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Research article

Allantoin reduces glucotoxicity and lipotoxicity in a type 2 diabetes rat model by modulating the PI3K and MAPK signaling pathways

Yao Zhao [a], Ming Qiao [b,c], Xiaomei Wang [a], Xinjie Luo [a], Jianhua Yang [b,c,**], Junping Hu [a,c,*]

[a] College of Pharmacy, Xinjiang Medical University, Urumqi, 830017, Xinjiang, China
[b] Department of Pharmacy, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, 830000, Xinjiang, China
[c] Xinjiang Key Laboratory of Clinical Drug Research, Urumqi 830011, Xinjiang, China

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ABSTRACT

Objective: The current study aimed to investigate the potential therapeutic impact of allantoin on diabetes produced by a high-fat diet (HFD) and streptozotocin (STZ) in rats.
Subjects and methods: Male Sprague-Dawley rats were fed a high-fat diet to induce insulin resistance, followed by streptozotocin injection to induce diabetes. The effect of oral treatment of allantoin (200, 400 and 800 mg/kg/day) for 8 weeks was evaluated by calculating the alteration in metabolic parameters, biochemical indicators, the oral glucose tolerance tests (OGTT) and hyperinsulinemic-euglycemic clamp tests were performed. Histopathological studies were performed in the liver, kidney and pancreas. Next, the expressions of the MAPK and insulin signaling pathway were measured by Western blot analysis to elucidate the potential mechanism underlying these antidiabetic activities.
Results: The administration of allantoin resulted in a significant decrease in fasting blood glucose (FBG) levels, glycogen levels, and glycosylated hemoglobin levels in diabetic rats. Additionally, allantoin therapy led to a dose-dependent increase in body weight growth and serum insulin levels. In addition, the administration of allantoin resulted in a considerable reduction in lipid profile levels and amelioration of histological alterations in rats with diabetes. The administration of allantoin to diabetic rats resulted in a notable decrease in Malondialdehyde (MDA) levels, accompanied by an increase in the activity of antioxidant enzymes in the serum, liver, and kidney. The findings of oral glucose tolerance and hyperinsulinemic-euglycemic clamp tests demonstrated a significant rise in insulin resistance following the administration of allantoin. The upregulation of IRS-2/PI3K/p-Akt/GLUT expression by allantoin suggests a mechanistic relationship between the PI3K/Akt signaling pathway and the antihyperglycemic activity of allantoin. Furthermore, it resulted in a reduction in the levels of TGF-β1/p38MAPK/Caspase-3 expression in the aforementioned rat tissues affected by diabetes.
Conclusions: This study implies that allantoin treats type 2 diabetes by activating PI3K. Additionally, it reduces liver, kidney, and pancreatic apoptosis and inflammation-induced insulin resistance.re

* Corresponding author. College of Pharmacy, Xinjiang Medical University, Urumqi, 830017, Xinjiang, China.
** Corresponding author. Department of Pharmacy, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, 830000, Xinjiang, China
E-mail addresses: yjh_yfy@163.com (J. Yang), hjp_yxy@163.com (J. Hu).

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1. Introduction

The prevalence of diabetes mellitus has attained epidemic proportions on a global scale, with projections indicating that the number of affected individuals will surpass 300 million by the year 2025. Type 2 diabetes mellitus (T2DM) is more prevalent in clinical practice compared to type 1 diabetes mellitus (T1DM) [1]. Within this cohort of individuals diagnosed with diabetes, it has been shown that a significant majority, ranging from 90 % to 95 %, exhibit T2DM [2]. Consequently, the prevention and management of T2DM has emerged as a prominent area of concern within the healthcare field. The management of diabetes mellitus encompasses several therapeutic modalities, including dietary interventions, physical activity, and pharmacological agents [3].At present, the pharmacological interventions for the management ofT2DM encompass the utilization of insulin as well as antihyperglycemic medications, including sulfonyl urea derivatives, biguanides, and thiazolidinediones [4]. Moreover, a number of herbal treatments offer a fresh approach for the treatment of T2DM, exhibiting a reduced incidence of adverse effects and a sustained moderate therapeutic impact [5].

As a standalone risk factor for T2DM, insulin resistance (IR) is the primary pathophysiology [6]. This phenomenon manifests as a decline in hepatic glycogen levels and a reduction in the expression of GLUT-4 in insulin receptor-positive cells [7]. Hepatic glycogen plays a crucial role in the regulation of blood glucose homeostasis. The GLUT-4 transporter facilitates the transportation of glucose molecules from the bloodstream to various tissues, enabling their use [8]. IR arises due to aberrant insulin levels and subsequent dysregulation of the PI3K/Akt signaling pathways. These pathways play a crucial role in regulating the transport of glucose, glycogen formation, glycolysis and the formation of proteins [9]. Therefore, the correction of IR represents a crucial therapeutic approach for the management ofT2DM. At present, a variety of oral hyperglycemia drugs, including rosiglitazone, metformin, and acarbose, have been made available in the marketplace. However, it is important to note that such synthetic substances have been shown to be linked with specific negative consequences [10]. Finding safer and more potent antidiabetic medications for T2DM is therefore important. The density of insulin receptors decreases in adipocytes, while there is an increase in the levels of proinflammatory cytokines such as TNF-a and IL-6 in both obese humans and rats. This observation suggests that these variables may contribute to IR [10,11].

Allantoin, also known as 5-ureidohydantoin or 5-ureidoacetolactam, is a diureide derived from glyoxylic acid. It is a chemical that is found abundantly in many plant sources, such as yam, beetroot, wheat sprouts, and maize [12]. This chemical, generated from purines, does not provide any discernible health hazards and is devoid of any harmful effects. The compound has a diverse range of pharmacological activities, including anti-inflammatory, wound healing, hypoglycemic, and antioxidant effects. The aforementioned substance is a widely used cosmetic ingredient that is now available in the commercial sector. It has the capacity to combat free radicals and mitigate inflammation. There have been assertions made about the potential antidiabetic properties of yam extract and allantoin. These purported benefits include enhancements in beta-cell function via antioxidative activity, the inhibition of lipid metabolites, and an augmentation in the synthesis of glucagon-like peptide-1 (GLP-1). The inclusion of a citation is necessary to support the information provided. Both yam extract and allantoin have been the subject of research about their possible therapeutic use in the treatment of diabetes [13].

Moreover, recent research has shown that the administration of allantoin may provide advantageous outcomes in mitigating the symptoms often associated with diabetes. The effects seen in streptozotocin (STZ)-induced diabetic rats are believed to be influenced by an upregulation of glucose transporter 4 (GLUT4) expression in skeletal muscle and the secretion of beta-endorphin from the adrenal gland. Moreover, previous studies have shown that the activation of the imidazoline I1 receptor in mice, which were induced to become obese by the consumption of a high-fat diet (HFD), results in the prevention of hyperleptinemia and an increase in calorie intake through the administration of allantoin [14,15]. Several studies have shown that the application of allantoin may induce the differentiation of myoblasts into myotubes. Additionally, it has been observed that allantoin can boost energy generation in C2C12 mouse skeletal muscle cells by upregulating mitochondrial biogenetic proteins [16].

Despite the demonstrated efficacy of allantoin in reducing lipid levels and promoting the regeneration of damaged pancreatic islet cells, the hypoglycemic mechanism of allantoin and its protective effect on organs under glucotoxic and lipotoxic damage remain unclear. This characteristic renders it a potential candidate for the formulation of a pharmaceutical intervention aimed at augmenting the metabolic processes involved in glucose and fat metabolism inside the body. The objective of this study was to investigate the potential hypoglycemic effects of allantoin by analyzing metabolism and biochemical and molecular mechanisms. The present study investigated many physiological parameters in streptozotocin-induced diabetic rats, including body weight, food and water consumption, fasting blood sugar levels, blood lipids, total cholesterol and protein levels, insulin levels, hepatic glycogen content, and protein expression in the insulin signaling pathway within the liver and muscle.

2. Materials and methods

2.1. Experimental animals

Male Sprague Dawley (SD) rats weighing between 120 and 130 g were procured from the Laboratory Animal Center at Xinjiang Medical University in Urumqi, Xinjiang, China. The acquisition of these rats was supported by Grant Number SCXK (xin) 2018–0002. Rats were used subsequent to a minimum of one week of quarantine and acclimatization. The subjects were housed inside the designated animal facility of the department, where they were subjected to controlled environmental conditions. These conditions included a temperature range of 23 °C ± 2 °C, a relative humidity range of 60 % ± 5 %, a light/dark cycle of 12 h each, and unrestricted access to a standard pellet meal and water. The research adhered to the rules established by the Committee for the Purpose of

Control and Supervision of Experiments on Animals (CPCSEA) under the Government of China.

Following a one-week period of acclimation to their surroundings, rats were divided into two groups. The first group received a standard diet consisting of 3.1 kcal with 14 % protein content. The second group was provided with a high-fat diet (HFD) sourced from Research Diets, USA, containing 4.25 kcal with 40 % fat content. This dietary intervention was carried out over a duration of eight weeks with the aim of inducing obesity, IR, and impaired glucose tolerance in the rats. To develop diabetes in each HFD rat, intraperitoneal injections of STZ (25 and 30 mg/kg body weight, respectively) were administered. STZ was dissolved in 0.1 M sodium citrate buffer with a pH of 4.5. Following the administration of STZ, the rats were provided with drinking water containing a 5 % sucrose solution for a duration of 48 h. This intervention aimed to mitigate premature mortality that may arise due to insulin secretion from partly damaged pancreatic islets. After a period of 72 h, the rats underwent blood sugar testing. To ensure the consistent presence of hyperglycemia, the rats with diabetes were then administered HFD for a duration of 2 weeks prior to the commencement of experimental protocols.

