Anti-diabetic Role of Adropin in Streptozotocin Induced Diabetic Rats via Alteration of PI3K/Akt and Insulin Signaling Pathway

Li He, Feng-Jiao Zhang, Hao-Yun Li, Lei Li, Li-Ge Song, Yu Mao, Jing Li, Hong-Mei Liu, Feng-Li Li, Ling-Yu Xu, Ya-Jie Huo, Huan-Huan Wang, Fang Luo, and Zhi-Qiang Kang*

Department of Endocrinology, Zhengzhou Central Hospital Affiliated to Zhengzhou University, Zhengzhou City, Henan Province, 450007, CHINA

1 Introduction

Worldwide, diabetes mellitus (DM) has become the 3rd most common disease that affected the large population after the cardiovascular disease and cancer. According to the study 5% of death induced by the DM recorded worldwide, and the figures reached almost 50% in the next few decades1-2. DM is considered as the most common disease whole over the world. DM is a multifactorial disease related with the hyperglycemia and enhanced risk of microvascular and micro-vascular complications associated with this disease3-4. DM and its complication major causes of mortality and morbidity. As pre the reports approximately 424.9 million people at an aged of 20-79 affected from the DM in 2017 worldwide5. DM is a metabolic disease characterized as inadequate secretion of insulin form the pancreatic β-cells in type I and if insulin release from the pancreatic β-cells and not utilize via related organ/tissue cells of the body during the type II6-7. Type II DM has been the most common widespread disease since most people are affected from this complex metabolic disease. Besides, almost large budget of health care and will became the higher financial burden on health in future. Clinical diagnosis study suggests that the 70% of host pancreatic β-cell is destroyed as a significance of immune related processes6-7. Hyperglycemia, inflammation, oxidative stress and hyperlipidemia are the significant characters of DM and show the major risk factor for the expansion of DM and its complication. Till date, available treatment for DM such as insulin therapy and numerous therapies include α-glucosidase inhibitors, sulfonylureas and thiazolidinediones. These treatments most use as monotherapy or in different combination to accomplish the improved glycemic control8.
Good glycemic control drug delays the expansion of diabetic complications, but does not minimize the diabetes\(^{10}\). The above discuss treatment having the limitation due to the serious side effects. Alternate therapy is the best approaches to treat the diabetes due to multiple effect such as antihyperlipidemic, antioxidant and antihyperglycemic effect along with long term safety\(^3,11\). Therefore, antidiabetic drug therapy focuses on the medicinal plants to offer the new promising efficient drugs with less or no side and adverse effect\(^7\). Previous research suggests that the phyto-constituents isolated from the herbal medicinal plants used in the treatment of numerous pathologies/diseases such as diabetes mellitus, cancer, high blood pressure and cardiovascular diseases\(^3,4\). Plant based phyto-constituents play a significant role in the discovery of new therapeutic agents and getting the more attention as a source of bioactive substance such as hypoglycaemic, hypolipidemic and anti-oxidant agents. Till date, more than 800 plant species already scrutinized against the diabetes mellitus\(^3,4,11\).

It is well known that adropin is the gene associated with energy homeostasis and that protein release has been suggested. Previous studies indicate that dietary nutrients can alter gene expression and the amount of adropin may also circulate. Studies also indicate that in obesity mice the adropin can improve dyslipidemia and glucose homeostasis. In addition, adropin has decreased the expression of PDK4 and increased the utilization of glucose. The current experimental study was to investigate the antidiabetic activity of adropin against diabetic rats caused by streptozotocin (STZ) by altering the signaling pathway of PI3K/Akt and insulin.

### 2 Material and Methods

#### 2.1 Chemical

Adropin, streptozotocin (STZ), glibenclamide were purchased from the Sigma Aldrich (St. Louis, U.S.A.). Lipid parameters were estimated using kits (Sigma Aldrich, USA). Primary antibodies include IR-2, IR-1, IR, Akt, P3K, p-Akt, AMPK and β-actin were procured from the Cell Signaling Technology (Danvers, MA, U.S.A.). All the chemical used in the experimental study was analytical grade.

