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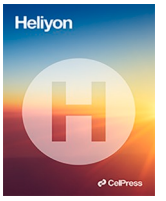

Research article

Comparison of the anti-diabetic effects of various grain and legume extracts in high-fat diet and streptozotocin-nicotinamide-induced diabetic rats

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ABSTRACT

The anti-diabetic properties of whole groats and dietary fibers from various grains and legumes are well known. Nevertheless, studies on the anti-diabetic effects of their extracts are limited, and it is difficult to compare their efficacy. This study investigated the anti-diabetic potential of ethanol extracts from oats (OE), sorghum (SE), foxtail millet (FE), proso millet (PE), adzuki bean (AE), and black soybean (BE) in a high-fat diet and streptozotocin-nicotinamide-induced diabetic rat models. The extracts, obtained using 99.9 % ethanol, were orally administered to diabetic rats for four weeks. Various parameters were evaluated, including fasting blood glucose levels, glucose tolerance, insulin sensitivity, serum insulin levels, and pancreas histological analysis. OE and SE effectively reduced fasting blood glucose levels and the area under the curve (AUC) in the oral glucose tolerance test. Only OE significantly decreased the AUC in the insulin tolerance test and increased insulin concentration and homeostatic model assessment of the β-cell function index, indicating improved insulin sensitivity and β-cell function. Histological and immunohistochemical analysis of the pancreas supported these findings, demonstrating that OE protected against pancreatic cell damage. In contrast, FE, PE, AE, and BE did not have a significant effect on diabetes-related parameters. These findings identify OE as the most promising natural intervention for diabetes management.

1. Introduction

Diabetes mellitus is a global health concern that poses significant challenges to individuals and healthcare systems worldwide [1]. The prevalence of diabetes has increased dramatically, rising from 180 million in 1980 to 422 million in 2014 [2]. In addition, the age mortality rate increased by 3 % from 2000 to 2019, with 48 % of deaths from diabetes occurring before the age of 70. Beyond mortality, diabetes is associated with severe health complications such as cardiovascular disease, kidney failure, vision loss, and foot ulcers [3,4]. Notably, type 2 diabetes (T2DM) overwhelmed type 1 diabetes in prevalence, with an incidence rate of 5.9 % in 2020, compared to 0.2 % for type 1 diabetes [5]. T2DM is characterized by hyperglycemia and abnormal glucose metabolism resulting from insulin resistance and pancreatic β-cell dysfunction [6]. Insulin resistance refers to reduced cell sensitivity to insulin, leading to increased insulin production and release in the pancreas. However, sustained high insulin demand can deplete β-cells, resulting in decreased insulin

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production and secretion.

Due to the increasing prevalence of T2DM, effective management strategies are urgently needed [7]. While oral anti-diabetic drugs, such as biguanides, sulphonylureas (metformin), and alpha-glucosidase inhibitors (acarbose and voglibose) are commonly used to manage T2DM, they can have adverse effects [8]. These medications may lead to weight gain and gastrointestinal side effects, including nausea, vomiting, and diarrhea [9]. Hence, there is growing interest in exploring natural resources with anti-diabetic effects as alternative approaches [10].

Grains and legumes, such as oats (*Avena sativa*), sorghum (*Sorghum bicolor*), foxtail millet (*Setaria italica*), proso millet (*Panicum millaceum*), adzuki bean (*Vigna angularis*), and black soybean (*Glycine max*), are recognized as valuable and nutrient-rich natural resources due to their bioactive compounds [11]. These crops have been reported to exhibit various health benefits, including antioxidant, anti-cancer, anti-obesity, and anti-diabetic effects [12,13]. Previous studies on their anti-diabetic effects have primarily focused on whole grains, highlighting the role of dietary fiber in reducing blood glucose levels by slowing down the emptying of the digestive tract [14].

Interestingly, these grains and legumes also contain abundant phenolic compounds with anti-diabetic effects [15]. For instance, ferulic acid, which is abundant in most grains, improves glucose homeostasis by regulating insulin signaling and genes involved in gluconeogenesis [16]. Additionally, catechin and vitexin in adzuki beans, as well as cyanidin-3-glucoside in black soybeans, are known to have anti-diabetic effects [17–19]. Organic solvent extraction is commonly used to obtain high concentrations of phenolic compounds from natural sources [20]. Therefore, extracts of these grains and legumes have potential as functional foods for the prevention and treatment of diabetes.

A few animal studies have investigated the anti-diabetic effects of organic solvent extracts from oats [21], sorghum [22–24], adzuki bean [25,26], and black soybean [27,28]. However, there is limited research comprehensively evaluating the effects of extracts on not only hyperglycemia but also insulin sensitivity and pancreas damage. Furthermore, comparing the anti-diabetic effects among different grains and legumes is challenging due to variations in extraction solvents, animal models, and measured outcomes across the previously reported studies.