The measurement of fasting blood sugar levels (FBG) was conducted using a glucometer. Rats exhibiting FBG values over 16.7 mmol/L were identified as diabetic and thereafter included in the research. A total of forty rats were effectively induced to develop diabetes. In the experimental design, the rats were allocated into six groups, each consisting of eight rats. These groups were as follows: the normal group, which consisted of rats fed a normal diet; the DM group, which consisted of diabetic rats fed HFD and served as the negative control; and the DM + A200, DM + A400, and DM + A800 groups, which consisted of diabetic rats fed a HFD and orally administered allantoin at doses of 200, 400, or 800 mg/kg body weight per day for a duration of 8 weeks, respectively. In this study, a group of rats with diabetes were treated with metformin (Met) by oral administration at a dosage of 200 mg/kg body weight per day for a period of 8 weeks. This treatment was used as a positive control in the experiment. Throughout the duration of the treatment period, the animals' weights were recorded, and their fasting blood glucose levels were assessed on a weekly basis using the Roche ACCU-CHEK Active glucometer. At the conclusion of the experiment, blood and tissue samples were collected from rats after deep anesthesia with pentobarbital sodium (30 mg/kg).

2.2. Oral glucose tolerance test (OGTT)

The oral glucose tolerance test (OGTT) was conducted at two time points, namely, 4 and 8 weeks prior to the conclusion of the investigation. Glucose (2 g/kg) was orally fed to rats that had undergone an overnight fast after the administration of allantoin. Blood samples were obtained from the tail vein at certain time intervals of 0, 30, 60, 90, and 120 min after the administration of glucose lavage.

2.3. Hyperinsulinemic-euglycemic clamp analysis

The hyperinsulinemic-euglycemic clamp test is widely regarded as the definitive technique for evaluating insulin sensitivity. Following an 8-week experimental period, rats were administered a hyperinsulinemic-euglycemic clamp study while under anesthesia, as previously outlined in the literature, with some adjustments [17]. In summary, after a 12-h period of fasting, catheters were surgically inserted into the left carotid artery of rats for the purpose of blood collection and into the right jugular vein for the administration of insulin and glucose. The commencement of measurements occurred 30 min after the surgical procedure. During the experiment, a continuous infusion of human recombinant insulin (Novolin R 100 IU/ml, Novo Nordisk, Copenhagen, Denmark) was administered to the subjects at a rate of 20 mU/kg/min for a duration of 120 min. Previously, it was discovered that there is cross-reactivity between the insulin of this particular species and that of the rat. The blood glucose concentration was maintained at its baseline level by periodically measuring the blood glucose concentration every 5 min and modifying the rate of glucose solution infusion accordingly. Blood samples were obtained at regular intervals of 5–10 min to measure the concentration of glucose. A glucometer was used for this purpose. Additionally, varying amounts of a solution containing glucose (10 %) were administered as necessary to maintain euglycemia at the baseline level.

Consequently, insulin-stimulated glucose uptake was estimated (by the rate of glucose infusion during the last 30 min of the clamp). The evaluation of whole-body insulin sensitivity was conducted using the glucose infusion rate (GIR: mg/kg/min), which may be calculated as follows: GIR (mg/kg/min) = rate (μl/min) × glucose concentration (g/ml) × 1000 ÷ body weight (g). The measurement of insulin levels was used to assess the reciprocal regulatory actions of islet β cells. Following a 120-min hyperinsulinemic-euglycemic clamp, blood samples were obtained by heart puncture and then transferred into a tube. Plasma separation was achieved using centrifugation at a force of 1200×*g* for a duration of 10 min. The liver and hind leg skeletal muscle were surgically removed and rapidly frozen by immersion in liquid nitrogen. The tissues and plasma samples were subjected to storage at a temperature of −80 °C to facilitate further analysis.

2.4. Blood glucose level and biochemical parameters measurement

Serum was extracted from the acquired blood sample and then used for the analysis of biochemical parameters. In addition to measuring the weights of the liver, kidney, and pancreas, the organ index for each organ was determined by calculating the ratio of the organ weight to body weight. Glucose levels were estimated using a glucometer. The assessment of beta cell activity and insulin resistance (known as HOMA-IR) was performed using the methodology developed by Matthews et al. (1985). Glucose levels were measured using a glucometer. The homeostatic model assessment (HOMA-IR), serving as an indicator for beta cell function and insulin resistance, was conducted using the method developed by Matthews et al. (1985). HOMA-IR was calculated using the following

formula: HOMA-IR = [Fasting glucose (mmol/L) × fasting insulin (mU/L)]/22.5. The levels of insulin, tumor necrosis factor (TNF-α), and interleukin 6 (IL-6) were measured using the solid phase enzyme-linked immunosorbent assay (ELISA) kit provided by R&D Systems (Minneapolis, MN), following the instructions provided by the manufacturer. Triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), free fatty acid (FFA), glycosylated hemoglobin (GHb), and glycosylated serum protein (GSP) were quantified utilizing commercially available kits provided by the Nanjing Jiancheng Institute of Bioengineering in China. The levels of serum enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen, and creatinine, were determined using commercially available kits obtained from the Nanjing Jiancheng Institute of Bioengineering in China. The manufacturer's recommendations were followed throughout the estimation process.

2.5. Determination of oxidative stress parameters

The kidney and liver samples were subjected to homogenization in a phosphate buffer solution, resulting in a 10 % homogenate. Subsequently, centrifugation was performed at 2500 revolutions per minute for a duration of 10 min at a temperature of 4 °C. The hepatic oxidative stress marker levels (SOD, GSH-Px, CAT, and MDA) in the supernatant collected from the liver/kidney homogenate and serum were determined using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

2.6. Determination of pro-inflammatory mediators

The blood concentrations of proinflammatory cytokines, including TNF-α and IL-6, were assessed using ELISA kits obtained from Elabscience Biotechnology in Wuhan, China.

2.7. Determination of liver and muscle glycogen

The rat liver and skeletal muscle tissues underwent a cold normal saline wash. The glycogen levels were quantified by a colorimetric test employing the anthracenone technique (Jiancheng Biotechnology, Nanjing, China).

2.8. Histopathological analysis of liver, kidney and pancreas tissues

The liver, kidney, and pancreatic tissues were submerged in a 10 % buffered formalin solution for fixation. Next, the specimens were subjected to dehydration using a series of alcohol solutions with increasing concentrations. Then, the tissues were subjected to paraffin embedding and sectioned into slices with a thickness of 5 μm. After that, the slices were further stained with hematoxylin & eosin (H&E) and Masson's trichrome, and the liver, kidney, and pancreatic tissues were evaluated under a Nikon light microscope (Tokyo, Japan) for histopathological changes.

2.9. Immunofluorescence staining to detect insulin expression in pancreatic tissue

Paraffin-embedded pancreatic tissue sections were deparaffinized, rehydrated, subjected to antigen retrieval, and quenched for autofluorescence. The sections were then incubated with 5 % BSA solution at room temperature for 30 min to block non-specific binding. Subsequently, the sections were incubated with an anti-insulin antibody (4590S, Cell Signaling Technology, USA; 1:500 dilution) at 4 °C overnight. After washing three times with PBS, fluorescently-labeled secondary antibodies were added and incubated at room temperature for 50 min. Following three additional washes with PBS, the sections were counterstained with DAPI (D9542, Sigma-Aldrich, Germany) nuclear stain and incubated in the dark at room temperature for 10 min. After washing three more times with PBS, the slides were cover slipped and imaged using Zeiss Axio-Imager fluorescence microscope (Carl Zeiss AG, Germany).

2.10. Western blot analysis

Western blotting was conducted following the protocol described by Martin et al., with minor adjustments. In summary, lysis buffer (R0010; Beijing Solarbio Science & Technology Co., Ltd.), protease inhibitor cocktail (G2007; Wuhan Servicebio Technology Co.,Ltd) and PMSF(G2008; Wuhan Servicebio Technology Co.,Ltd) was employed to create a 10 % w/v tissue homogenate. The resulting homogenate was then solubilized at a temperature of 4 °C for a duration of 1 h, followed by centrifugation at a force of 12000×*g* for a period of 20 min. The supernatants were collected, and the protein content was determined using the BCA method. The samples were thereafter kept at a temperature of −20 °C until further analysis. In this experiment, protein samples weighing 40 μg were loaded onto distinct lanes of a 10 % polyacrylamide gel. The samples were then subjected to sodium dodecyl sulfate–polyacrylamide acrylamide gel electrophoresis (SDS–PAGE) using a Bio-Rad Mini-PROTEAN Tetra Cell. The proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore Corporation, USA) using a Mini *Trans*-Blot Electrophoretic Transfer Cell (Bio-Rad, USA). The membranes were blocked using bovine serum albumin (5 %) in TBS-T (0.1 % Tween 20) for a duration of 2 h. Subsequently, they were subjected to overnight incubation at a temperature of 4 °C with primary antibodies, namely, IRS-2 (CST #4502, 1/1000, Boston, USA), PI3 kinase-p85, Akt (CST #4685, 1/1000, Boston, USA), phospho-Akt (CST #4058, 1/1000, Boston, USA), GLUT-4 (Abcam #ab654, 1/2000, London, England), TGFβ-1 (Abcam #ab215715, 1/1000, London, England), p38MAPK (CST #8690, 1/2000, Boston, USA), and caspase-3 (Abcam #ab13847, 1/1000, London, England). The β-actin antibody (CST #4970, 1/1000 dilution, Boston, USA) was used

as an internal control in this study. The membranes were then rinsed with a TBS-T solution in a gentle manner and subjected to incubation with either a secondary antibody, specifically HRP-linked, IgG anti-mouse or anti-rabbit, for a duration of 1 h. The needed blots underwent a washing process with TBS-T solution, repeated three times for a duration of 15 min each. Subsequently, the blots were visualized by using enhanced chemiluminescent substrates. The densitometric study was conducted using the ImageJ (NIH) analysis program. The quantification of band intensity was performed using densitometric analysis, and the results are presented in relation to the intensity of β-actin.

