative properties. In all, the summary of the FT-IR analysis may be attributed to the cleavage of hydrogen bonds between cellulose and hemicellulose due to downsizing and strong disruptive forces generated in the process that could have led to the breakdown of some chemical bonds in the OF chains. Nevertheless, the micronization process had no significant effect on the chemical structure and functional groups of OF.

The analyzed MOF showed free-radical scavenging activities equivalent to that of Trolox (52.2 μmol for ABTS, 2.4 μmol for DPPH and 7.2 μmol for FRAP, Table 1). An overall improvement in the antioxidant capacity of MOF could be attributed to increased amounts of total flavonoid contents remaining in okara fiber after its micronization. Previous studies that utilize micronization of agro-based products in antioxidant capacity have indicated that micronization potentiates antioxidant capacity as well as flavonoid content. Radical scavenging activity is of immense importance to the healthcare sector. Biologically, chronic exposure to reactive oxygen radicals has been linked to various diseases including diabetes, multiple sclerosis, arthritis, and ulcerative colitis. Nitric oxide toxicity increases exponentially when it reacts with superoxide radicals to form the highly reactive peroxynitrite anion (ONOO$^-$).

Moreover, MOF had higher ABTS** radical scavenging values than FRAP, which is not unusual because reducing power is denoted by the reaction of electron donors or antioxidant compounds with free radicals. In addition, the OH$^-$ groups of MOF could have contributed to its reducing activity. The low values of DPPH free radical scavenging activity exhibited by MOF might be due to the inability of hydrophobic radicals to attack the MOF macromolecules in the solution.

STZ is a toxic DNA alkylating agent obtained from Streptomyces achromogenes and it initiates beta cells apoptosis by stimulating oxidative stress via excessive generation of reactive oxygen species (ROS), resulting in alteration in insulin biosynthesis. On the other hand, a high fat/high sugar diet has been expensively used to induce experimental models of insulin resistance, a condition that predisposes to type 2 DM. As such the combination of HFD and low dose STZ has been widely accepted and used to induce experimental type 2 diabetes because it can reproduce the pathogenesis of human type 2 diabetes in animal models. Insulin resistance caused by HFD makes the pancreatic β-cells easily susceptible to the diabetogenic effect of STZ at low doses.

In this study, rats that were intraperitoneally administered with STZ (35 mg/kg) after 4 weeks of high fat/high sugar diet
displayed a marked reduction in body weight, high food and water intakes as well as a drastic increase in blood glucose levels. Insufficient insulin levels experienced in DM hinder the transport of glucose from the blood into the cells as an energy source, leading to the burning of fats and muscle as an energy source for the body cells. This ultimately leads to body weight loss, increased hunger (polyphagia), and thirst (polydipsia) in diabetes. Interestingly, we observed that treatment with 100 and 400 mg/kg MOF for 28 days after DM induction displayed a significant glucose-lowering effect. MOF dose dependently reduced FBG levels, which was almost comparable to the FBG of the normal control animals. Additionally, a concomitant increase in the serum insulin levels were also observed in the MOF-treated groups compared to the diabetic control group. These results demonstrated the antihyperglycemic properties of MOF and its impact on the pancreas in relation to insulin secretion, which obviously justifies the marked reduction in blood glucose level after treatment and also in line with previous studies. Histopathological screening of pancreas tissues of the diabetic rats and treated diabetic rats supported these biochemical findings and revealed the pancreatic and insulin-secreting effects of MOF. Accumulating research reports have demonstrated that one of the proposed antidiabetic mechanisms of natural products is the alleviation and restoration of pancreatic function. Because the mechanism of HFD/STZ-induced diabetes has been linked to ROS and oxidative damage of the pancreatic beta-cell, it is envisaged that the antioxidative capabilities of MOF might have restored the integrity and capacity of the beta-cell to produce insulin which is needed for stabilizing blood glucose levels.

In this study, marked increase in the serum concentrations of AST and ALT was observed in the untreated diabetic rats, which is characteristic of gross hepatic damage, corroborating previous studies by Makinde et al. and Olatunji et al. Furthermore, hyperglycemia-induced kidney dysfunction was vivid in this study as indicated by significant increases in serum levels of creatinine and blood urea nitrogen of the diabetic rats, which agrees with previous investigations. The liver and kidney are two vital organs that are critically affected by diabetes. Previous studies have implicated insulin deficiency in hepatic injury due to the initiation of gluconeogenesis, leading to increased levels of serum markers of hepatic injury including AST, ALP, and ALT. In addition, the liver plays an undisputable role in insulin clearance, glucose regulation, and lipid and carbohydrate metabolism. These hepatic functions are however grossly impeded in diabetes. The administration of MOF alleviated and suppressed the increased level of markers of hepatorenal dysfunction, suggesting that MOF has protective effects against hyperglycemia-induced hepatorenal damage.

