Zuogui Jiangtang Jieyu Decoction Ameliorates Depression-Like Behavior in Diabetic Rats via Activation of Neuronal and Astrocytic IR/IRS-1 Signaling Pathway

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Research

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Abstract

Background: Zuogui Jiangtang Jieyu decoction(ZJJ) is a Chinese herbal formulation on the strength of Zuogui Wan which recorded in the “Jing Yue Quan Shu” in the Ming dynasty. It is mainly used for treatment of diabetes-related depression in current clinical applications and researches. This study aims to investigate whether brain IR/IRS-1 signaling pathway involved in the therapeutic effect of ZJJ on depression-like behavior in diabetic rats.

Methods: Sprague Dawley rats were fed with high fat diet, and subjected to streptozotocin injection to establish the diabetes animal model. After treatment with different doses of ZJJ (20.530 g/kg or 10.265 g/kg) for 4 weeks, blood glucose level and peripheral insulin resistance were measured. Forced-swimming test (FST) and Morris water maze test (MWMT) were applied for mood and cognitive function assessment. Then western blot was used to analyze the protein levels of insulin receptor (IR), insulin receptor substrate-1 (IRS-1), phosphatidylonositol-3-kinase (PI3K), and protein kinase B (AKT) in the hippocampus of diabetic rats. Meanwhile, immunofluorescence and double-labeling immunofluorescence were performed to analyze the above proteins expression in the neuron and astrocyte, respectively. At last, energy metabolism and glycogen metabolism were tested by using ELISA. Additionally, the insulin-sensitive glucose transporter 4 (GLUT4) and the lactate transporter monocarboxylate transporter 4 (MCT4) were analyzed by using western blot.

Results: ZJJ administration significantly decreased blood glucose and improved peripheral insulin resistance of diabetic rats. Besides, ZJJ improved the depression-like behavior and cognitive dysfunction caused by diabetes. Furthermore, result of the western blot analysis showed that ZJJ treatment increased the expression of phospho-IR, phospho-IRS-1, phospho-PI3K, and phospho-AKT in the hippocampus of diabetic rat. Moreover, result of immunofluorescence showed that the above proteins were increased not only in the neuron but also in the astrocyte after ZJJ administration. In addition, ZJJ increased the content of ATP, glycogen and lactate, as well as the expression of GLUT4 and MCT4 in the hippocampus of diabetic rats.

Conclusions: These findings suggested that ZJJ improves the depression-like behavior of diabetic rats by activating both neuronal and astrocytic IR/IRS-1 signaling pathway. And the brain IR/IRS-1 signaling pathway plays an important role in astrocyte-neuron metabolic coupling, providing a potential mechanism by which IR/IRS-1 signaling pathway may contribute to the treatment of ZJJ on diabetes related depression.

Introduction

Depressive symptoms, including sadness, cognitive dysfunction, disrupted sleep, appetite changes, or suicidality may occur in people with diabetes[1]. Subsequently, depression can exacerbate and accelerate adverse clinical profiles of diabetes[2]. However, the underlying molecular mechanism between diabetes and depression remains elusive. Clinical report indicates that individuals with diabetes and insulin

resistance has a higher risk of suffering from depression[3]. Conversely, patients with major depressive disorder were 1.5 times more likely to develop insulin resistance than those whom without depression[4]. In fact, insulin resistance, as a hallmark of diabetes exists not only in the periphery but also in the brain. And the defective brain insulin signaling induced depression-like behavior may involve in neurogenesis, synaptic plasticity, hypothalamic-pituitary-adrenal (HPA) axis regulation, energy metabolism, neuroinflammation and so on. Thus, brain insulin resistance may play a critical role in the development of diabetes-associated depression[5].

Insulin and its specific receptor (insulin receptor, IR) is highly expressed in the hippocampus, the core area of emotional regulation and cognitive abilities. And the binding between insulin and IR leads to recruitment and phosphorylation of the insulin receptor substrates 1 (IRS-1), which then activates PI3K/AKT pathway and apply to downstream nodes, such as glycogen synthase kinase 3β (GSK-3β)[6]. The above molecules regulate the survival and conduction functions of hippocampal neurons. A previous study indicated that an abnormal IR/IRS-1 signaling in hippocampus was closely related to cognitive disorder caused by diabetes, while PI3K/AKT signal in hippocampus could also regulate emotional impairment[7]. And the deletion of insulin receptor (IR) in brain (NIRKO) could lead to an array of metabolic abnormalities and mental disorders[8]. Therefore, neuronal IR/IRS-1 signaling pathway and its downstream PI3K/AKT signaling may play a key role in diabetes-associated depression.