#### 2.2 Experimental animals

For the experimental protocol, Swiss Albino Wistar rats (6-8 weeks old, 125-150 g body weight, male) were used. All the rats were kept under the specific pathogen free conditions in standard laboratory environment at 22-25°C (temperature) with a 40-60% (relative humidity) and 12/12 h dark/light cycle. The rats were received the water *ad libitum* and standard rodent chow. The rats were acclimated for 2 weeks before the experiment. The whole protocol follows the “Principles of laboratory animal care”.

#### 2.3 Induction of diabetes

Freshly prepared 60 mg/kg streptozotocin (STZ) single intraperitoneal injection into a 0.1 M citrate buffer (pH = 4.5)\(^8,12\). With the exception of normal monitoring, all rats obtained a single dose of STZ for the induction of diabetes with an equivalent volume of the vehicle (0.1 M citrate buffer). After 7 days, treated the STZ, blood glucose level (BGL) were estimated using the glucose estimation kits. Rats with BGL greater than 350 mg/dL were known to be diabetic and were used for further testing.

#### 2.4 Experimental study

In short, the rats were split into six groups, each group containing six rats. The rats were group as follow:

- **Group A**: normal control received (0.25 % carboxymethyl cellulose)
- **Group B**: diabetic control
- **Group C**: diabetic control received glibenclamide (10 mg/kg)
- **Group D**: diabetic control received adropin (20 mg/kg)
- **Group E**: diabetic control received adropin (40 mg/kg)
- **Group F**: diabetic control received adropin (80 mg/kg), respectively.

After successfully administration of STZ, the rats were received the orally administration of above mention treatment for 28 days\(^8\). The BGL and body weight of all rats in the group were estimated at regular time intervals. At the end of the experimental research, blood samples of the entire group of rats were collected and centrifuged for 15 minutes at 15000 rpm to separate the serum and processed for further biochemical assays at \(-20°\)

#### 2.5 Estimation of liver glycogen and blood glucose levels

For the estimation of the blood glucose level, a single touch glucometer was used. Anthrone was used to estimate liver glycogen using the previously described procedure with minor modifications\(^8,12\).

#### 2.6 Estimation of lipid profile

Lipid parameters including high density lipoprotein (HDL), total cholesterol (TC) and triglyceride (TG) were estimated using the standard kits (Sigma Aldrich, USA) according to following the manufacture instruction. For the estimation of very low density lipoprotein (VLDL) and low density lipoprotein (LDL), the following formula

\[
\text{LDL (mg/dL)} = \text{TC} - \text{HDL} - (\text{TG}/5)
\]

\[
\text{VLDL (mg/dL)} = \text{TC} - \text{HDL} - \text{LDL}
\]

#### 2.7 Estimation of oxidative stress

Oxidative stress markers such as thiobarbituric acid reactive substances (TBARS) and protein carbonyl (PC) have been measured in liver tissue\(^13,14\). The oxidative stress...
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marker such as reduced glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx) and super oxide dismutase (SOD) were estimated in the pancreas tissue \(^{9,12,16}\).

### 2.8 qRT-PCR analysis

10 mg of pancreatic tissue samples from each group were used to test the mRNA expression of the target gene and the total mRNA was isolated using the TriZol reagent. The mRNA purification was done using the RNeasy mini kit. The primer sequences were as follows: β-actin, 5’-CCT-GAGCAGGAATCTGCTGT-3’ (forward), 5’-GCTGATCCACATCTGCTGGAA-3’ (reverse); PPARγ, 5’-CCAGAGCTGCTGATCTGCG-3’ (forward), 5’-GCCACCTTGTTCCTCTGCTC-3’ (reverse) and GLUT4, 5’-GACATTTGGCGGAGCCTAAC-3’ (forward) and 5’-TAACTCCAGGAGGTGACACAG-3’ (reverse).

### 2.9 Statistical analysis

The information is expressed as mean ± SEM and evaluated by one-way variance analysis (ANOVA), followed by a multiple Tukey comparison test or an unpaired Student t-test using GraphPad Prism 5.0 software (GraphPad, San Diego, CA, USA). There were called statistically relevant differences of \(p < 0.01\).

### 3 Results

#### 3.1 Blood glucose level and plasma insulin

During the DM, glucose level considerably boosted and insulin level decreased due to expansion of disease. Figure 1a showed the blood glucose level of all group of rats. STZ induced DM rats demonstrated the augmented blood glucose level throughout the experimental study and glibenclamide treatment significantly \((p < 0.001)\) suppress the blood glucose level. Adropin (20 and 40 mg/kg) treatment significantly \((p < 0.001)\) decreased the blood glucose level. Adropin (80 mg/kg) decreased the blood glucose level and reached almost near to the normal group rats (Fig. 1a).