Therefore, this study aimed to investigate the anti-diabetic effects of 99 % ethanol extracts derived from oats (OE), sorghum (SE), foxtail millet (FE), proso millet (PE), adzuki bean (AE), and black soybean (BE) in a high-fat diet and streptozotocin (STZ)-nicotinamide (NA)-induced diabetic rat model. In this study, we evaluated the potential of these extracts to control hyperglycemia and improve glucose tolerance and insulin resistance. OE, which exhibited the most potent anti-diabetic effects, was also assessed for its ability to mitigate pancreatic cell damage.

2. Materials and methods

2.1. Sample preparation

Oat (Daeyang), sorghum (Sodamchal), foxtail millet (Samdachal), proso millet (Geumsilchal), adzuki bean (Arari), and black soybean (Chungja #4), harvested in 2018, were provided by the National Institute of Crop Science, Rural Development Administration (Suwon, Korea). The grains and legumes were then ground and stirred in 99.9 % ethanol (Duksan Pure Chemicals Co., Ansan, Korea) at a ratio of 1 L per 100 g for 24 h at room temperature. The extracts were filtered and concentrated using a rotary vacuum evaporator (Eyela, Tokyo, Japan) at 50 °C. Each extract was stored at −80 °C for further experiments.

2.2. Quantification of phenolic compounds

The composition of phenolic compounds in each extract was determined through high-performance liquid chromatography [29]. Utilizing an ODS column (5 μm, 4.6 × 250 mm; Agilent Technologies, Santa Clara, CA, USA), a gradient elution was executed using solvent A (water with 0.1 % (v/v) acetic acid) and solvent B (acetonitrile with 0.1 % (v/v) acetic acid). The flow rate was 1 mL/min, and the injection volume was 20 μL. UV detection was set at 280 nm. Quantification of each phenolic compound involved referencing a standard curve constructed by injecting varying concentrations of standards into the HPLC system. Peak identification was facilitated by introducing standards to the sample, and peak areas were calculated by comparing them to the standard peaks.

2.3. Animals and diets

The animal experiments were approved by the Animal Ethics Committee of Woojung Bio (WJIACUC20190624-2-40). Five-week-old male Sprague-Dawley rats were obtained from Dooyeol Biotech (Seoul, Korea). All rats were housed in a controlled environment (22 ± 2 °C, 50 ± 15 % humidity, and 12:12 light/dark cycle). Rats in a normal group were fed either a normal diet (SAFE A40, SAFE Inc., Augy, France) and rats in other groups a high-fat diet (45 % kcal from fat, D12451, Research Diets, New Brunswick, NJ, USA) throughout the experiment. The composition of the normal diet and high-fat diet is as follows: normal diet - protein (25 % kcal), carbohydrate (62 % kcal), and fat (13 % kcal); high-fat diet - protein (20 % kcal), carbohydrate (35 % kcal), and fat (45 % kcal). Distilled water was purified using UV sterilization and provided in a polysulfone drinking bottle for free ingestion. After a week of acclimation, rats were intraperitoneally injected with 60 mg/kg body weight (bw) of STZ in 0.1 M acetate buffer and 120 mg/kg bw of NA with the exception of the normal group. After a week, rats with fasting blood glucose levels ≥200 mg/dL were considered to have successfully established a diabetic model.

The rats were divided into nine groups ($n = 8$); (1) NC (saline), a normal control group, (2) MC (saline), a diabetic model control

group, (3) PC (200 mg/kg bw of metformin), a positive control group, (4) OE (500 mg/kg bw of oat extract), (5) SE (500 mg/kg bw of sorghum extract), (6) FE (500 mg/kg bw of foxtail millet extract), (7) PE (500 mg/kg bw of proso millet extract), (8) AE (500 mg/kg bw of adzuki bean extract), and (9) BE (500 mg/kg bw of black soybean extract). All treatments were dissolved in 0.5 % (w/v) carboxymethyl cellulose in saline (Sigma-Aldrich, St. Louis, MO, USA), and orally administered to the rats daily for four weeks. The dosage of the extract (500 mg/kg body weight) was determined based on the absence of heaptoxocity in our previous study evaluating the antihypertensive activity of the legumes, which is equivalent to 4.836 g for a 60 kg person [30,31]. Body weight and feed intake were measured weekly throughout the experiment. Rats were fasted for 12 h and fasting blood glucose levels were measured in the fourth week.

2.4. Oral glucose tolerance test and insulin tolerance test

The oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) were performed during the fourth week. For the OGTT, rats were fasted for 16 h and orally administered 2 g/kg bw of glucose (Sigma-Aldrich). Blood glucose levels were measured from tail veins using a glucometer (Green Cross, Seoul, Korea) at 0, 30, 60, 90, 120, and 180 min after administration. Five days later, the ITT was conducted in a similar manner. After fasting for 16 h, the rats were intraperitoneally injected with insulin (2 U/kg bw), and blood glucose levels were measured at 0, 30, 60, 90, and 120 min. The area under the curve (AUC) for blood glucose levels was calculated using Prism 9 software (GraphPad Software, San Diego, CA, USA). In the period between the OGTT and ITT, the NC group was fed a normal diet, and the other groups were maintained on a high-fat diet. During this period, each group received oral administrations of saline, metformin, and the respective extracts.