2.11. Statistical analysis

The data are provided as the mean ± standard error of the mean (SEM). Statistical analysis of the data was conducted using GraphPad Prism 5.0(GraphPad Software, United States). The statistical analysis for multiple groups was conducted using a one-way analysis of variance (ANOVA) followed by post hoc Tukey's testing. The post hoc tests were performed only if the F statistic gained statistical significance ($p < 0.05$) and there was no substantial violation of the assumption of homogeneity of variance. A significance level of $p < 0.05$ was deemed to be statistically significant. All research included in the analysis did not exclude any data.

3. Results

3.1. Effects of allantoin on body weight, food and water intake

Fig. 1A is depicting the alterations in body weight of control and experimental rats. In comparison with normal rats, there was a significant decrease ($p < 0.05$) in the body weights of diabetic rats. On the other hand, treatment of diabetic rats with allantoin (200, 400 and 800 mg/kg) or metformin caused a significant increase ($p < 0.05$) in the body weights when compared to body weights of diabetic rats. Diabetic condition has resulted in drastic increase ($p < 0.05$) in daily food (Fig. 1B) and water intake (Fig. 1C) which upon treatment with 200, 400 and 800 mg/kg allantoin or metformin decreased significantly ($p < 0.05$) when compared to diabetic rats.

3.2. Effects of allantoin on the fasting blood glucose, glycated hemoglobin, glycogen, glycated serum albumin levels

There was a significant increase ($p < 0.05$) in weekly fasting blood glucose, glycated hemoglobin, glycogen, glycated serum albumin in diabetic rats which did not reduce till 8 weeks when compared to normal rats. Whereas 200, 400 and 800 mg/kg allantoin or metformin treatment for 8 weeks significantly reduced ($p < 0.05$) the fasting glucose, glycated hemoglobin, glycogen, glycated serum albumin levels to near normal levels (Fig. 2A–E).

3.3. Effects of allantoin on the lipid profiles and FFA levels

Since diabetes is linked to changes in lipid metabolism, our analysis focused on that variable. A considerable ($p < 0.05$) Fig. 3(A-E) rise in TC, TG, LDL-c, and FFA in diabetic rats compared to control rats, whereas HDL-c levels were considerably (p 0.05) lower in diabetic rats. All treated groups showed substantial ($p < 0.05$) decreases in TC, TG, LDL-c, and FFA after 8 weeks, whereas all groups

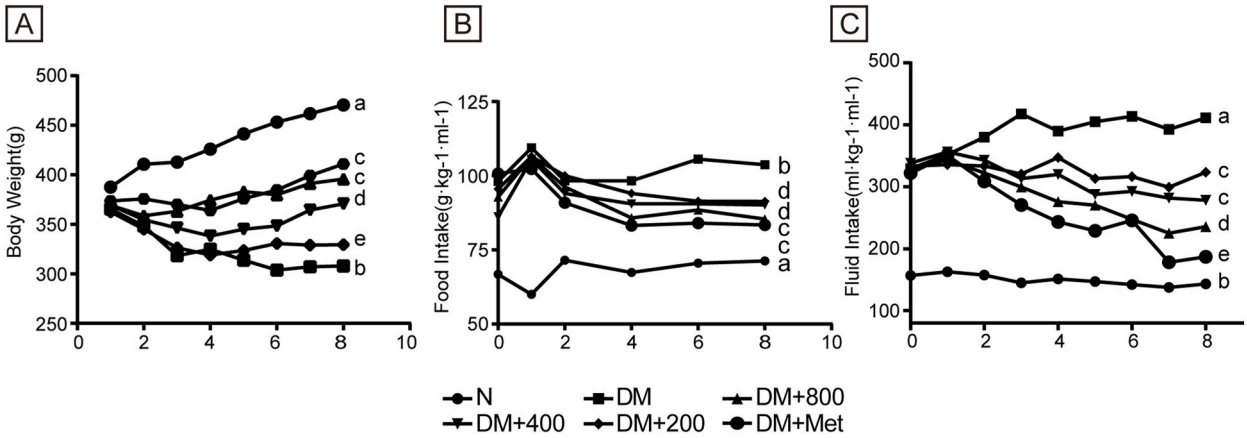

Fig. 1. Effects of allantoin on body weight (A), food (B) and fluid (C) intake. The results presented as mean ± SEM (n = 8) were evaluated by one-way ANOVA followed by post hoc Tukey's tests (statistically significant if $p < 0.05$). Values which have the same superscript letter(s) in each graph are not significantly different. When no letters are present in the superscript, the ANOVA test was significant. N= Normal rats; DM = type 2 diabetes mellitus rats; DM+800 = type 2 diabetes mellitus rats +800 mg/kg bw of allantoin; DM+400 = type 2 diabetes mellitus rats +400 mg/kg bw of allantoin; DM+200 = type 2 diabetes mellitus rats +200 mg/kg bw of allantoin; DM + Met = type 2 diabetes mellitus rats +200 mg/kg bw of metformin.

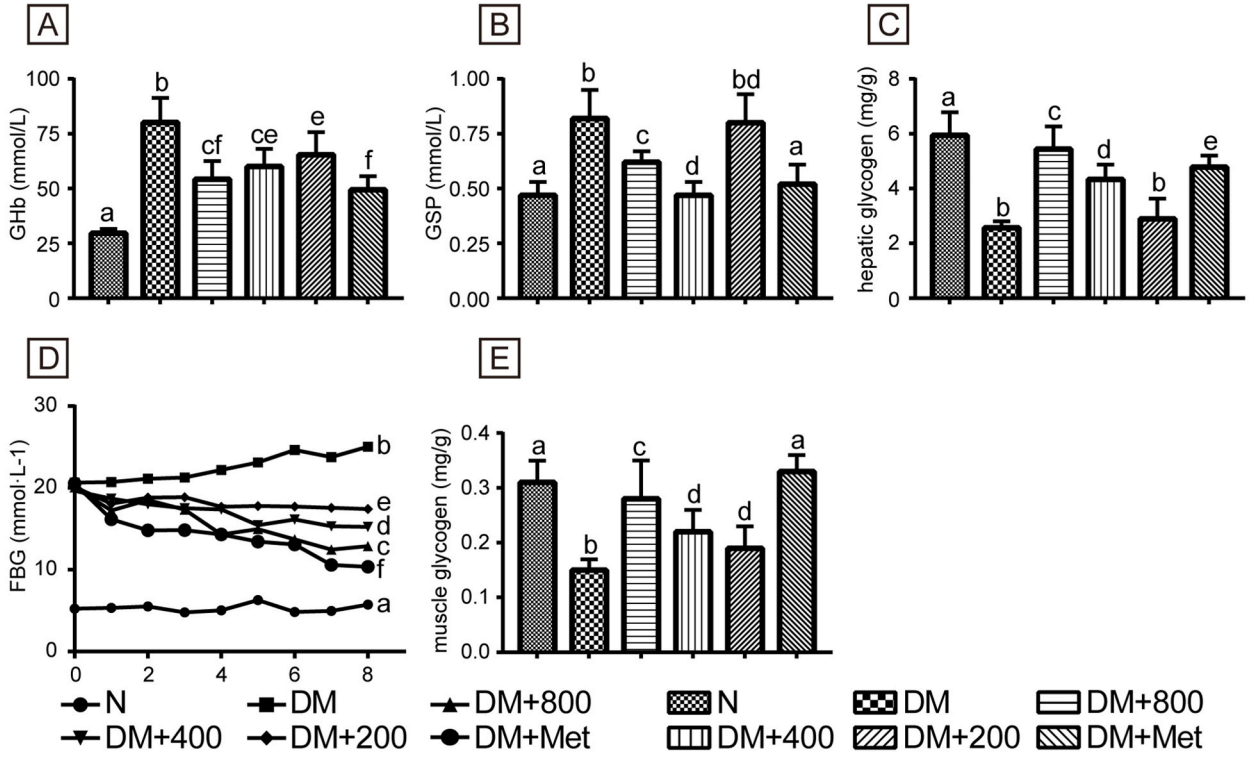

Fig. 2. Effects of allantoin on the glycated hemoglobin (GHb) (A), glycated serum albumin (GSP) (B), hepatic glycogen (C), fasting blood glucose (FBG)(D), muscle glycogen (E) levels. The results presented as mean ± SEM (n = 8) were evaluated by one-way ANOVA followed by post hoc Tukey's tests (statistically significant if $p < 0.05$). Values which have the same superscript letter(s) in each graph are not significantly different. When no letters are present in the superscript, the ANOVA test was not significant. N= Normal rats; DM = type 2 diabetes mellitus rats; DM+800 = type 2 diabetes mellitus rats +800 mg/kg bw of allantoin; DM+400 = type 2 diabetes mellitus rats +400 mg/kg bw of allantoin; DM+200 = type 2 diabetes mellitus rats +200 mg/kg bw of allantoin; DM + Met = type 2 diabetes mellitus rats +200 mg/kg bw of metformin.

showed significant increases in HDL-c. Additionally, compared to metformin, allantoin 800 mg/kg had more favorable effects on blood TC and HDL-c levels in diabetic rats. After being given allantoin orally for 8 weeks, lipid metabolism was significantly improved in diabetic rats.

3.4. Effects of allantoin on insulin resistance and pancreatic functions

The histological examination of the pancreas using H&E staining provided insights into the structural characteristics of the pancreas. The results revealed that diabetic rats had diminished islets of Langerhans with distorted morphology. However, treatment with either 400 or 800 mg/kg allantoin or metformin resulted in the restoration of the size and shape of the islet cells. Compared to the diabetic rats, the allantoin-treated group or the metformin-treated group showed an enhancement in insulin fluorescence staining in the pancreatic islet tissue (Fig. 4A). The key characteristic of DM is a large decrease in blood insulin levels in diabetic rats. However, the administration of allantoin or metformin to diabetic rats resulted in a notable improvement in insulin levels compared to those seen in diabetic controls (Fig. 4B). The advantageous impacts of allantoin exhibit similarities to those of metformin.