Plasma insulin level notably decreased in the STZ induced DM group rats. Adropin significantly \((p < 0.001)\) boosted the plasma insulin level and reached near to the normal control group rats (Fig. 1b). Glibenclamide treated rats exhibited the increased level of plasma insulin level.

### 3.2 Body weight

Decrease the body weight is the main complication of diabetes mellitus. STZ induced rats demonstrated the reduced body weight (final) as compared to the initial body weight. Normal control and treated group rats (adropin and glibenclamide) significantly \((p < 0.001)\) increased the body weight (final) as compared to final body weight (Fig. 2).

### 3.3 Food intake

Due to break the pancreatic β-cells, the diabetes patient and rodent increased the food intake. Normal group rats exhibited the normal pattern of food intake throughout the complete experimental study. STZ induced DM rats demonstrated the enhanced food intake as compared to initial food intake (compared with the initial food intake). Adropin (20 and 40 mg/kg) significantly \((p < 0.001)\) reduced the food intake as compared to STZ induced DM rats. Adropin significantly \((p < 0.001)\) down-regulated the food intake and exhibit the normal pattern of food intake (Fig. 3). Glibenclamide significantly \((p < 0.001)\) decreased the food intake.

### 3.4 Carbohydrate enzymes

The increased level of glucose-6-phosphatase, fructose-1,6 biphosphatase and decreased level of hexokinase, glycogen, G-6-PDH were reported by STZ induced control group rats compared to normal control rats. Adropin sig-

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**Fig. 1** The effect of adropin on the blood glucose and plasma insulin level of STZ induced DM rats. a: blood glucose level and b: plasma insulin. One way-ANOVA followed by Dennett’s comparison test; \(* p < 0.05\), \(** p < 0.01\) and *** \(p < 0.001\).
nificantly \( p < 0.001 \) reduced the amount of glucose-6-phosphatase, fructose-1,6 biphosphatase and increased dose-dependent levels of hexokinase, glycogen, and G-6-PDH (Fig. 4).

3.5 Lipid parameter
The main complications of diabetes mellitus are hypertriglycemia and hypercholestemia. During DM, the lipid profile changed and insulin decreased due to a rise in blood glucose. Compared to normal control levels, triglycerides, cholesterol, low-density lipoprotein, very low-density lipoprotein, and high-density lipoprotein levels were elevated in the STZ mediated control group. Adropin significantly \( p < 0.001 \) decreased triglyceride, cholesterol, low-density lipoprotein, very low-density lipoprotein levels, and increased high-density lipoprotein dose-dependent levels. A similar result was reported in the lipid profile group of rats treated with glibenclamide (Fig. 5).

3.6 Antioxidant
During the diabetes mellitus, reduced the endogenous antioxidant parameters due to expansion of disease. STZ induced rats demonstrated the increased level of TBARS, protein carbonyl and reduced level of GSH, SOD, CAT as compared to other group (normal and treated group rats). Adropin significantly \( p < 0.001 \) decreased the TBARS, protein carbonyl level and boosted the level of GSH, SOD, CAT at dose dependently (Fig. 6).

3.7 HbA1c and C. peptide level
The STZ-induced rats showed an increase in HbA1c and a decrease in C levels. As opposed to control group mice, peptide. Adropin substantially \( p < 0.001 \) decreased the level of HbA1c and increased the level of C. peptide as a dose dependent (Fig. 7).

Fig. 3  The effect of adropin on the food intake of STZ induced DM rats. One way-ANOVA followed by Dennett’s comparison test; * \( p < 0.05 \), ** \( p < 0.01 \) and *** \( p < 0.001 \).

Fig. 4  The effect of adropin on the carbohydrate enzymes of STZ induced DM rats. a: Fructose 1-6-biphosphatase, b: Glucose-6-phosphatase, c: G-6-PHD, d: hexokinase and e: glycogen. One way-ANOVA followed by Dennett’s comparison test; * \( p < 0.05 \), ** \( p < 0.01 \) and *** \( p < 0.001 \).