2.5. Blood biochemical analysis

At the end of the experimental period, rats were fasted overnight and anesthetized with 2 % isoflurane (2 L/min). Blood was collected from the abdominal aorta vein and serum was isolated by centrifugation at 3000×*g* for 20 min at 4 °C. Serum insulin levels were measured using an ELISA kit (Elabscience, Bethesda, MD, USA), following the manufacturer's protocol. Homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-β) indexes were calculated from fasting blood glucose level and insulin levels. Both indexes were computed using the subsequent formulas: HOMA-IR = (fasting blood glucose level × insulin level)/405, and HOMA-β = (20 × insulin level)/(fasting blood glucose level - 63).

2.6. Histological and immunohistochemical analysis of the pancreas

Pancreatic tissues from rats in the NC, MC, PC, and OE groups were fixed in a 10 % formalin solution. The fixed tissue was embedded in a paraffin block and sectioned at a 4 μm thickness. For histological analysis, sections were stained with hematoxylin and eosin (H&E). For insulin immunohistochemical (IHC) staining of pancreatic β-cells, sections were incubated with an insulin antibody (Cell Signaling Technology, Danvers, MA, USA). The stained area and total area of the islets were measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Table 1
Profiles of phenolic compounds in oat, sorghum, foxtail millet, proso millet, adzuki bean, and black soybean extract (mean ± SD).

Classification	Compound (μg/g)	OE	SE	FE	PE	AE	BE
Phenolic acid	Ferulic acid	22.60 ± 2.28	28.35 ± 0.11	N.D.	N.D.	9.09 ± 0.51	N.D.
	Caffeic acid	9.86 ± 1.38	166.74 ± 4.00	3.27 ± 0.63	5.69 ± 3.10	1.70 ± 0.10	N.D.
	Vanillic acid	8.93 ± 1.35	N.D.	8.06 ± 0.27	N.D.	13.76 ± 0.29	16.36 ± 0.97
	2-Hydroxy cinnamic acid	8.50 ± 0.38	N.D.	N.D.	N.D.	5.97 ± 0.59	N.D.
	t-3-Hydroxy cinnamic acid	N.D.	N.D.	N.D.	0.69 ± 0.98	26.22 ± 0.47	21.21 ± 0.97
	Sinapic acid	8.21 ± 2.95	N.D.	8.89 ± 0.42	N.D.	7.93 ± 0.26	9.83 ± 1.17
	Syringic acid	5.60 ± 1.17	N.D.	N.D.	N.D.	5.39 ± 1.94	N.D.
	4-Hydroxy benzoic acid	5.25 ± 1.01	9.52 ± 1.53	N.D.	0.89 ± 1.25	95.48 ± 10.31	86.51 ± 2.63
	p-Coumaric acid	5.17 ± 0.59	32.75 ± 2.38	1.91 ± 0.90	4.94 ± 2.90	79.01 ± 3.78	16.12 ± 1.00
	t-Cinnamic acid	4.58 ± 0.48	N.D.	N.D.	3.77 ± 2.98	1.26 ± 0.16	N.D.
	Chlorogenic acid	N.D.	44.13 ± 1.08	N.D.	3.48 ± 3.08	4.09 ± 0.35	N.D.
	Total	78.68 ± 9.48	281.48 ± 0.99	22.13 ± 1.30	19.45 ± 11.78	249.90 ± 11.14	150.04 ± 3.17
Flavonoid	Naringin	8.13 ± 4.57	N.D.	N.D.	1.85 ± 1.24	11.25 ± 1.18	N.D.
	Rutin hydrate	N.D.	495.00 ± 7.09	9.10 ± 3.68	59.25 ± 44.70	32.58 ± 0.75	N.D.
	Naringenin	N.D.	159.01 ± 9.89	N.D.	N.D.	N.D.	N.D.
	Myricetin	N.D.	132.47 ± 6.32	16.87 ± 2.90	N.D.	N.D.	N.D.
	Protocatechuic acid	N.D.	N.D.	2.47 ± 1.60	10.86 ± 6.78	N.D.	N.D.
	Quercetin	N.D.	N.D.	N.D.	0.59 ± 0.83	30.07 ± 2.02	N.D.
	Total	8.13 ± 4.57	786.48 ± 8.89	25.41 ± 9.47	72.55 ± 53.55	73.90 ± 1.42	N.D.
Phenolic amide	Avenanthramide A	50.64 ± 1.79	–	–	–	–	–
	Avenanthramide B	75.90 ± 0.31	–	–	–	–	–
	Avenanthramide C	99.87 ± 1.00	–	–	–	–	–

2.7. Statistical analysis

All data were analyzed using GraphPad Prism (GraphPad Software). Statistical analyses included one-way analysis of variance (ANOVA), followed by Fisher's LSD test. *P*-values <0.001, 0.01, and 0.05 were considered statistically significant. Data are expressed as mean ± standard error of the mean (SEM) ($n = 8$).