Fig. 4C, Ddepict glucose tolerance, as assessed using OGTT, either four weeks or one week prior to the conclusion of the trial. The blood glucose levels of typical rats exhibited an elevation within a 30-min timeframe, which subsequently returned to baseline levels under the influence of physiological hormonal regulation. On the other hand, diabetic rats exhibited impaired glucose tolerance and did not return to baseline levels. In comparison to the control group consisting of normal rats, the diabetic rats exhibited a statistically significant increase in the area under the curve (AUC) ($p < 0.05$), as shown in Fig. 4E and F. The rats with diabetes had impaired glucose tolerance and could not achieve spontaneous recovery, as shown by their final glucose level of 16.7 mmol/L at the conclusion of the trial. The administration of allantoin or metformin to diabetic rats resulted in a considerable improvement in glucose tolerance, comparable to that seen with the standard medication ($p < 0.05$).

At the conclusion of the eighth week of therapy, rats performed a hyperinsulinemic-euglycemic clamp test to evaluate the overall insulin sensitivity of their whole bodies. The administration of allantoin or metformin resulted in a considerable increase in the rate of glucose infusion (Fig. 4G) required to maintain euglycemia during the insulin infusion compared to diabetic rats. The findings of the study also demonstrated a significant rise in HOMA-IR, ISI and pancreas index (Fig. 4H–J) among the diabetic rats, demonstrating the presence of IR. However, this resistance was shown to diminish with the administration of doses of 200, 400, and 800 mg/kg

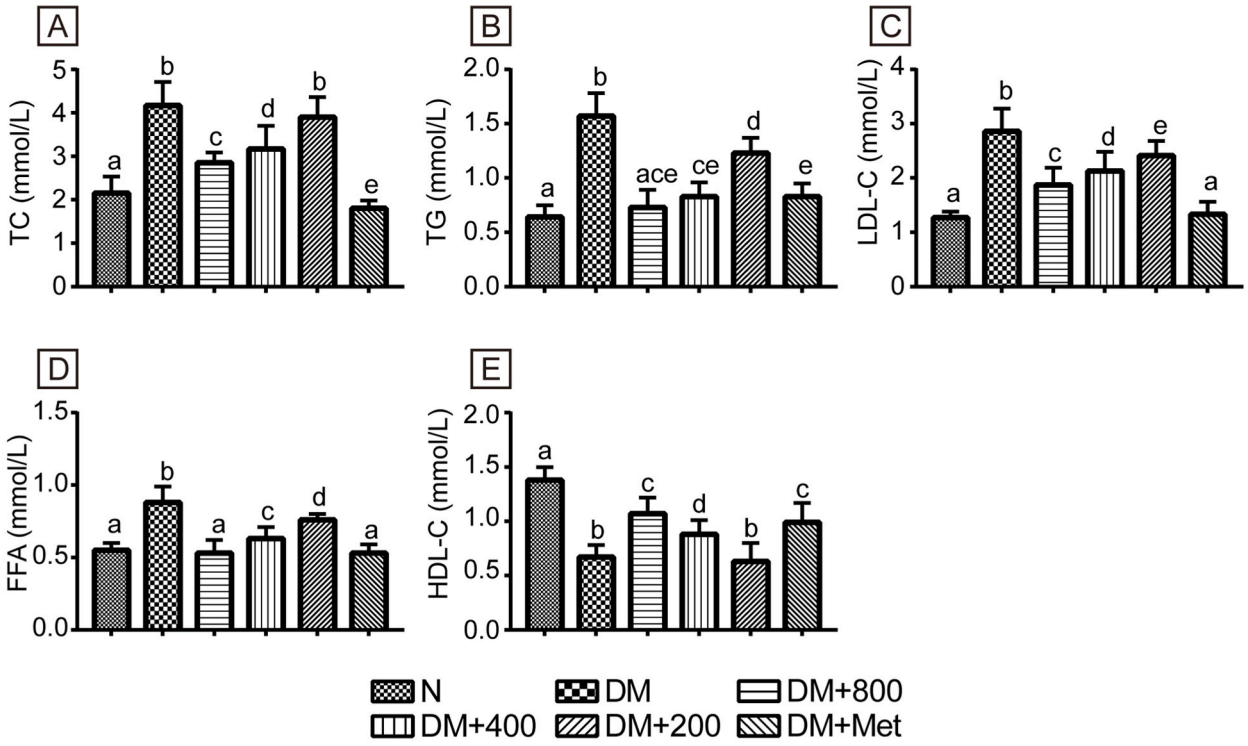

Fig. 3. Effect of allantoin on total cholesterol (TC) (A), triglycerides (TG) (B), low-density lipoprotein (LDL-C) (C), free fat acid (FFA) (D), high-density lipoprotein (HDL-C) (E) levels. The results presented as mean ± SEM (n = 8) were evaluated by one-way ANOVA followed by post hoc Tukey's tests (statistically significant if $p < 0.05$). Values which have the same superscript letter(s) in each graph are not significantly different. When no letters are present in the superscript, the ANOVA test was not significant. N= Normal rats; DM = type 2 diabetes mellitus rats; DM+800 = type 2 diabetes mellitus rats +800 mg/kg bw of allantoin; DM+400 = type 2 diabetes mellitus rats +400 mg/kg bw of allantoin; DM+200 = type 2 diabetes mellitus rats +200 mg/kg bw of allantoin; DM + Met = type 2 diabetes mellitus rats +200 mg/kg bw of metformin.

metformin, as evidenced by statistical significance ($p < 0.05$). Additionally, it was shown that metformin had greater efficacy in mitigating insulin resistance in rats with diabetes.

3.5. Effect of allantoin on kidney function

Diabetic nephropathy is a common complication associated with the kidneys, which are especially susceptible to high levels of glucose. The histological sections of the kidneys of diabetic rats (Fig. 5A–C) displayed modified glomerular architecture, suggesting renal dysfunction. In contrast, the administration of allantoin or metformin at doses of 200, 400, and 800 mg/kg to diabetic rats resulted in a significant amelioration of glomerular damage compared to diabetic rats. Additionally, urine volume (Fig. 5D and E) and urinary protein excretion (Fig. 5H–J), which serve as indicators of polyuria and proteinuria, respectively, were found to be significantly elevated in diabetic rats compared to normal rats ($p < 0.05$). However, treatment with allantoin at doses of 200, 400, and 800 mg/kg, as well as metformin, resulted in a significant reduction in urine volume and urinary protein levels in diabetic rats when compared to the diabetic group ($p < 0.05$). Additionally, it was noted that serum creatinine and BUN (Fig. 5F and G) exhibited a considerable increase, suggesting impaired renal function in diabetic rats compared to nondiabetic rats, leading to an inability to effectively eliminate waste products. The administration of allantoin at doses of 200, 400, and 800 mg/kg, as well as metformin, resulted in a substantial decrease in serum creatinine and BUN levels in diabetic rats. This suggests that both allantoin and metformin have a beneficial effect on renal damage ($p < 0.05$).

3.6. Effect of allantoin on liver function

The liver serves as the primary location for glucose metabolism, namely, facilitating the process of glycogenolysis. The liver histological observations in diabetic rats revealed aberrant morphology characterized by hepatocellular necrosis and vacuolization. However, diabetic rats that received treatment with allantoin or metformin at doses of 400, or 800 mg/kg exhibited few changes and exhibited liver structures that were comparable to those of healthy rats (Fig. 6A). In diabetic rats, the hepatic cholesterol (Fig. 6B), hepatic TG levels (Fig. 6C) and liver index (Fig. 6D) were considerably elevated, whereas ALT levels (Fig. 6E) and AST levels (Fig. 6F) were dramatically reduced ($p < 0.05$). The administration of allantoin at doses of 400 or 800 mg/kg to diabetic rats resulted in a