Although most studies suggest that the cell respond to insulin in hippocampus is mainly neurons, recent study showed that insulin can act on astrocytes (a gliocyte in hippocampus) and produces physiological effects[9]. And astrocyte may be a “hopeful” target for intervention of emotional abnormalities[10]. Clinical study reveals that astrocyte is relevant to reduced volume of hippocampus in subjects with major depression[10]. In fact, the damage of astrocyte is not only existed in depression, but also found in mood disorder and cognitive impairment in diabetes. The expression of glial fibrillary acidic protein (GFAP) was increased in hippocampus of streptozotocin (STZ)-induced diabetic mice which accompany with deficits on memory and cognitive dysfunction[11]. And knockdown of IR in astrocyte, the mice showed the anxiety and depressive-like behavior[9]. Therefore, IR/IRS-1 signaling in astrocyte may also involve in the comorbid depression in diabetes.

Zuogui Jiangtang Jieyu decoction (ZJJ) is a traditional Chinese medicine (TCM), which consists of eleven herbs-namely, *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao (astragali radix, 18 g), *Hypericum perforatum* L. (hyperici perforati herba, 3 g), *Curcuma longa* L. (curcumae longae rhizoma, 9 g), *Rehmannia glutinosa* Libosch. (rehmanniae radix praeparata, 15 g), *Cornus officinalis* Sieb. et Zucc.(corni fructus, 12 g), *Lycium barbarum* L. (lycii fructus, 12 g), *Cuscuta australis* R. Br. (cuscutae semen, 9 g), *Eucommia ulmoides* Oliv. (eucommiae cortex, 9 g), *Salvia miltiorrhiza* Bge. (salviae miltiorrhizae radix et rhizoma, 12 g), *Paeonia suffruticosa* Andr. (moutan cortex, 6 g), *Achyranthes bidentata* Bl. (achyranthis bidentatae radix, 9 g). Our previous works reported the hypoglycemic and antidepressant effect of ZJJ[12, 13]. And ZJJ has also been shown to regulate the level of glucocorticoid in diabetic rats with depression[13]. Given that diabetes-induced brain insulin resistance is associated with the elevated level of glucocorticoid caused by alteration of HPA axis[14], the effects of ZJJ on

diabetes-induced depression may be related with the hippocampal IR/IRS-1 signaling. Thus, in this study, we investigated the effect of ZJJ on IR/IRS-1 signaling pathway in the hippocampal neuron and astrocyte.

Materials And Methods

Animal Materials.

Six-week-old male Sprague Dawley (SD) rats, weighing 180 to 200 g, were purchased from Hunan Slack Scene of Laboratory Animal Company (Hunan, China). All rats were maintained in the SPF laboratory animal center in the first affiliated hospital of Hunan university of Chinese medicine. And the temperature in the room is maintained at 23 ± 1℃ with a 12 h light/dark cycle. All laboratory procedures were performed accoding to the guidelines established by the Animal Ethics Welfare Committee of the First Affiliated Hospital of Hunan University of Chinese Medicine.

Experimental Groups and Drug Administration.

Rats were fed with high-fat diet (HFD) for four weeks to inducing obesity, and then they were injected with STZ (38 mg/kg, i.p.) which dissolved in sodium citrate buffer (pH 4.9, 4℃) to establish diabetes. Rats were considered diabetic if the fasting plasma glucose levels > 16 mmol/L. Then the experimental subjects were divided into 5 groups, consisting of control group (Control), diabetic group (DM), metformin (200 mg/kg) and fluoxetine (30 mg/kg) group (MET/FLX), high dose (20.530 g/kg) of Zuogui Jiangtang Jieyu decoction group (ZJJ-H), low dose (10.265 g/kg) of Zuogui Jiangtang Jieyu decoction group (ZJJ-L). Treatment groups were administrated with ZJJ or MET/FLX for 4 weeks starting from the eighth week. After the treatment, the depression-like behavior and cognitive function of rats were tested by forced-swimming test (FST) and morris water maze test (MWMT), respectively.

Animal Behavior Test.

Forced-swimming test (FST).