3. Results

3.1. Quantification of phenolic compounds using HPLC

The concentrations of phenolic compounds in six grains and legume extracts are presented in Table 1. OE exhibited various phenolic acids and phenolic amides, including ferulic acid (22.60 ± 2.28 μg/g), caffeic acid (9.86 ± 1.38 μg/g), avenanthramide (99.87 ± 1.00 μg/g). SE demonstrated high levels of flavonoids and phenolic acids, including rutin hydrate (495.00 ± 7.09 μg/g), caffeic acid (166.74 ± 4.00 μg/g), and naringenin (159.01 ± 9.89 μg/g). FE showed a moderate content of myricetin (16.87 ± 2.90 μg/g) and rutin hydrate (9.10 ± 3.68 μg/g), while PE contained 2-hydroxy cinnamic acid (5.97 ± 0.59 μg/g) and t-3-hydroxy cinnamic acid (0.69 ± 0.98 μg/g). AE presented abundant 4-hydroxy benzoic acid (95.48 ± 10.31 μg/g) and *p*-coumaric acid (79.01 ± 3.78 μg/g). BE was characterized by significant amounts of 4-hydroxy benzoic acid (86.51 ± 2.63 μg/g) and vanillic acid (16.36 ± 0.97 μg/g). SE and AE had the highest total phenolic content (281.48 ± 0.99 μg/g, 249.90 ± 11.25 μg/g), while SE had the highest total flavonoid content (786.48 ± 8.89 μg/g). Rutin hydrate was prominent in SE, and avenanthramides were exclusive to OE.

3.2. Growth performance

The growth performance of the rats is shown in Fig. 1A–C. Final body weight, feed intake, and water intake were significantly different between the NC and MC groups ($p < 0.01$). None of the treatments significantly changed feed intake; however, all treatments significantly reduced water intake compared with the MC group ($p < 0.01$). Notably, the PC and OE groups significantly increased weight compared with the MC group from the second week to the final week ($p < 0.05$).

3.3. Fasting blood glucose level

As shown in Fig. 2, the fasting blood glucose level in the MC group (271.0 ± 15.4 mg/dL) was significantly higher than in the NC group (97.6 ± 1.43 mg/dL), indicating the successful establishment of the diabetic model. Notably, a significant decrease in blood

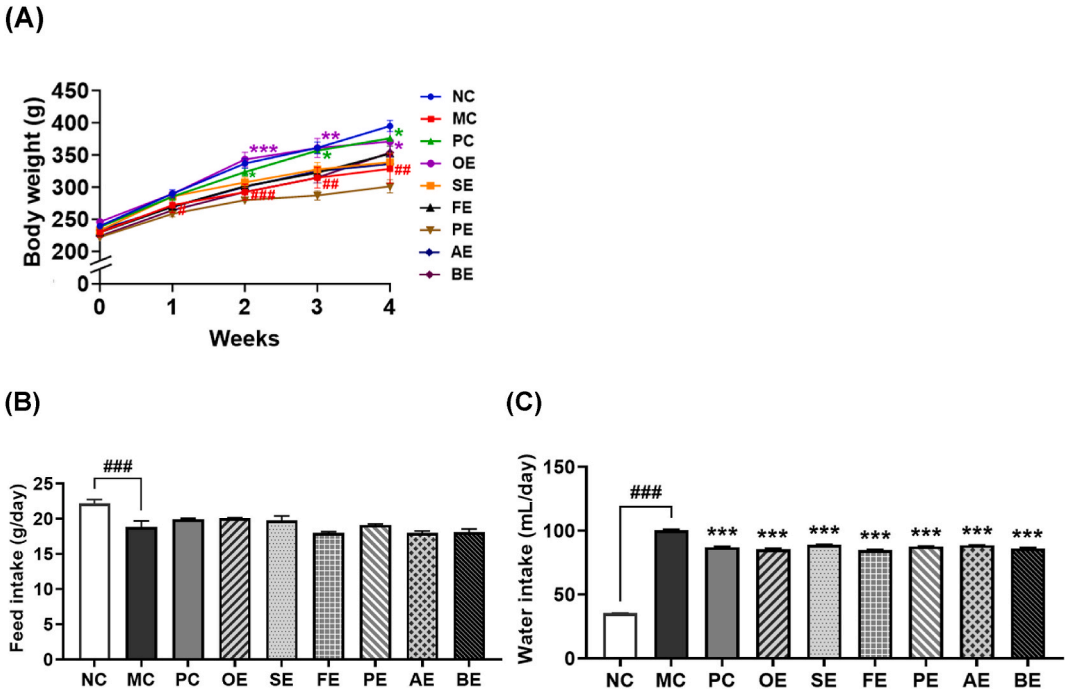

Fig. 1. (A) Body weight changes, (B) feed intake, and (C) water intake. NC: normal control; MC: model control; PC: positive control (metformin); OE: oat extract; SE: sorghum extract; FE: foxtail millet extract; PE: proso millet extract; AE: adzuki bean extract; BE: black soybean extract. Data are expressed as mean ± SEM ($n = 8$). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs NC. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs MC.

glucose was observed in the PC (185.8 ± 29.2 mg/dL), OE (192.8 ± 23.6 mg/dL), and SE (206.4 ± 25.4 mg/dL) groups compared with the MC group, showing a considerable improvement in glycemic control in diabetic rats ($p < 0.05$). In summary, OE and SE were as effective in lowering blood glucose levels as metformin.