Fig. 5  The effect of adropin on the lipid parameters of STZ induced DM rats. a: LDL, b: HDL, c: triglyceride, d: total cholesterol and e: VLDL. One way-ANOVA followed by Dennett’s comparison test; * \( p < 0.05 \), ** \( p < 0.01 \) and *** \( p < 0.001 \).
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3.8 AMPK pathway

Figure 8 showed the effect of adropin on the AMPK pathway and insulin signalling in the STZ induced DM rats. The expression of IR, IRS-2, PKB, IRS-1, Akt, AMPK and p-AMPK altered in the STZ induced DM rats and dose dependently treatment of adropin significantly \( p < 0.001 \) modulated the AMPK and insulin signalling pathway.

3.9 GLUT4 and PPAR\( \gamma \)

During the diabetes mellitus, increased the level of GLUT4 and PPAR\( \gamma \) and similar result was found in the STZ induced DM rats. Adropin significantly \( p < 0.001 \) decreased the level of GLUT4 and PPAR\( \gamma \) at dose dependent manner (Fig. 9).

4 Discussion

Diabetes mellitus has metabolic disorder that is largely heterogeneous. To estimate the DM, a number of drug and rodent models have been produced to date. Till date, streptozotocin (STZ) induced diabetic model gain more popularity due to effective action with low toxicity\(^{13} \). Consequently, STZ induced diabetic model commonly used to
check the DM. The mechanism involved in STZ-induced diabetes operates on the pancreatic β-cells as follows, firstly, via the glucose transporter (GLUT2) STZ reaches the β-cells and induces alteration of the DNA in the form of alkylation and, eventually, initiates ADP-ribosylation and induces ATP and NAD+ reduction. Finally, single injection of STZ, partially induced the destruction of β-cells.

Hepatic tissue is well known to play an important role in protecting the synthesis of glucose metabolism and post-prandial hyperglycemia. The glucose homeostasis was maintained via insulin through controlling the activities of G6PDH and hexokinase. Hexokinase considerably reduced the level during the DM, which further reduced the exchange of glucose utilization. Hepatic tissue utilizes the glucose to convert into the G6PDH by the help of hexokinase and finally generate the energy. Deficiency of hexokinase, induce the various alterations such as glycolysis, reduced energy production and glucose utilization. During the DM, boost the level of G6PDH, which augment the fat production to carbohydrates and finally start the deposition of fat into the renal and hepatic tissue. Hexokinase (a rate limiting enzymes) involved in the metabolism of carbohydrate, which further generates the G-6PDH upon glucose phosphorylation. STZ induced rats showed the reduced level of hexokinase and increased the level of G6PDH and adropin significantly increased the level of hexokinase and reduced level of G6PDH. Upon the adropin treatment, diabetic rats exhibited the crucial improvement the enzymes activity may be due to the ability to reduce the insulin level and almost bring back near to normal level. Gluconeogenesis is the mechanism of synthesizing the glucose from sources other than carbohydrates. The enzyme involved in the process of gluconeogenesis is G-6-pase, which exaggerated during the diabetic condition and starts the conversion of glucose-6-phosphate to glucose. During the diabetic condition, an insulin deficiency occurs which further lead the over-production of these enzymes and ultimately leads to the inflated glucose into the hepatic tissue. In the current study, adropin significantly decreased the level of G-6-pase almost near to the normal level. Adropin decreased these enzymes may be due to increase the level of insulin.

It is well proved that hyperglycaemia during the diabetes induce the oxidative stress and injury in the pancreatic β-cells leading to their dysfunction and insulin resistance in tissue. During the DM, increased the level of HbA1c and reduced the level of insulin and c-peptide due to availability of increase glucose level. HbA1c level increase in the STZ group rats due to availability of high blood glucose normal haemoglobin to transformation. Adropin significantly reduced the HbA1c level and increased level of c-peptide. The antidiabetic activity of adropin may be due to insulin potentiation from the current β-cells of the Langerhans islets. The restoration of above mention parameters by adropin might be due to increase the secretion of insulin from the pancreatic β-cells or via regenerating the pancreatic islets of Langerhans.