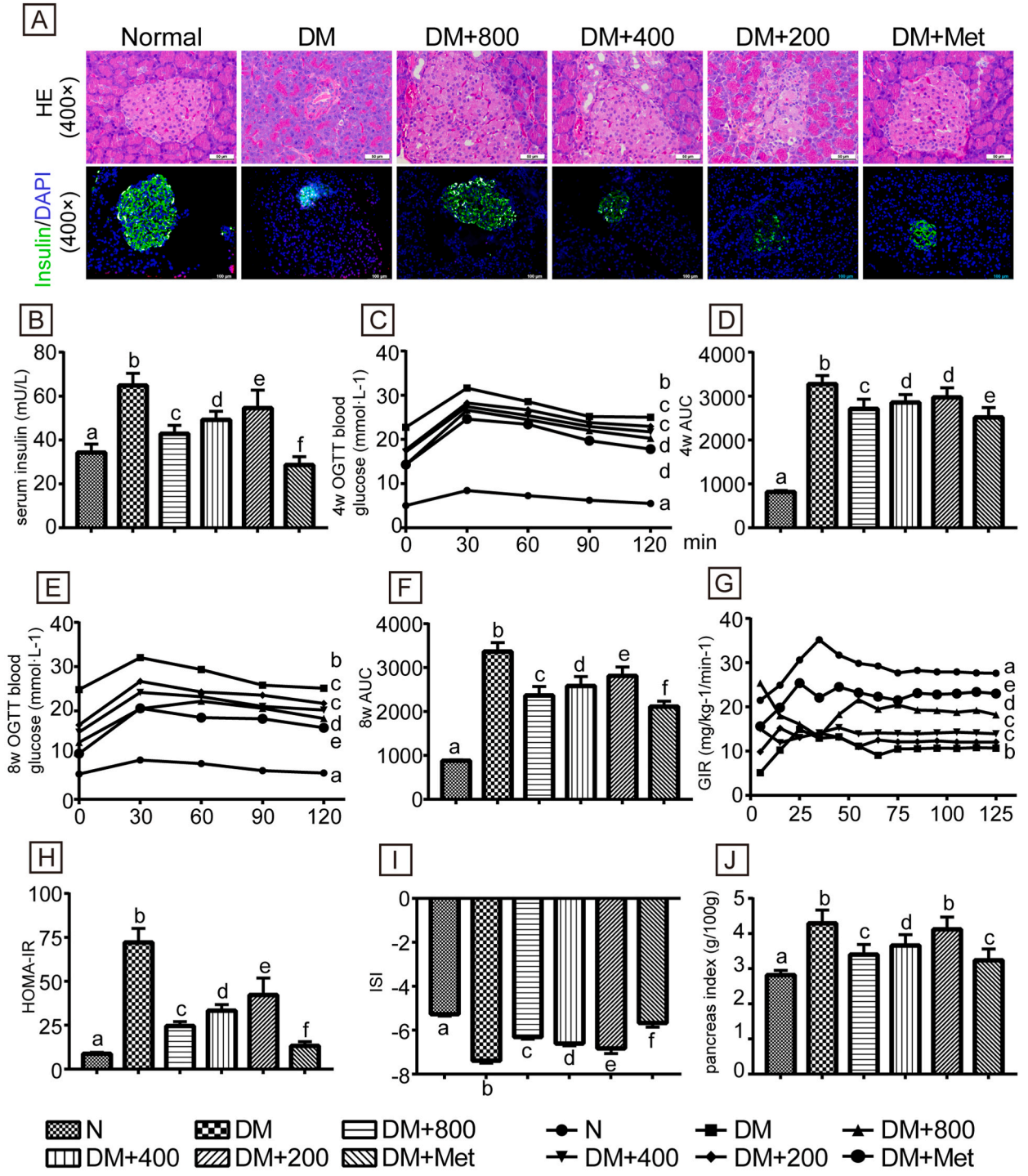

Fig. 4. Effects of allantoin on insulin resistance and pancreatic functions, including histology of pancreas (A), serum insulin (B), 4w OGTT blood glucose (C), 4w AUC (D), HOMA-IR (E), 8w OGTT blood glucose (F), 8w AUC (G), ISI (H), GIR (I), pancreas index (J). The results presented as mean ± SEM (n = 8) were evaluated by one-way ANOVA followed by post hoc Tukey's tests (statistically significant if $p < 0.05$). Values which have the same superscript letter(s) in each graph are not significantly different. When no letters are present in the superscript, the ANOVA test was not significant. N= Normal rats; DM = type 2 diabetes mellitus rats; DM+800 = type 2 diabetes mellitus rats +800 mg/kg bw of allantoin; DM+400 = type 2 diabetes mellitus rats +400 mg/kg bw of allantoin; DM+200 = type 2 diabetes mellitus rats +200 mg/kg bw of allantoin; DM + Met = type 2 diabetes mellitus rats +200 mg/kg bw of metformin.

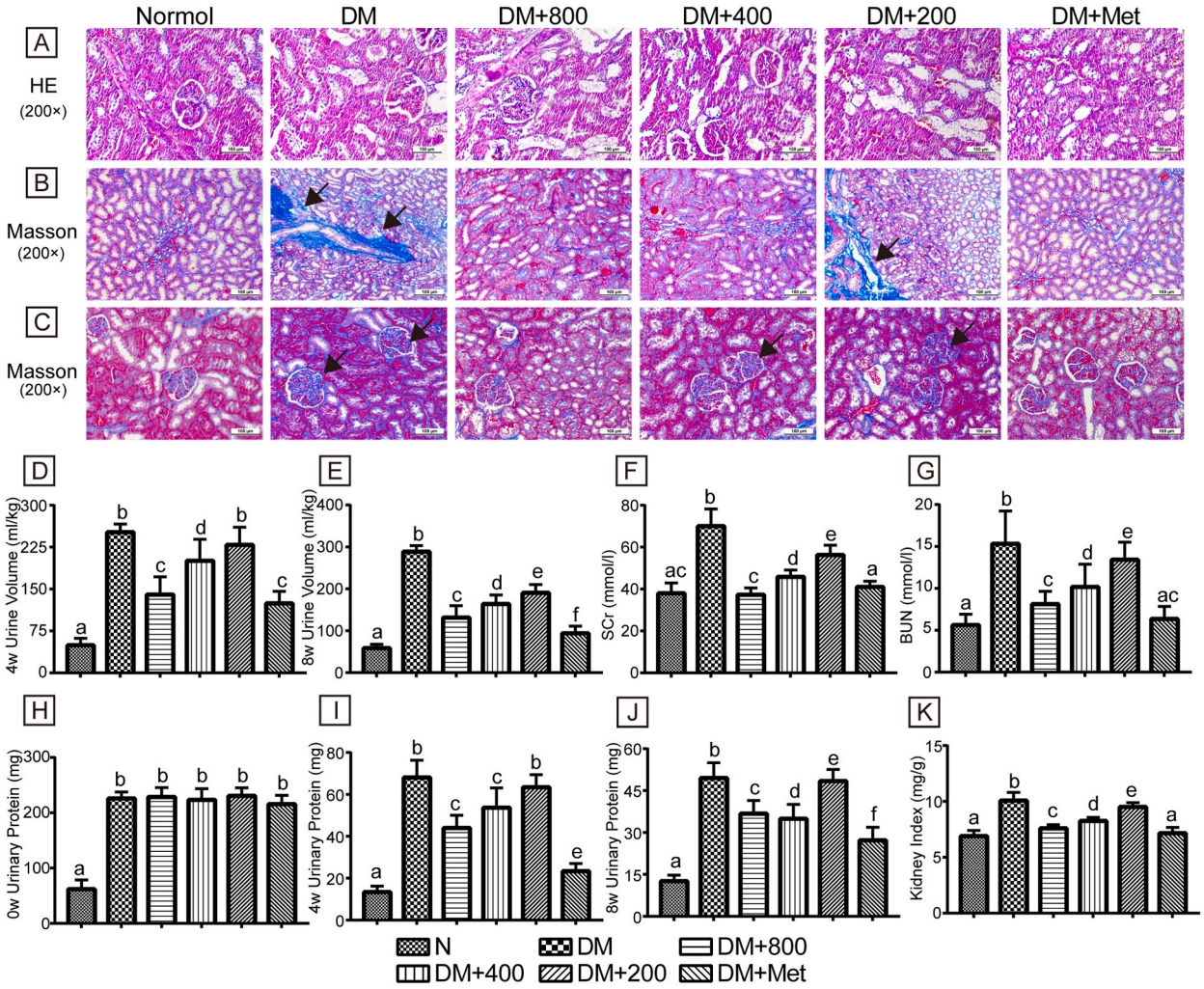

Fig. 5. Effect of allantoin on kidney function, including histology of kidney (A, B, C), 4w urine volume (D), 8w urine volume (E), SCr (F), BUN (G), 0w urinary protein (H), 4w urinary protein (I), 8w urinary protein (J), kidney index(K).The black arrow indicates the site of collagen deposition. The results presented as mean ± SEM (n = 8) were evaluated by one-way ANOVA followed by post hoc Tukey's tests (statistically significant if $p < 0.05$). Values which have the same superscript letter(s) in each graph are not significantly different. When no same letter(s) are present in the superscript, the ANOVA test was significant. N= Normal rats; DM = type 2 diabetes mellitus rats; DM+800 = type 2 diabetes mellitus rats +800 mg/kg bw of allantoin; DM+400 = type 2 diabetes mellitus rats +400 mg/kg bw of allantoin; DM+200 = type 2 diabetes mellitus rats +200 mg/kg bw of allantoin; DM + Met = type 2 diabetes mellitus rats +200 mg/kg bw of metformin.

substantial improvement in all measured parameters compared to diabetic rats ($p < 0.05$).

3.7. Effect of allantoin on anti-oxidant enzymes

Oxidative stress is the main mechanism leading to the pathogenesis of diabetic nephropathy. A large number of oxidative stress products accumulate in the body and damage organs. Fig. 7 depicts activities of enzymatic antioxidants such as SOD (Fig. 7A–C) and GSH (Fig. 7G–I) along with the levels of MDA (Fig. 7J-L) in serum, liver and kidney of normal and experimental rats. The results revealed significantly increased levels of MDA and significantly decreased activities of SOD, CAT and GSH in serum, liver and kidney of diabetic rats. However, after treatment of diabetic rats with 200, 400 or 800 mg/kg allantoin or metformin for 8 weeks significantly improved the oxidative status by means of significant decrease in levels of MDA and significant increase in activities of SOD and GSH in serum, kidney and liver ($p < 0.05$).

3.8. Effect of allantoin on pro-inflammatory parameters

Inflammatory response plays a key role in the occurrence and progression of diabetes mellitus. During IR, the secretion of TNF-α, IL-