3.4. Oral glucose tolerance test and insulin tolerance test

To assess the ability to efficiently eliminate glucose after glucose intake, we conducted an OGTT by measuring glucose levels at various time points (Fig. 3A and B). All groups showed an initial increase in blood glucose levels after glucose administration, peaking at 30 or 60 min. Throughout the OGTT, the MC group consistently exhibited significantly elevated blood glucose levels compared with the NC group. Notably, the PC, OE, and SE groups displayed lower blood glucose levels than the MC group. In addition, the NC group showed a decrease in blood glucose levels, returning to baseline levels (0 min) at 120 min, while all other groups, except MC, reached baseline levels at 180 min. As shown in Fig. 3B, the AUC data from the OGTT revealed that MC had a significantly higher OGTT AUC compared with the NC group ($p < 0.001$). Interestingly, the AUC of the OGTT in the PC, OE, and SE groups was significantly reduced by 16.8 %, 19.6 %, and 19.9 %, respectively, compared with the MC ($p < 0.05$) group, whereas there was no significant reduction in the FE, PE, AE, and BE groups.

To assess the ability to respond to insulin, we performed an ITT to measure glucose levels at various time points after insulin injection (Fig. 3C and D). Remarkably, significant decreases in the AUC of the ITT were observed in the PC and OE groups compared with the MC group. However, changes in glucose levels in the SE group were not as significant as those in the OGTT, implying that SE was insufficient to regulate insulin resistance. These results suggest that the oral administration of OE and SE for 4 weeks effectively improved glucose tolerance and, more importantly, OE decreased insulin resistance in diabetic rats.

3.5. Insulin, HOMA-IR, and HOMA-β

Fig. 4A–C shows the insulin concentration in serum, HOMA-IR and HOMA-β indexes of the rats treated with various grain and legume extracts. The MC group showed a significantly lower insulin concentration compared with the NC group. Among the treatments, only OE significantly increased insulin concentration compared with the MC group. In addition, HOMA-IR, which indicates the degree of insulin resistance, showed that the MC group was more insulin resistant than NC. While both PC and OE reduced HOMA-IR in comparison with MC, these reductions were not statistically significant. The HOMA-β index indicates the functionality of the β-cells in the pancreas. β-Cells in the MC group were destroyed by STZ, resulting in reduced functionality to secrete insulin in comparison to the NC group. Notably, both PC and OE displayed significantly higher HOMA- β indexes compared with the MC group ($p < 0.05$).

3.6. H&E and IHC staining of the pancreas

Histological analysis of the pancreas (H&E staining and IHC staining) are presented in Fig. 5A and B. The cell number in the pancreas of the MC group was lower compared with that in the NC group. In addition, degenerative and necrotic changes in the islets of the rats were observed in the MC group. Both PC and OE treatments rescued the pancreatic damage compared with the MC group. Moreover, a pronounced reduction in the yellow-brownish staining, which signifies insulin immunoreactivity, was evident in the MC group ($p < 0.001$). Metformin and OE treatment significantly increased insulin immunoreactive area (%) compared to the MC group, indicating that they ameliorated the STZ-induced damage to pancreatic islet cells, in particular, insulin-secreting β-cells ($p < 0.05$). In addition, there was no significant difference between PC and OE. Thus, OE effectively protected islets from STZ-induced pancreatic damage.

4. Discussion

The anti-diabetic properties of whole grains and dietary fibers derived from various grains and legumes are well-documented. However, research on the anti-diabetic effects of their extracts is still limited, making it challenging to compare their efficacy

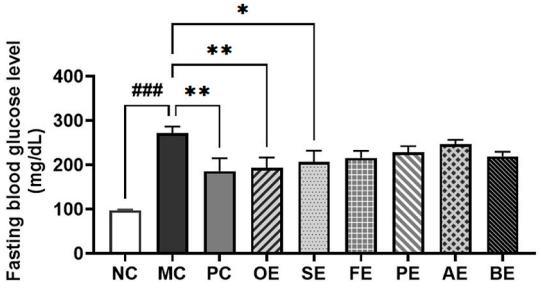

Fig. 2. Fasting blood glucose levels. NC: normal control; MC: model control; PC: positive control (metformin); OE: oat extract; SE: sorghum extract; FE: foxtail millet extract; PE: proso millet extract; AE: adzuki bean extract; BE: black soybean extract. Data are expressed as mean ± SEM ($n = 8$). $^{###}p < 0.001$ vs NC. $^{*}p < 0.05$, $^{**}p < 0.01$ vs MC.