Forced-swimming test (FST) was detected to assess the depressive-like behavior of rats[15]. In this experiment, rats were placed in a circular fiberglass pool, containing 30 cm of water at 25 ± 1 ℃. The duration of observed immobility time was recorded during the last 3 min of the whole 4 min testing period. And decreased immobility time was used as the index of antidepressant-like efficacy.

Morris water maze test (MWMT).

Morris water maze test was used to assess the cognitive function of rats in this study[15]. Each rat was placed in a circle water tank (200 cm in diameter) filled with water (25 ± 1℃). And the container was divided into four equal quadrants. For place navigation test, rats were daily introduced to different quadrant of the water tank on the first four days. And the time of rats to find the submerged platform was recorded as the escape latency time, which was used as the index of learning function. In the spatial probe test, rats were placed in the water tank where the platform was removed. The time of rats spent in target quadrant and the number of times that the rats crossed the platform were recorded to assess the memory function of rats.

ELISA Analysis

Fasting blood glucose (FBG) was detected by using fasting blood samples from rat tail veins. The levels of fasting insulin (FINS)(Nanjing Jiancheng, China), ATP(Nanjing Jiancheng, China), glycogen(Feiya Biotechnology, China) and lactate(Feiya Biotechnology, China) were tested by using enzymatic kitsaccording to the manufacturer's instructions. The assessment of insulin resistance was calculated as followed: (HOMA-IR) = (FBG × FINS) / 22.5. HOMA-IR refers to homeostasis model assessment of insulin resistance.

Western Blot Analysis

Western blot analysis was performed as described in our previous study[13]. Briefly, equal amounts of proteins were separated by SDS-PAGE and transferred to Polyvinylidene fluoride (PVDF) membranes (Millipore Corporation, USA). After the membranes were blocked by TBST (TBS plus 0.05% Tween 20) plus 1% non-fat dry milk for 60 min, proteins were incubated with primary antibody overnignt at 4℃. And the following antibodies were used: anti-IR (1:1000, Affinity biosciences, USA), anti-phospho-IR (1:2000, Affinity biosciences, USA), anti-IRS-1 (1:1000, Cell signaling, USA), anti-phospho–tyr-IRS-1 (1:1000, Cell signaling, USA), anti-PI3K (1:1000, Affinity biosciences, USA), anti-phospho-PI3K (1:2000, Affinity biosciences, USA), anti-AKT (1:1000, Cell signaling, USA), anti-phospho-AKT (1:2000, Affinity biosciences, USA), anti-MCT4(1:2000, proteintech, USA), and anti-GLUT4(1:1000, proteintech, USA), anti-GAPDH(1:1000, Cell signaling, USA). PVDF membranes were then washed with TBST and incubated with HRP-linked antibody(1:3000, Cell signaling, USA) at room temperature for 60 min. PVDF membranes were then washed with TBST and developed using Enhanced Chemioluminescence Reagents (ECL, New Cell & Molecular Biotech, China). At last, the protein bands were analyzed by using Image Lab system.

Immunofluorescence Analysis

Rats were perfused sequentially with (1) 250 mL of saline solution; (2) 250 mL of 4% paraformaldehyde in 0.1M phosphate buffer saline (PBS). The slices of brain were cut by a microtome and incubated with primary antibody for the phospho-IR (1:200, Affinity biosciences, USA), phospho-IRS-1 (1:200, Bioss antibodies, China), phospho-PI3K (1:100, Affinity biosciences, USA), phospho-Akt (1:100, Affinity biosciences, USA), or GFAP (1:200, Cell signaling, USA). Then sections were washed and incubated with

anti-IgG secondary antibody coupled to FITC (green) or CY3(red). Tissue sections were examine on a fluorescence microscope (Nikon Eclipse C1).

Statistical Analysis.

All the data were presented as mean ± standard error of the means (S.E.M.). Two- way ANOVA was performed to detect the differences between two groups, and Turkey's post hoc analysis was performed when appropriate. P values < 0.05 was considered to be statistically significant.

Results

Zuogui Jiangtang Jieyu Decoction Treatment Improves Peripheral Insulin Resistance in Diabetic Rats

First, the effect of Zuogui Jiangtang Jieyu decoction (ZJJ) on peripheral glucose metabolism was investigated in diabetic rats. As shown in Fig. 1, the levels of blood glucose and insulin were significantly increased in diabetic rats when compared with those in the control group ($P < 0.01$). The high dose (20.530 g/kg) of ZJJ effectively decreased the blood glucose and insulin levels ($P < 0.01$), while the low dose (10.265 g/kg) only lowered the blood glucose level in diabetic rat ($P < 0.05$). Furthermore, two doses of ZJJ (20.530 g/kg and 10.265 g/kg) treatment significantly reduced the value of HOMA-IR in diabetic rats ($P < 0.01$).