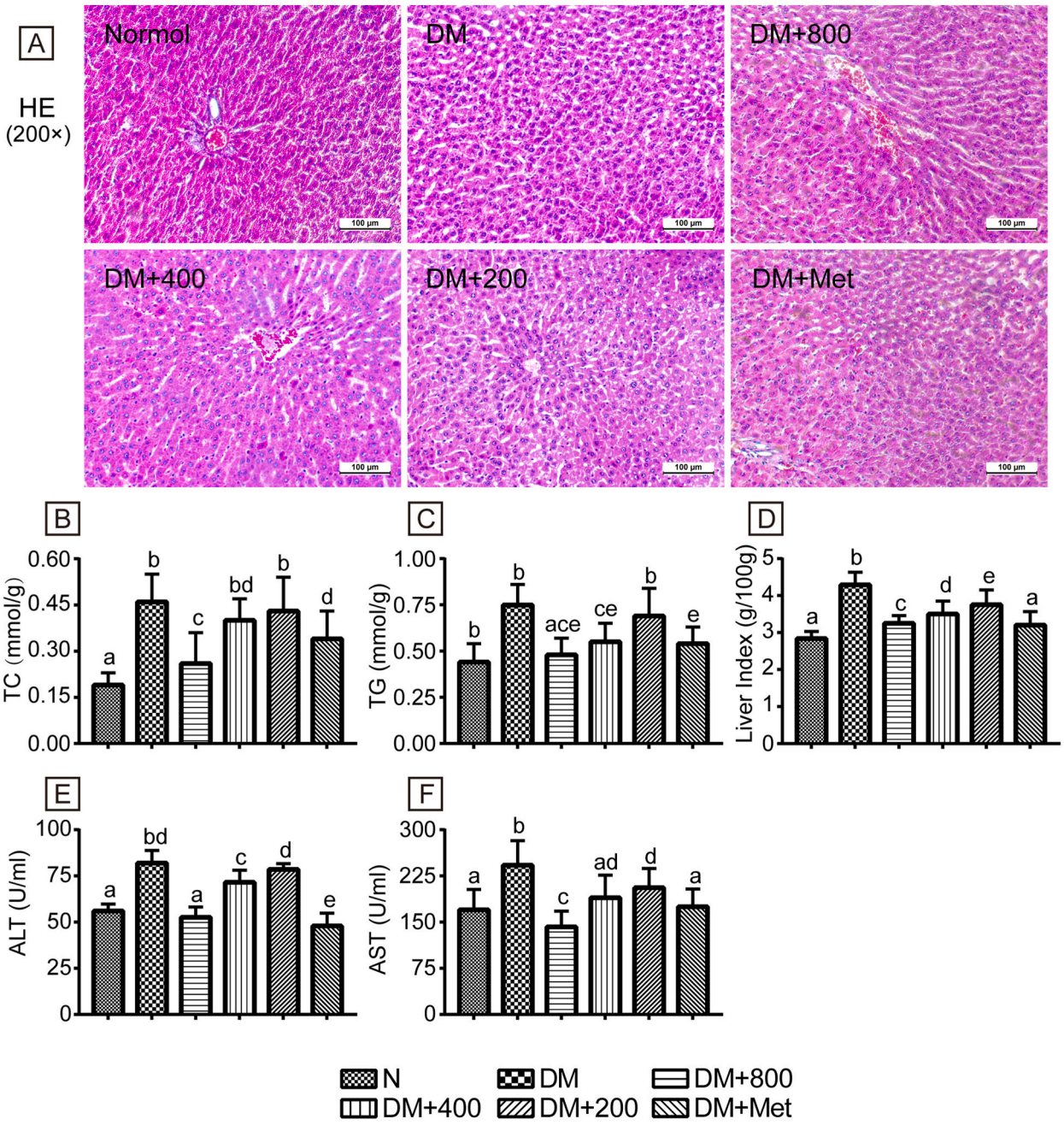

Fig. 6. Effect of allantoin on histology of liver (A), total cholesterol (TC) (B), (TG) (C), liver index, ALT (E), AST (F) level. The results presented as mean ± SEM (n = 8) were evaluated by one-way ANOVA followed by post hoc Tukey's tests (statistically significant if $p < 0.05$). Values which have the same superscript letter(s) in each graph are not significantly different. When no letters are present in the superscript, the ANOVA test was significant. N= Normal rats; DM = type 2 diabetes mellitus rats; DM+800 = type 2 diabetes mellitus rats +800 mg/kg bw of allantoin; DM+400 = type 2 diabetes mellitus rats +400 mg/kg bw of allantoin; DM+200 = type 2 diabetes mellitus rats +200 mg/kg bw of allantoin; DM + Met = type 2 diabetes mellitus rats +200 mg/kg bw of metformin.

1, IL-6, etc. increases. These factors can proliferate smooth muscle cells and increase endothelial permeability, and further secrete IL-1 and TNF-α to act on organs such as liver and kidney. According to the data shown in Fig. 7K-L, the levels of the proinflammatory cytokines IL-6 and TNF-α were considerably elevated in diabetic rats compared to normal rats ($p < 0.05$). In contrast, the concentration of IL-6 exhibited a significant decrease in diabetic individuals treated with allantoin at doses of 400 and 800 mg/kg in a dose-dependent pattern. Additionally, the concentration of TNF-α showed a significant reduction ($p < 0.05$) in allantoin-treated diabetic individuals, except for those receiving a dose of 200 mg/kg, when compared to the diabetic control group ($p < 0.05$).

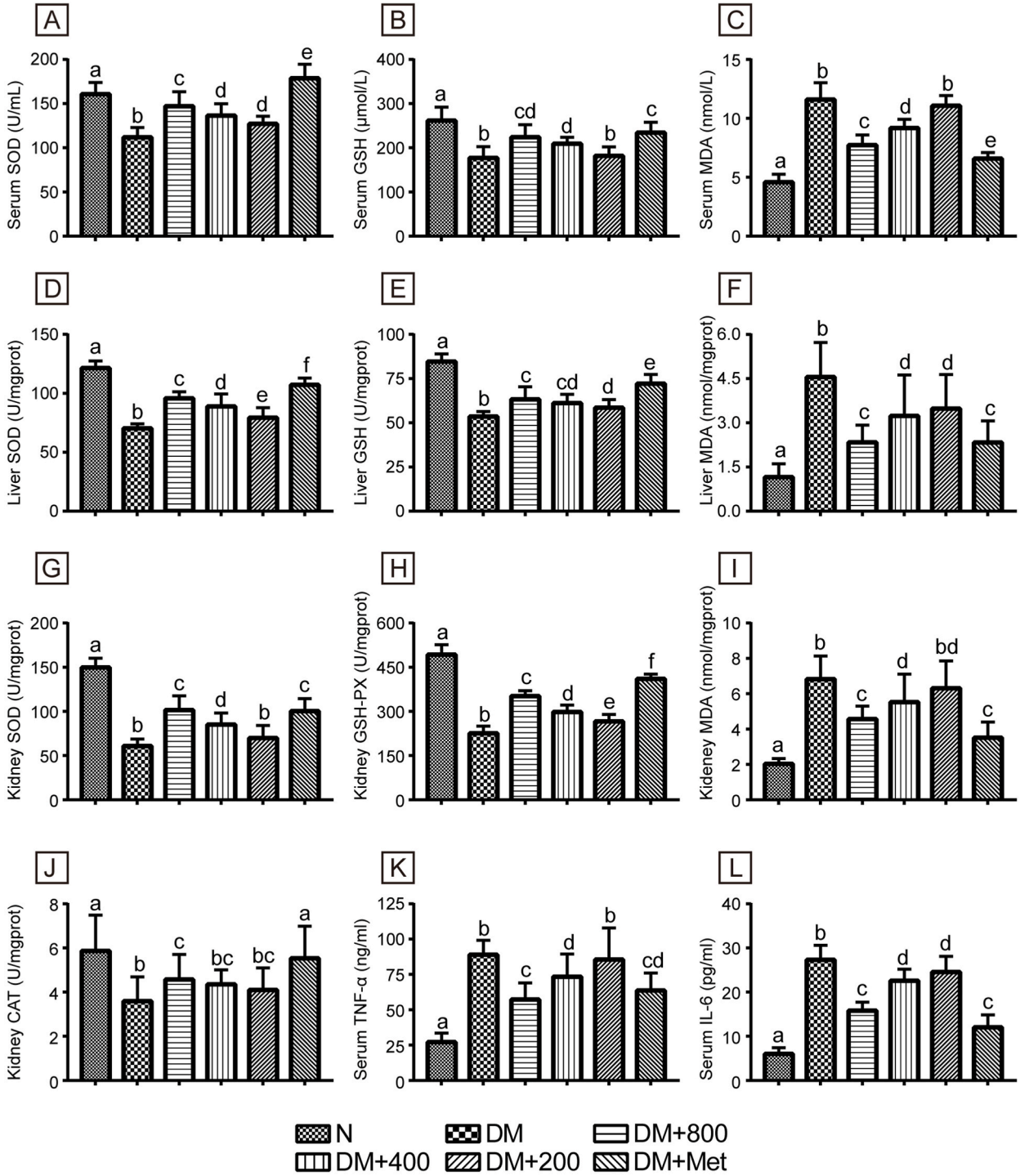

Fig. 7. Effect of allantoin on serum SOD (A), GSH (B), MDA (C), liver SOD (D), liver GSH (E), liver MDA (F), kidney SOD (G), kidney GSH-PX (H), kidney MDA (I), kidney CAT (J), serum TNF-α (K), serum IL-6 (L). The results presented as mean ± SEM (n = 8) were evaluated by one-way ANOVA followed by post hoc Tukey's tests (statistically significant if $p < 0.05$). Values which have the same superscript letter(s) in each graph are not significantly different. When no letters are present in the superscript, the ANOVA test was not significant. N= Normal rats; DM = type 2 diabetes mellitus rats; DM+800 = type 2 diabetes mellitus rats +800 mg/kg bw of allantoin; DM+400 = type 2 diabetes mellitus rats +400 mg/kg bw of allantoin; DM+200 = type 2 diabetes mellitus rats +200 mg/kg bw of allantoin; DM + Met = type 2 diabetes mellitus rats +200 mg/kg bw of metformin.