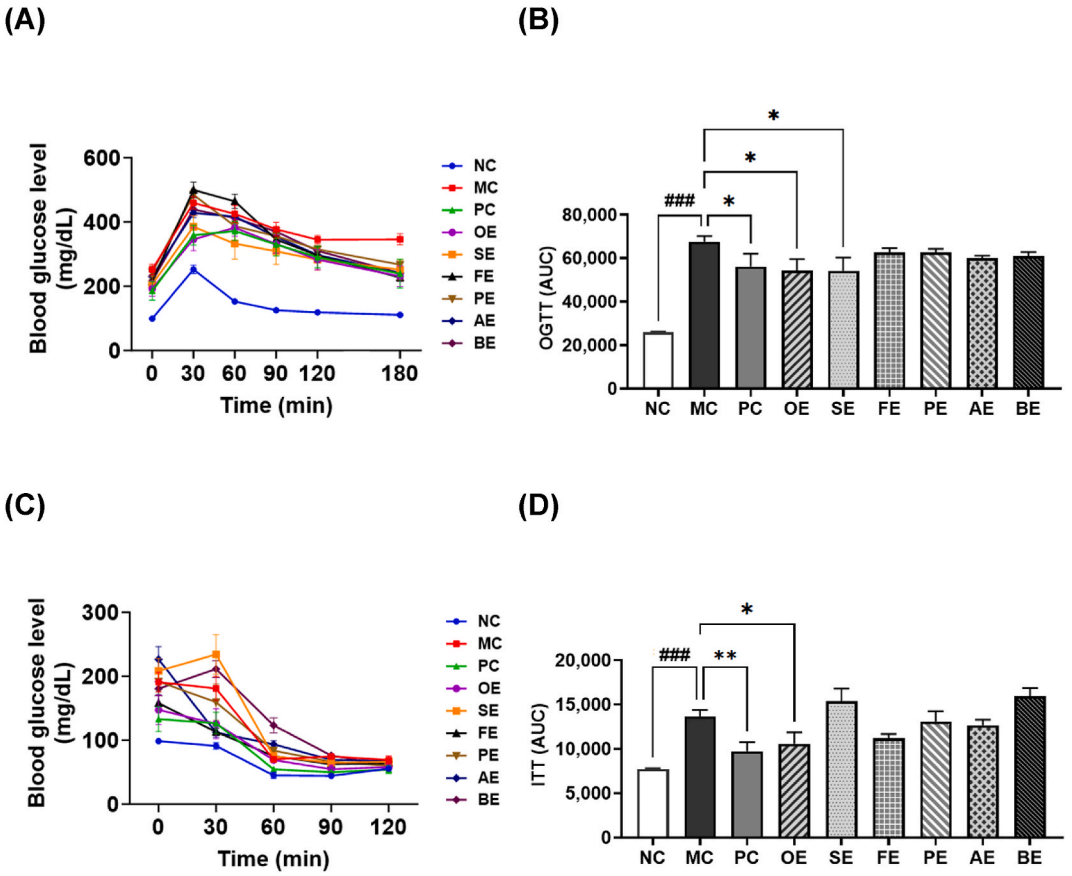

Fig. 3. (A) Blood glucose levels and (B) area under the curve (AUC) in the oral glucose tolerance test, (C) blood glucose level and (D) AUC in the insulin tolerance test. NC: normal control; MC: model control; PC: positive control (metformin); OE: oat extract; SE: sorghum extract; FE: foxtail millet extract; PE: proso millet extract; AE: adzuki bean extract; BE: black soybean extract. Data are expressed as mean ± SEM ($n = 8$). $^{###}p < 0.001$ vs NC. $^{*}p < 0.05$, $^{**}p < 0.01$ vs MC.

across previous studies. In this study, six different extracts were orally administered to diabetic rats for four weeks to assess their anti-diabetic effects. Among these extracts, OE exhibited the most significant anti-diabetic properties. Notably, OE effectively lowered blood glucose levels by 28.9 %, a reduction comparable to that achieved with metformin. OE also improved impaired glucose tolerance and insulin resistance and protected β-cell function in diabetic rats. Subsequently, SE displayed hypoglycemic effects and improved glucose tolerance.

Oats are a fiber-rich crop, well-known for their ability to regulate postprandial blood glucose levels. While previous studies have provided strong evidence of the anti-diabetic effects of oats primarily due to β-glucan, a soluble dietary fiber, our study revealed the novel perspective for anti-diabetic effects of oat phenolic rich extract [32–35]. Despite using 99.9 % ethanol for extraction, theoretically eliminating β-glucan, OE displayed potent blood glucose-lowering effects. These findings suggest that phenolic compounds in oats, beyond β-glucan, may contribute to their hypoglycemic activity.