During the diabetic condition, alter the lipid metabolism due to change in the carbohydrate metabolism. During the DM condition, increased the level of TG, TC, LDL, VLDL and reduced the level of HDL and similar result was observed in diabetic control rat. Increased the level of serum lipids due to owing to the unreduced action of lipolytic hormones on adipose tissues. This further activates the peripheral fat deposition for fatty acid mobilization. Triacylglycerols synthesis via glycerol esterification might be use for this and start the secretion the VLDL into the serum. It is well documented that insulin secretion influence the cholesterol synthesis. In the current study, we found that the adropin considerably reduce the TG, TC, LDL, VLDL and increase the HDL.

Increasing the blood glucose level and decreasing the insulin level during DM activates PPARγ. PPARγ is an adi-

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**Fig. 8** The effect of adropin on the insulin signalling and AMPK pathway of STZ induced DM rats. a: AKT, b: IRS-2, c: IRS-1, d: IR, e: p-AKT and f: PI3K. One way-ANOVA followed by Dennett’s comparison test; * p<0.05 and *** p<0.001.

**Fig. 9** The effect of adropin on the GLUT4 and PPARγ expression of STZ induced DM rats. a: GLUT4 and b: PPARγ. One way-ANOVA followed by Dennett’s comparison test; * p<0.05, ** p<0.01 and *** p<0.001.
pogenesis activator which further increases the development of small insulin-sensitive adipocytes\textsuperscript{23, 25}. Mechanistically, PPAR\gamma ligands bind with the PPAR/RXR heterodimer, a conformational alteration occurs, which further leads to the transcription of gene responsible for adipocyte production\textsuperscript{24, 25}. Various previous studies suggest that the over-production of GLUT4 in either adipose tissues or muscles increase the insulin dependent glucose uptake and glucose tolerance\textsuperscript{24, 25}. In particular, the enhance expression of GLUT4 in these tissues reduces the insulin resistance along with increase insulin sensitization and ameliorates diabetes with metabolic perturbations\textsuperscript{24–26}. Subsequently, we have found that adropin reduced the expression of both enzymes mRNA and suggesting the antidiabetic effect at molecular level\textsuperscript{24, 25, 27}. Hyperglycemic rats displayed increased expression of GLUT4 and PPAR\gamma, indicating increased glucose levels and decreased serum insulin levels. The outcome indicates that the mRNA expression of GLUT4 and PPAR\gamma in STZ induced DM rats was considerably reduced by adropin.

GLUT2 play a significant role in the glucose transportation in the hepatic tissue. Glucose such as fatty acid synthase and pyruvate kinase, are the up-regulators of GLUT2\textsuperscript{28}. The modulated glucose level homeostasis during the diabetic condition is avoided via GLUT2 action\textsuperscript{28}. Collectively, lipogenic factor and glucose play a crucial role in the co-regulation of GLUT2 and indicate that transcription of GLUT2 is involved in glucometabolism.

An enzyme known as the master regulator of catabolic and metabolism pathways is AMP-activated protein kinase (AMPK)\textsuperscript{29, 30}. ACC protein altered the AMPK via regulation of fatty acid and glucose metabolism. AMPK activation brings the reduction the glucose production, triglycerides, fatty acid and boosted the fatty acid oxidation in diabetic rats\textsuperscript{29–31}. Adropin substantially (p < 0.001) increases the function of AMPK and also participates in elevations within the level of AMPK, ACC phosphorylation in diabetic rat liver\textsuperscript{29, 30, 32}. Our experimental result clearly suggests that the adropin regulate the AMPK and also bring almost near to the normal level and provide the beneficial effect.

It is well proved that PI3K/Akt signalling pathway involved in the cellular survival and proliferation\textsuperscript{33}. The activation of Akt play a significant role in the regulating various metabolic processes via PI3K\textsuperscript{33, 34}. Akt joins with other enzymes and stimulates insulin secretion and is also active in the exocytosis of granule-containing insulin by acting on the filaments of actin\textsuperscript{35}. The current experimental study showed that the adropin significantly (p < 0.001) regulated the PI3K/Akt pathway and suggesting the antidiabetic effect.

5 Conclusion
Collectively, we can say that adropin having a potent antidiabetic drug. Our result suggest that the adropin significantly (p < 0.001) restored the blood glucose level, body weight, plasma insulin, carbohydrate enzymes and hepatic glycogen and reach almost near to normal control. To promote glucose in the hepatic tissue, adropin can increase GLUT-4 and PPAR\gamma translocation from cytoplasm to the membrane. Adropin significantly altered the AMPK and insulin signalling pathway and suggesting the antidiabetic effect.

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