Zuogui Jiangtang Jieyu Decoction Treatment Attenuates Depressive-Like Behavior and Cognitive Dysfunction in Diabetes Rats

Then, the effect of Zuogui Jiangtang Jieyu decoction (ZJJ) on cognitive performance (including learning and memory) and mood was investigated by the Morris water maze test and forced swimming test, respectively. In Morris water maze test, as shown in Fig. 2A, the escape latency was decreased during four training days in all groups. More concretely, there was no significantly different escape latency time between the control group and DM group on the first training days, while the DM rats showed significantly increased latency time on the last three days compared with the normal rats (Fig. 2B, P < 0.01). Treatment of the high dose of ZJJ (20.530 g/kg) obviously reduced latency time of diabetic rats on day 3 and 4 during training trials (Fig. 2B, day 3:$P < 0.05$, and day 4:$P < 0.01$). Furthermore, ZJJ treatment increased the swimming time in target quadrant (Fig. 2C, 20.530 g/kg: $P < 0.01$, and 10.265 g/kg: $P < 0.05$) and the times that rats crossing the platform (Fig. 2D, 20.530 g/kg: $P < 0.05$). The above results suggested that ZJJ improved the cognitive function of diabetic rats. In addition, in the forced swimming test, which uses reduced immobility time as the index of antidepressant-like efficacy, the DM rats showed a dramatic increase in the immobility time when compared with control group (Fig. 2E, P < 0.01). Conversely, both two doses of ZJJ decreased the immobility time of diabetic rats (Fig. 2E, 20.530 g/kg: $P < 0.01$, and 10.265 g/kg: $P < 0.05$).

Zuogui Jiangtang Jieyu Decoction Treatment Activates the Hippocampal IR/IRS-1 Signaling in Diabetic Rats

IR/IRS-1 signaling pathway was recognized as an association between diabetes and depression[16]. And aberrant IR/IRS-1 signaling may be related to mood and cognitive impairment[17]. In this study, western blot analyses showed that compared with control rats, the phosphorylation of IR, IRS-1, PI3K and AKT were significantly decreased in the hippocampus of diabetic rats (Fig. 3, $P < 0.05$ or $P < 0.01$). And the reduction of these proteins were reversed by treated with the high dose (20.530 g/kg) of Zuogui Jiangtang Jieyu decoction ($P < 0.05$ or $P < 0.01$).

Zuogui Jiangtang Jieyu Decoction Treatment improves the neuronal IR/IRS-1 Signaling in the Hippocampus of Diabetic Rats

To further investigate the regulation of Zuogui Jiangtang Jieyu decoction on neuronal IR/IRS-1 signaling, the immunofluorescence analysis was used in this study. As shown in Fig. 4, the expression of p-IR, p-IRS-1, p-PI3K, p-AKT were significantly decreased in the diabetic rat ($P < 0.01$). And 20.530 g/kg Zuogui Jiangtang Jieyu decoction treatment increased the above four proteins level, while 10.265 g/kg Zuogui Jiangtang Jieyu decoction only increased the expression of p-IR ($P < 0.01$ or $P < 0.05$).

Zuogui Jiangtang Jieyu Decoction Treatment Regulates the Neuronal Energy Metabolism in Diabetic Rats

Brain IR/IRS-1 signaling in the hippocampus may contribute to neuronal activity by effecting the glucose utilization and energy metabolism[18]. In this study, an insulin-sensitive glucose transporter, GLUT4 was obviously reduced in the hippocampus of diabetic rat, and the ATP release was decreased as well (Fig. 5, $P < 0.05$). Zuogui Jiangtang Jieyu decoction treatment (20.530 g/kg) significantly increased the expression of GLUT4 ($P < 0.05$) and the level of ATP ($P < 0.01$), suggesting its potential effect on neuronal energy metabolism.