A detailed analysis of phenolic compounds in each extract revealed that despite having lower phenolic acid and flavonoid contents than SE and AE, OE exhibited a high avenanthramide content, a group of phenolamides found only in oats. In a previous study, dihydroavenanthramide, a synthetic analog of avenanthramide, had a protective effect against STZ-induced pancreatic injury and played a crucial role in maintaining insulin levels and enhancing immune responsiveness to insulin [36]. Additionally, avenanthramide inhibited the production of advanced glycation end products and lowered blood glucose levels by modulating gut microbiota in obese rats [26,37]. These results suggest that avenanthramides, specifically found in OE, contribute significantly to its glucose-lowering effects.

Further elucidating the potential mechanisms, the hypoglycemic effects of OE might be linked to the mitigation of pancreatic cell destruction confirmed by H&E and insulin IHC results. Previous studies on oat bran and oat extract using 90 % methanol in diabetic rats emphasized the regenerative potential of oats on pancreatic islets, reducing blood glucose levels and improving insulin resistance [21,38]. Taken together, the ability of OE to increase insulin concentration and regenerate islet cells may have contributed to the dramatic decrease in fasting blood glucose levels. Further research is needed to identify the antidiabetic mechanisms and specific active compounds of OE.

Following OE, SE also exhibited hypoglycemic effects and improved glucose tolerance in rats, aligning with previous studies

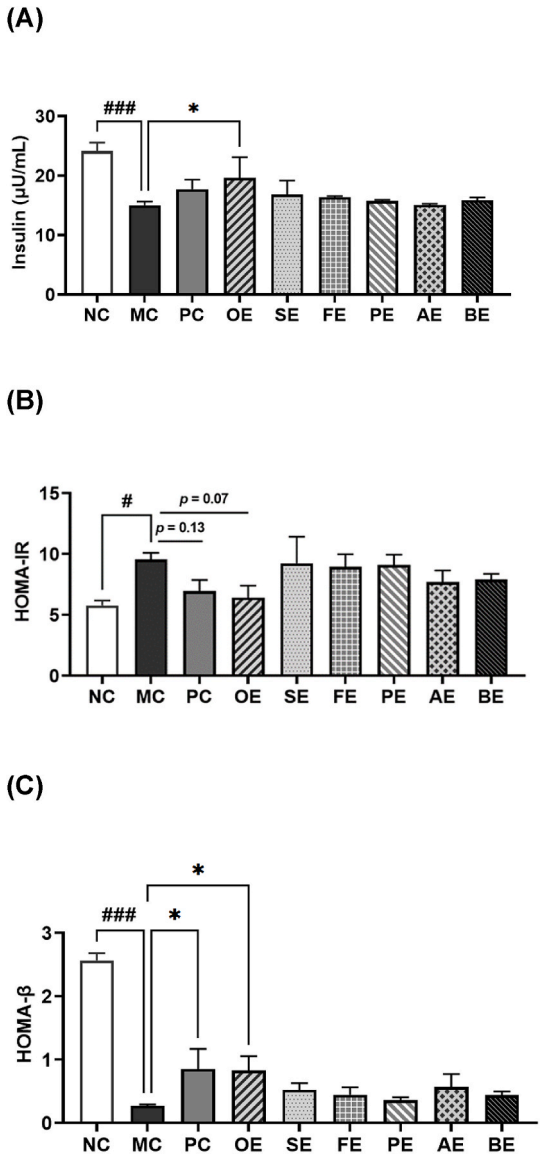

Fig. 4. (A) Insulin concentration, (B) HOMA-IR, and (C) HOMA-β. NC: normal control; MC: model control; PC: positive control (metformin); OE: oat extract; SE: sorghum extract; FE: foxtail millet extract; PE: proso millet extract; AE: adzuki bean extract; BE: black soybean extract. Data are expressed as mean ± SEM ($n = 8$). $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs NC. $^{*}p < 0.05$ vs MC.

demonstrating that SE significantly lowered blood glucose levels by inhibiting the mRNA expression related to hepatic gluconeogenesis [23,24]. However, SE did not affect insulin concentration and did not exhibit a comprehensive anti-diabetic effect beyond OE in this model. The animal model in this study may have contributed to these results. A high-fat diet and STZ-NA-induced diabetes animal model used in this study has the characteristics of severe T2DM, including not only high blood glucose levels and insulin resistance but also insulin insufficiency due to damaged pancreatic β-cells [39]. In this model, the restoration of pancreatic β-cells can play an important role in helping to normalize blood sugar metabolism and alleviate diabetes symptoms.

Contrary to expectations, FE, PE, AE, and BE did not exhibit significant effects on blood glucose levels in this study. Previous studies have shown that foxtail millet water extract and adzuki bean water extract reduced fasting blood glucose [26,40]. These inconsistent results may be due to variations in grain and legume varieties and extraction solvents. Additionally, it is also speculated that a longer duration of intervention is required to observe significant changes in diabetes-related parameters.