Several studies have reported abnormalities in lipid metabolism in diabetes, which is symbolized by increased levels of TGs, TC, LDL-C, and free fatty acids, as well as reduced level of HDL-C. These anomalies increase the predisposition to coronary heart diseases and aggravate several other complications of diabetes. Increased concentration of LDL-C can accumulate on the blood vessel walls, leading to the development of atherosclerotic plaque. On the other hand, several literature have highlighted the prominent role of the “good cholesterol” (HDL-C). It enhances the absorption and efflux of TG and TC to the liver for further breakdown and elimination from the body. Furthermore, insulin deficiency impedes the activation of lipoprotein lipase, an enzyme that facilitates the hydrolysis of TGs, as such the impairment in the activation of this enzyme increases the level of TG in the blood, leading to hypertriglyceridemia. In this study, the serum concentrations of TG, TC, and LDL-C were markedly increased in the non-treated diabetic group compared to the normal control, corroborating previous literature. On the contrary, treatment with MOF doses for 28 days resulted in significant decreases in serum TG, TC, and LDL-C. Our results are consistent with an earlier study that indicated that micronized okara ameliorated lipid dysfunction in BALB/c mice.

Several bodies of evidence have suggested that hyperglycemia-induced oxidative stress instigated by excessive generation of reactive oxygen species is a major contributor to the onset and progression of diabetic complications. Increased oxidative stress is one of the major prevailing factors in DM which ultimately leads to several other factors including inflammation and dyslipidemia. Sustained high glucose level leads to ROS overproduction via the enhancement of mitochondrial oxygen consumption and mitochondrial dysfunction. The increased generation of ROS ultimately reduces the capacity of endogenous antioxidant enzymes, resulting in oxidative stress, beta-cell dysfunctions, and insulin resistance. In particular, pancreatic tissues are highly susceptible to oxidative stress due to their inability to effectively mobilize innate antioxidant enzymes. Increasing evidence suggests that hyperglycemia-induced ROS accumulation impairs beta-cell function due to the inadequate levels of beta-cells antioxidant enzymes, notably, SOD, CAT, and Gpx. In addition, the exposure of beta-cells to oxidative stress impedes insulin secretion through various mechanisms including opening ATP-sensitive K+ channels and suppressing calcium influx. MDA, a byproduct of lipid peroxidation is frequently used as an indicator of oxidative stress, and it is remarkably elevated in various organs in diabetes. Antioxidant enzymes protect against the deleterious effects of oxidative stress and ROS by converting them into non-reactive oxygen molecules and water, whereas antioxidant enzymes including SOD, CAT, and GSH-Px antagonize the impact of ROS and oxidative stress in organs and tissues. SOD acts by reducing superoxide to hydrogen peroxide by spontaneous dismutation, while catalase and GPx catalyze the conversion of hydrogen peroxide to water. As such, oxidative stress parameters were assessed in the pancreas of untreated and treated diabetic rats in this study. Consistent with several other earlier reports, DM significantly increased pancreas MDA levels with a corresponding reduction in the activities of CAT, SOD, and GSH-Px in comparison to normal rats. The ROS accrued as a result of HFD-/STZ-induced hyperglycemia might have overwhelmed the antioxidant homeostasis in the pancreas leading to the results obtained. Our results agree with previous studies indicating the oxidative reduction in the activities of antioxidant defense enzymes in the pancreas of diabetic models, whereas the alterations observed in the antioxidant parameters were significantly and concentration-dependently abrogated by MOF in the treated diabetic rats. MOF reinforced the activities of CAT, SOD and GSH-Px, while lipid peroxidation was reduced as expressed by the abated MDA concentration in the pancreas tissues.
CONCLUSIONS

This study provided pieces of evidence on the hypoglycemic, antihyperlipidemic, and antioxidant activity of MOF, as evidenced by its ability to improve pancreatic β-cell function and stimulate insulin secretion, leading to a decrease in the blood glucose level. Furthermore, it was revealed that MOF ameliorated oxidative pancreatic damage by increasing the activities of SOD, CAT, and GPx while reducing the MDA level. Moreover, the changes in the serum concentration of hepatorenal function enzymes as well as lipid profiles were ameliorated in the MOF-treated diabetic rats. MOF at a dose of 400 mg/kg was the most potent. Overall, this study further strengthens the idea that food and agricultural wastes contain bioactive metabolites with antidiabetic and antioxidant properties and it also lays the groundwork for future research into unravelling the mechanism of action of the antidiabetic effects of MOF.

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