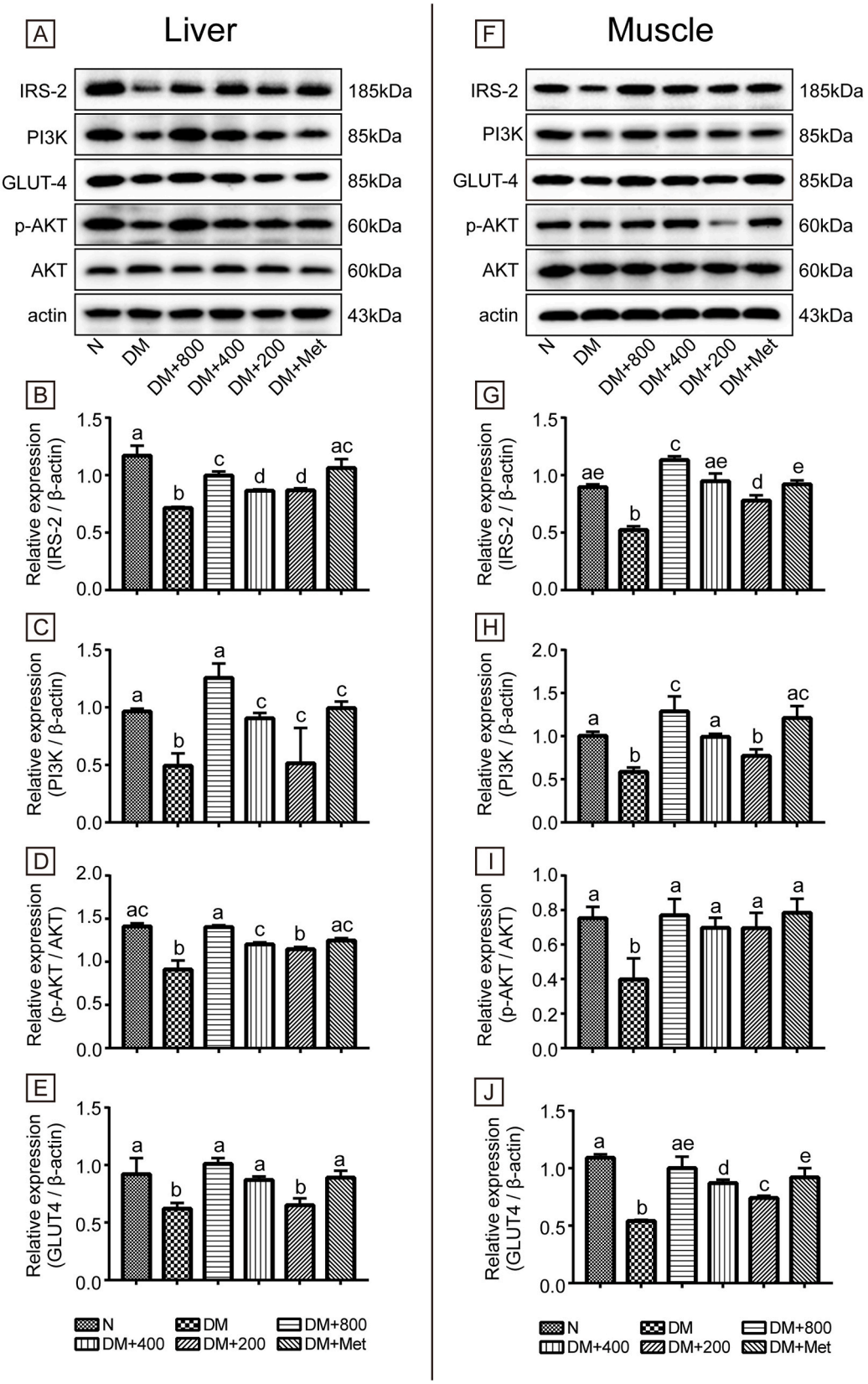

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Fig. 8. Western bolt analysis of IRS-2, PI3K, *p*-AKT, AKT and GLUT4 expression in the liver (A–D) and skeletal muscles (E–H). The results presented as mean ± SEM (n = 3) were evaluated by one-way ANOVA followed by post hoc Tukey's tests (statistically significant if $p < 0.05$). Values which have the same superscript letter(s) in each graph are not significantly different. When no letters are present in the superscript, the ANOVA test was not significant. N= Normal rats; DM = type 2 diabetes mellitus rats; DM+800 = type 2 diabetes mellitus rats +800 mg/kg bw of allantoin; DM+400 = type 2 diabetes mellitus rats +400 mg/kg bw of allantoin; DM+200 = type 2 diabetes mellitus rats +200 mg/kg bw of allantoin; DM + Met = type 2 diabetes mellitus rats +200 mg/kg bw of metformin.

3.9. *Effect of allantoin on the PI3K/AKT pathway in liver and skeletal muscle in diabetic rats*

Western blot studies were performed to evaluate proteins extracted from the liver and skeletal muscle. In our study, the levels of IRS-2, PI3K *p*-Akt/Akt, and GLUT4 expression were downregulated in the liver (Fig. 8A–E) and skeletal muscle (Fig. 8F–J) of diabetic rats compared to normal rats. Following an 8-week treatment period, the observed levels of GLUT-4 protein in various allantoin groups were found to be significantly greater than those in the diabetic group ($p < 0.05$, as seen in Fig. 6). The protein levels of IRS-2, PI3K and *p*-Akt exhibited a significant rise in the groups treated with allantoin at doses of 400 and 800 mg compared to the T2DM group ($p < 0.05$).

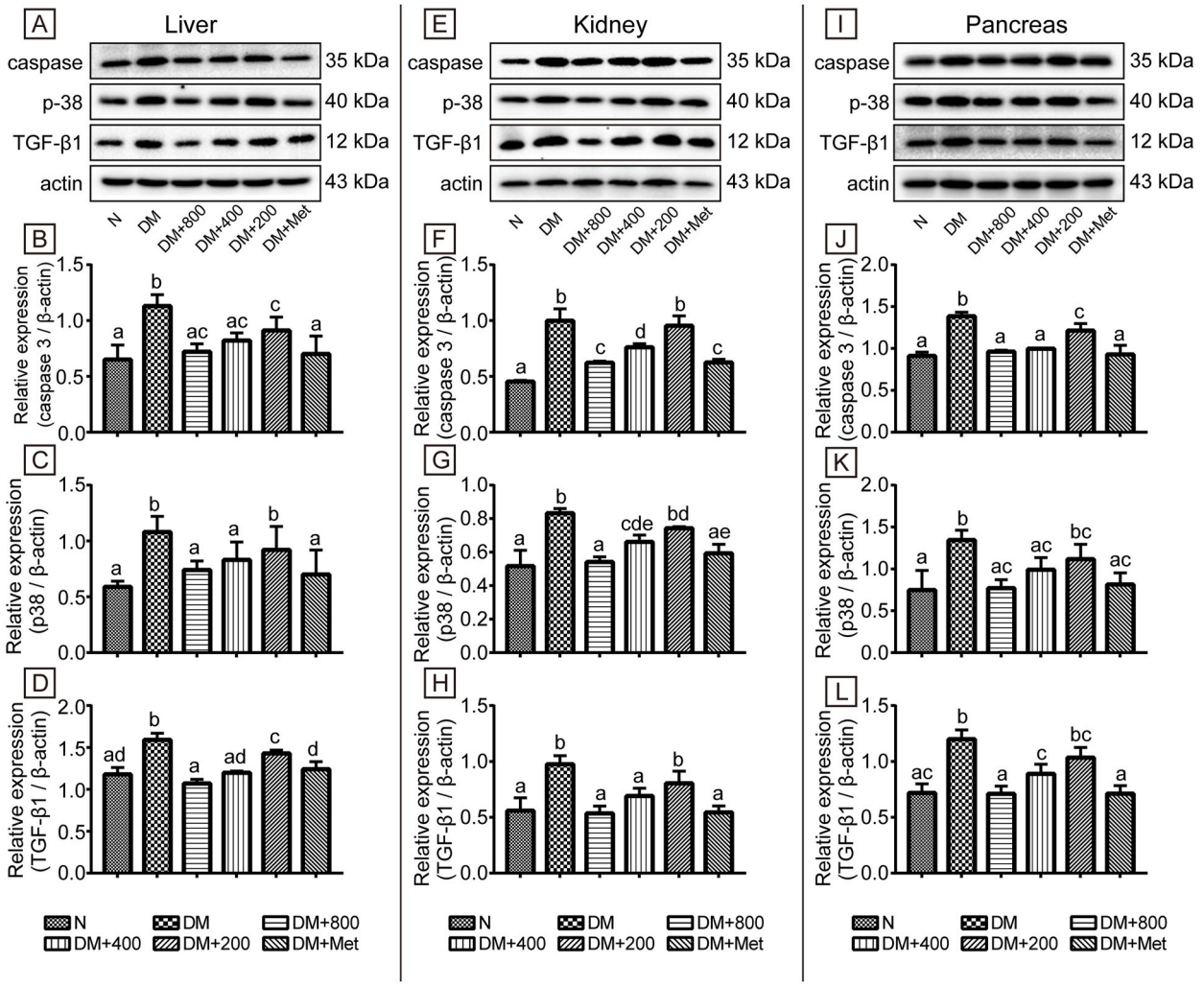

Fig. 9. Western bolt analysis of caspase, p-38, and TGF-β in the liver (A–D), kidney (E–H) and pancreas (I–L). The results presented as mean ± SEM (n = 3) were evaluated by one-way ANOVA followed by post hoc Tukey's tests (statistically significant if $p < 0.05$). Values which have the same superscript letter(s) in each graph are not significantly different. When no letters are present in the superscript, the ANOVA test was not significant. N= Normal rats; DM = type 2 diabetes mellitus rats; DM+800 = type 2 diabetes mellitus rats +800 mg/kg bw of allantoin; DM+400 = type 2 diabetes mellitus rats +400 mg/kg bw of allantoin; DM+200 = type 2 diabetes mellitus rats +200 mg/kg bw of allantoin; DM + Met = type 2 diabetes mellitus rats +200 mg/kg bw of metformin.

3.10. Effect of allantoin on the TGF-β/p38/caspase-3 pathway in liver, kidney and pancreas in diabetic rats

To ascertain the potential correlation between the hypoglycemic effects of allantoin and the regulation of the p38 MAPK pathway, we conducted an analysis of the protein expression levels of TGF-β, p38, and caspase-3 in the liver (Fig. 9A–D), kidney (Fig. 9E–H), and pancreas (Fig. 9I-L) using Western blot analyses. The findings of the study indicate that diabetic rats exhibited a significant increase in the protein expression of TGF-β, p38, and caspase-3 in comparison to normal rats. However, administration of allantoin at doses of 400 and 800 mg/kg effectively reduced the production of TGF-β, p38, and caspase-3 at the protein level, with statistical significance ($p < 0.05$).