Zuogui Jiangtang Jieyu Decoction Treatment Improves the Astrocytic IR/IRS-1 Signaling in Diabetic Rats

Given that insulin may act on astrocyte affecting both energy homeostasis and neurobehaviors, we further investigated the effect of Zuogui Jiangtang Jieyu decoction on astrocytic IR/IRS-1 signaling by using double-labeling immuno- fluorescence method. As shown in Fig. 6, compared with the control rat, p-IR, p-IRS-1, p-PI3K, and p-AKT expression were significantly reduced in the astrocyte of diabetic rat ($P < 0.01$). After the high dose (20.530 g/kg) of Zuogui Jiangtang Jieyu decoction treatment, the expression of p-IR, p-IRS-1, p-PI3K, and p-AKT were significantly increased ($P < 0.05$ or $P < 0.01$). And the low dose (10.265 g/kg) of this decoction obviously increased the p-IRS-1 and p-PI3K levels ($P < 0.05$ or $P < 0.01$).

Zuogui Jiangtang Jieyu Decoction Treatment Modulates the Astrocytic Glycogen Metabolism in Diabetic Rats

Astrocyte glycogen and lactate play a key role in supporting neuronal function by providing energy[19]. As shown in Fig. 7A and 7B, the content of glycogen and lactate was reduced in the hippocampus of diabetic rats ($P < 0.05$ or $P < 0.01$). In addition, western blot results showed the obviously decrease in the expression of MCT4 in diabetic rats, which was responsible for exporting lactate out of astrocyte (Fig. 7C, $P < 0.01$). Zuogui Jiangtang Jieyu decoction treatment increased the levels of glycogen and lactate as well as the expression of MCT4 ($P < 0.05$ or $P < 0.01$).

Discussion

In this study, after 4 weeks of HFD and a single injection of STZ, SD rats exhibited the significantly periphery insulin resistance. And the depression-like behavior and cognitive dysfunction were found in diabetic rats after 12 weeks of diabetes duration. Zuogui Jiangtang Jieyu decoction showed the hypoglycemic and anti-depression effect on diabetic rats with depression-like behavior, which is consistent with our previous studies[13]. However, different from the earlier studies, the method of chronic unpredictable mild stress (CUMS) was not used in diabetic rats in this study. In fact, clinical study reported that the duration of diabetes was associated with the depression in later life of people with diabetes[20]. And similarly, STZ-injected rats could exhibit depression-like behavior after a period of diabetes[21]. Kamal et al.[22] reported that the damaged hippocampal synaptic plasticity, which is a key pathophysiological mechanism of depression was observed after a diabetes duration of six or eight weeks in STZ-induced diabetic rats. After 12 weeks of diabetes, the deficit of synaptic plasticity reached a maximum and remained stable thereafter. CUMS exposed to rats accelerates or exacerbates the depression-like behavior of diabetic rats[15]. But after subjected to CUMS, the depression-like behavior in diabetic rats may partly because of diabetes and partly due to CUMS. So in this study, the effect of Zuogui Jiangtang Jieyu decoction on depression-like behavior of diabetic rats was investigated once again without the interfering of CUMS. Diabetic rats received the Zuogui Jiangtang Jieyu decoction administration for 4 weeks starting from the 8th week of diabetes. And the depression like behavior and cognitive deficit in STZ-induced diabetic rats were reversed by Zuogui Jiangtang Jieyu decoction.

In recent years, some findings suggested that aberrant hippocampal IR/IRS-1 signaling was associated with depressive disorders[23, 24]. And mice with a brain-specific knockout of the insulin receptor (NIRKO mice) exhibited the age-related anxiety and depression-like behaviors[25]. In this study, phosphorylation of IR and IRS-1 was decreased in the hippocampus of diabetic rats. It is worth noted that there are numerous residues on IRS-1, including tyrosine, serine, threonine and so on[26]. Insulin-stimulated IR mediates the different sites phosphorylation of IRS-1, with both positive and negative effects on insulin sensitivity. For instance, as a negative mediators of insulin signaling, ser $(P)^{307}$-IRS−1 blocks the interaction between the IRS−1 and IR, which may lead to the insulin resistance[27]. On the contrary, insulin-initiated the increase in tyrosine phosphorylation of IRS−1 exhibited a positive effects on insulin

sensitivity in C57BL/6J mice[28]. In our previous study, the level of ser (P)^{307}-IRS−1 was increased in the hippocampus of diabetic rats with depression[15]. And in this study, the level of tyr (P)-IRS−1 was decreased in the same brain area. Thus, the above findings suggest that aberrant IR/IRS-1 signaling exits in STZ-induced diabetic rats with depression-like behavior. In addition, the downstream molecules of IRS-1^{tyr}, including PI3K and AKT were also reduced in diabetic rats. Therefore, different sites of IRS−1 may involve in the brain insulin resistance in diabetic rats. And future research will be require to clarify the relationship between ser(P)-IRS−1 and tyr(P)-IRS−1 and their impact on the regulation of brain insulin resistance .