5. Conclusion

In summary, we comprehensively evaluated the anti-diabetic effects of various grain and legume extracts in a high-fat diet and STZ-

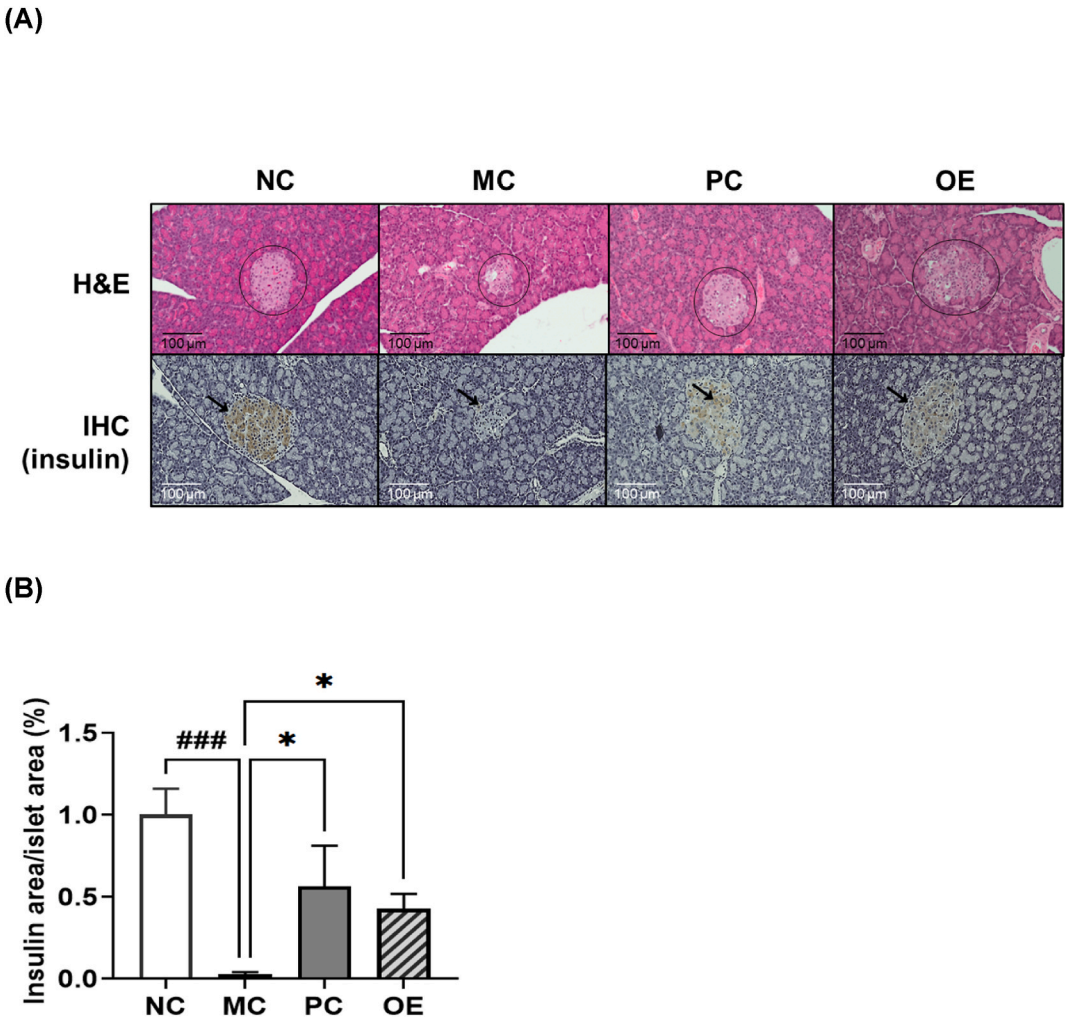

Fig. 5. (A) Hematoxylin and eosin (H&E) staining of the pancreas and insulin immunohistochemical (IHC) staining of pancreatic β-cells and (B) the ratio of insulin immunoreactive area to islet area (%). Circles indicate pancreas Langerhans islets and arrows indicate insulin immunoreactivity. NC: normal control; MC: model control; PC: positive control (metformin); OE: oat extract. Data are expressed as mean ± SEM ($n = 4$). $^{###}p < 0.001$ vs NC. $^{*}p < 0.05$ vs MC.

NA-induced diabetic rat model. Among these extracts, OE emerged as the most potent, significantly reducing fasting blood glucose levels and improving glucose tolerance and insulin resistance. Notably, OE exhibited protective effects on pancreatic β-cells, indicating a potential mechanism for its hypoglycemic effects. While SE also demonstrated glycemic-lowering effects, the overall findings highlight the promising anti-diabetic potential of OE. This study contributes valuable insights into the comparative efficacy of various grain and legume extracts, emphasizing their potential, in particular OE, as functional foods in managing T2DM.

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Data availability statement

Data will be made available on request.

Declarations

The animal experiments were approved by the Animal Ethics Committee of Woojung Bio (WJIACUC20190624-2-40).

CRediT authorship contribution statement

Eunwoo Jeong: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Youjin Baek:** Writing – review & editing, Investigation, Conceptualization. **Hyun-Joo Kim:** Writing – review & editing, Resources, Conceptualization. **Hyeon Gyu Lee:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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