4. Discussion

In this study, we utilized a rat model of type 2 diabetes generated by a high-fat diet to examine the effect of allantoin on decreasing insulin resistance and compared it to the effect that metformin had on the same rats. The results showed that allantoin was more effective than metformin at lowering insulin resistance. This model of type 2 diabetes in rats is an excellent depiction of human insulin resistance and an accurate picture of type 2 diabetes in humans. In the current study, rats that were fed a diet high in fat were given injections of beta cell toxin at low dosages together with STZ. The injection of these substances induced the development of type 2 diabetes in the rats. Numerous previous studies have demonstrated that insulin resistance is attributable to a high-fat diet, while hyperglycemia is induced by a low dose of STZ injection. This consistent correlation between the two conditions was confirmed [18].

The results of this study demonstrate that rats with diabetes exhibited significantly elevated blood insulin levels compared to non-diabetic rats. However, after the administration of allantoin, there was a marked decrease in blood insulin concentrations in diabetic rats, along with a significant reduction in HOMA-IR and a substantial increase in ISI. To induce changes in insulin resistance within an animal model of type 2 diabetes, the gold standard hyperinsulinemic-euglycemic clamp test was employed in this investigation [19]. The clamp approach is a commonly used technique in both clinical and experimental settings to assess the ability of a substance to influence insulin receptor responsiveness [20]. Additionally, diabetic rats exhibited a significant decline in glucose tolerance and GIR, which serves as a confirmation of their insulin resistance. The findings of the OGTT and hyperinsulinemic-euglycemic clamp test indicated significant improvements in diabetic rats following therapy with allantoin. The AUC results obtained from the OGTT and GIR indicate that therapy with allantoin, similar to metformin, resulted in significant enhancements in insulin sensitivity. This improvement may be attributed to an increase in glucose uptake and utilization. In general, these findings suggest that the improvement in glucose tolerance observed with allantoin administration is mostly attributed to an increase in insulin sensitivity in peripheral tissues. The findings combined suggest that allantoin has the potential to improve insulin resistance and exhibits strong insulin-sensitizing effects.

Insulin resistance, a defining feature of T2DM, can lead to the disruption of insulin-dependent metabolic pathways, such as glycogen production and glucose transportation [21,22]. Glycogen is a significant indicator of glycometabolism, especially in terms of glucose distribution and uptake. It is the primary storage form of glucose within living organisms [23]. It has been reported that impaired glycogen synthesis could promote the occurrence of hyperglycemia and diabetes [24,25]. The primary strategy for managing T2DM involves activating glucose uptake in tissues responsive to insulin, such as the liver, skeletal muscle, and adipose tissue, which are responsible for synthesizing glycogen [26]. In the present investigation, it was revealed that rats with diabetes exhibited a reduction in muscle glycogen content. Additionally, a drop in hepatic glycogen content was observed in diabetic rats, which is likely attributable to the diminished responsiveness of target tissues to insulin. The glycogen level in the liver and skeletal muscle was significantly increased with the administration of allantoin. In summary, our findings demonstrated that the administration of allantoin resulted in a reduction in blood glucose levels, suggesting a hypoglycemic impact. This effect was likely mediated by an increase in glycogen synthesis. In the context of diabetes, the presence of insulin resistance and/or insufficiency results in the inactivation of lipoprotein lipase, causing a state of hyperglyceridemia [27]. The present study found that the induction of type 2 diabetes in rats led to elevated levels of total cholesterol, triglycerides, and LDL while simultaneously reducing levels of HDL. These observed results align with previous studies reported in the literature. The application of allantoin resulted in a reduction in serum and hepatic TC and TG, as well as serum FFA and LDL-C. The groups who received allantoin treatment also showed a significant improvement in HDL-C levels.

Furthermore, prior studies have established a correlation between oxidative stress agents and the accumulation of excessive levels of fat [28,29]. Oxidative stress possesses the capacity to induce an inflammatory response, hence promoting IR. This is achieved by the overexpression of several signaling molecules associated with inflammatory pathways in adipose tissues and the liver, which subsequently hinder the proper functioning of insulin signaling [30]. In reaction to the initiation of persistent inflammation, a variety of inflammatory mediators, including TNF-α and IL-6, are secreted. These mediators have the potential to exacerbate IR and diminish tissue sensitivity to insulin, thus restricting the uptake of glucose [31,32]. This study observed a considerable increase in indicators of oxidative state, namely, higher levels of MDA (a measure of lipid peroxidation), along with a significant decrease in the activity of SOD, CAT, and GSH-Px in the blood, liver, and kidney of rats with diabetes. The current findings align with previous observations, allantoin had a mitigating effect on oxidative stress and inflammation through its ability to enhance the activity of antioxidant defense mechanisms and decrease the secretion of cytokines associated with inflammation.

The PI3K/AKT signaling pathway is considered a canonical insulin route due to its involvement in facilitating glucose absorption in hepatic and skeletal muscle tissues. The reduction or inhibition of this route has the potential to mitigate the physiological consequences of insulin, hence potentially mitigating IR. The hormone insulin binds to the insulin receptor (InsR) located on the surface of hepatocytes [33]. The tyrosine kinase domain of the InsR inner-membrane region is responsible for phosphorylating IRS-2. IRS-2 may

be involved in several liver pathways, namely, in the activation of PI3K. This activation subsequently leads to the translocation of Akt from the cytoplasm to the cytomembrane [34]. Akt is activated by PDK1, either directly or indirectly [35]. The activation of Akt facilitates the transmission of signals to the receptor substrate located downstream, resulting in the generation of several biological consequences, including glucose absorption, glycolysis, glycogen synthesis, and protein synthesis [36]. To obtain deeper insights into the effects of allantoin on IR, PI3K/Akt signaling pathway-related proteins, namely, IRS-2, PI3K, *p*-AKT, and Glut4, were examined in the liver and skeletal muscle using Western blot analysis. The protein expression levels showed a substantial drop in diabetic rats. Following the administration of allantoin, there was an observed rise in the levels of protein expression. This increase suggests that allantoin may serve as a mitigating agent for IR in all tissues impacted by insulin. Hence, it is justifiable to propose that the potential enhancement of IR by allantoin might be attributed to its influence on the PI3K/Akt pathway.

The potential mechanism by which allantoin exerts its effects may involve suppressing proinflammatory cytokines through the regulation of the p38 MAPK pathway [37]. Activation of p38 MAPK has been reported in various organs, including the liver, kidney, and pancreas, in individuals with T2DM [38]. There is evidence supporting the idea that elevated glucose levels can initiate the activation of the p38 MAPK signaling pathway within various tissues, p38 activation leads to an upregulation of caspase-3, an enzyme involved in programmed cell death. Elevated levels of this enzyme have been observed in rats with diabetes, indicating the initiation of apoptosis in tissues [39,40]. The aforementioned results were validated in our investigation, wherein elevated glucose levels were shown to activate the cascade of oxidative stress, hence inducing the inflammatory process and apoptosis. These effects are mediated by the upregulation of TGF-β, p38 MAPK, and caspase-3 expression. In this study, it was shown that the suppression of p38 MAPK by allantoin led to a substantial decrease in the expression of TGF-β, p38 MAPK, and caspase-3 activity. These findings provide evidence for the antiapoptotic effects of allantoin in diabetic rats.

5. Conclusions

This study aimed to investigate the antidiabetic effects of allantoin on rats with a high-fat diet and streptozotocin-induced type 2 diabetes. Additionally, it examined the potential mechanism of action involving the PI3K/Akt/GLUT-4 signaling pathway. The findings of the study indicate that allantoin has hypoglycemic action, which is dosage-dependent, as evidenced by the analysis of numerous biochemical markers. Moreover, the study observed that allantoin can enhance the expression of key proteins involved in the PI3K/Akt signaling pathway, including IRS-2, PI3K-p85, *p*-Akt, and GLUT-4. This suggests that allantoin effectively stimulates the insulin signaling pathway. Additionally, allantoin demonstrated the ability to decrease the expression of proinflammatory cytokines, such as IL-6 and TNF-α, by enhancing insulin sensitivity and regulating blood glucose levels. This mechanism ultimately prevents the exacerbation of insulin resistance caused by inflammation. Thus, it can be asserted that allantoin exhibits antidiabetic effects in type 2 diabetes mellitus by activating the PI3K/Akt/GLUT-4 signaling pathway and mitigating inflammation-induced insulin resistance. Furthermore, the study showed that allantoin attenuates liver, kidney, and pancreatic injury under conditions characterized by excessive sugar and fat intake, mainly through its effect on the MAPK signaling pathway, reducing tissue cell apoptosis.

Limitation

Despite conducting animal trials to assess the therapeutic impact of allantoin on diabetic rats, there is still a lack of comprehensive understanding of the particular pathways via which allantoin exerts its effects. Additional investigation is warranted to explore the potential of allantoin in exerting comparable protective effects on hepatocytes or myocytes in an in vitro setting, as well as its potential to ameliorate IR. The present work has demonstrated that the therapeutic efficacy of allantoin is, in part, ascribed to its modulation of the insulin signaling pathway and MAPK pathway. However, the specific molecular target of allantoin has yet to be identified. Subsequent investigations may prioritize the elucidation of the specific targets of allantoin inside an organism, thereby establishing a foundation for preclinical research aimed at developing therapeutic interventions for diabetes.

Ethics approval

All the experiments were conducted in strict compliance with the animal welfare and ethical principles of the center of animal experiments of the Xinjiang Medical University and approved by the Ethics Committee of the Animal Experimental Center of the center of animal experiments of the Xinjiang Medical University (confirmation number: IACUC-20220620-5).

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Data availability statement

Data included in article.

CRediT authorship contribution statement

Yao Zhao: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Ming Qiao:** Methodology, Investigation, Data curation. **Xiaomei Wang:** Visualization, Supervision, Software, Data curation. **Xinjie Luo:** Writing – review & editing, Conceptualization. **Jianhua Yang:** Validation, Supervision, Investigation. **Junping Hu:** Writing – review & editing, 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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