It has become clear that the neuron is an insulin-sensitive cell. Insulin action on the neuronal cell has been shown to regulate systemic energy homeostasis[29]. Moreover, neuronal IR/IRS-1 signaling has also been found to involve in some neurobehaviors including cognitive and mood[30, 31]. In the present study, western blot analysis showed that Zuogui Jiangtang Jieyu decoction activated the hippocampal insulin signaling pathway by increased the phosphorylation of IR, IRS-1, PI3K and AKT. At the same time, immunofluorescence analysis showed that the expression of p-IR, p-IRS-1, p-PI3K and p-AKT were increased in hippocampal neurons, which was consistent with the result of western blot. In fact, IR/IRS-1 signaling regulates neurobehaviors partly dependent on its regulation on brain glucose utilization[32], which play an important role in major depressive disorder. Grillo et al[33] reported that insulin-stimulated translocation of glucose transporter 4 (GLUT4) to the membrane rapidly increased glucose utilization of neuron. Furthermore, the process of GLUT4 translocated to membrane is related with neurocognitive improvement[34]. On the contrary, impaired IR/IRS-1 signaling leads to the reduced levels of GLUT4[35]. In the present study, Zuogui Jiangtang Jieyu decoction increased the GLUT4 expression in the hippocampus, implying that the positive effect of this decoction on glucose uptake. In addition, the reduced ATP content in the hippocampus of diabetic rats was reversed by Zuogui Jiangtang Jieyu decoction administration. Therefore, Zuogui Jiangtang Jieyu decoction regulates the energy metabolism in the hippocampus of diabetic rats with depression, which may related to its regulation on neuronal IR/IRS-1 signaling.

Brain IR/IRS-1 signaling is important for energy homeostasis, cellular metabolism and mood disorders, while most of these effects are thought to occur in neurons. As a matter of fact, brain cells consist of not only neuron cells, but also glial cells. Notably, a previous study indicates that IR/IRS-1 signaling in the astrocyte is involved in regulating neural behavior, including mood and cognition[9]. Besides, astrocyte may be a crucial target for IR/IRS-1 signaling. Interestingly, in this study, Zuogui Jiangtang Jieyu decoction has been found to regulate astrocytic IR/IRS-1 signaling by increased the expression of major proteins. In fact, it is generally accepted that astrocyte is the main cells to store glycogen[36], which is involved in maintaining neuronal activity in the brain[19]. And IR/IRS-1 signaling could phosphorylate the downstream molecule AKT, which regulate the primary enzyme in glycogen synthesis[37, 38]. In this study, Zuogui Jiangtang Jieyu decoction increased the content of glycogen in astrocyte may be related to its activation of astrocytic IR/IRS-1 signaling pathway. Moreover, administration of Zuogui Jiangtang Jieyu decoction increased the levels of lactate, accompanied with increased the expression of GLUT4.

Indeed, when the energy demand exceeds supply in the nervous system, glycogen is used to generate lactate, some of which is released from astrocyte by MCT4 and transported to the neighboring neurons[39]. Thus the above findings suggest that Zuogui Jiangtang Jieyu decoction may improve the astrocytic glycogen metabolism to provide energy support for neuron in the hippocampus of diabetic rats with depression.

Therefore, our findings suggested that both neuronal and astrocytic IR/IRS-1 signaling pathway make important contribution to the treatment of Zuogui Jiangtang Jieyu decoction on diabetes-induced depression. Furthermore, the effect of Zuogui Jiangtang Jieyu decoction on the astrocyte-neuron metabolic coupling will be investigated in our further research.

Conclusion

In summary, the hypoglycemic and anti-depression effect of Zuogui Jiangtang Jieyu decoction were investigated in the present study. Western blot and immunofluorescence method demonstrated that Zuogui Jiangtang Jieyu decoction activated both neuronal and astrocytic IR/IRS-1 signaling pathway in the hippocampus of diabetic rats. Moreover, the decreased expression of GLUT4 and the content of ATP in diabetic rats were reversed by Zuogui Jiangtang Jieyu decoction, providing the potential regulation of this decoction on neuronal energy metabolism. And Zuogui Jiangtang Jieyu decoction increased the expression of MCT4 and the levels of glycogen and lactate, suggesting that astrocytic glycogen metabolism may participate in the function of Zuogui Jiangtang Jieyu decoction on diabetes-related depression. These findings provide evidence that Zuogui Jiangtang Jieyu decoction not only impacts on energy metabolism by activating neuronal IR/IRS-1 signaling but also regulates glycogen metabolism by activating astrocytic IR/IRS-1 signaling. And regulation on Astrocyte-neuron metabolic coupling may play a critical role in the therapy of Zuogui Jiangtang Jieyu decoction.

Abbreviations

ZJJ, Zuogui Jiangtang Jieyu decoction; FST, forced-swimming test; MWMT, morris water maze test; IR, insulin receptor; IRS-1, insulin receptor substrate-1; PI3K, phosphatidylonositol-3-kinase; AKT, protein kinase B; STZ, streptozotocin; GFAP, glial fibrillary acidic protein; HPA, hypothalamic-pituitary-adrenal; GLUT4, glucose transporter 4; MCT4, monocarboxylate transporter 4.

Declarations

Ethics approval and consent to participate

This study was carried out in accordance with the principles of the Basel Declaration and recommendations of Animal Experimental Ethics, Animal Ethics Welfare Committee of the First Affiliated Hospital of Hunan University of Chinese Medicine. The protocol was approved by the Animal Ethics Welfare Committee of the First Affiliated Hospital of Hunan University of Chinese Medicine.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and material

The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HY JLing and PM participated in the design of this study. JLiu helped to accomplish the animal experiments. HY, XL and WL were responsible for the analyses of the samples. YW designed the study and supervised all work. All authors approved the final manuscript.

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Figures

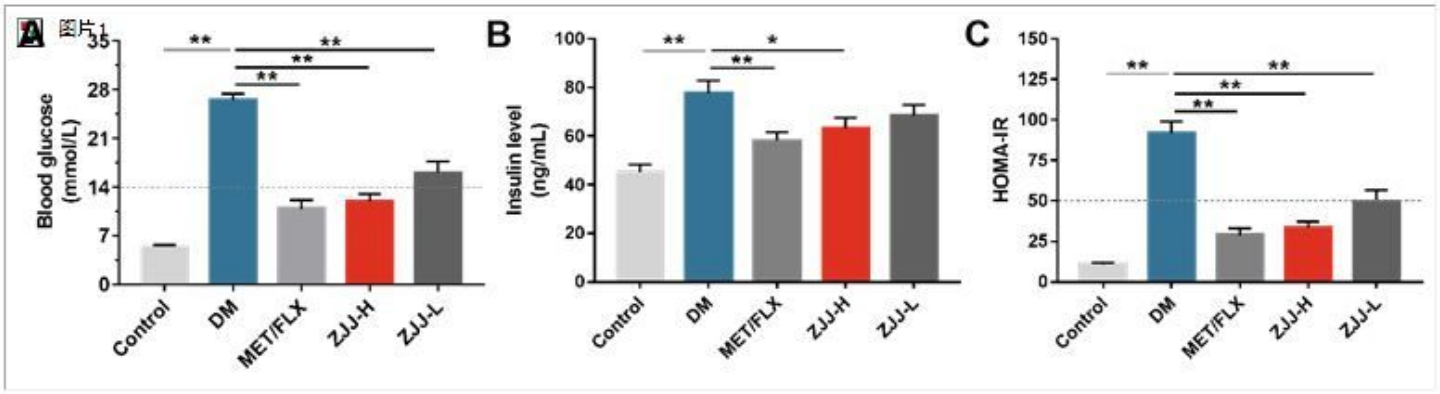

Figure 1

Effect of Zuogui Jiangtang Jieyu decoction (ZJJ) on peripheral insulin resistance in diabetic rats. (A) Both high and low dose of ZJJ reduced the levels of blood glucose. (B) High dose of ZJJ reduced the levels of blood insulin. (C) Both high and low dose of ZJJ reduced the value of HOMA-IR. n=8 in each group. **P<0.01 and *P<0.05. Data are presented as the mean ± S.E.M. HOMA-IR: homeostasis model assessment of insulin resistance.

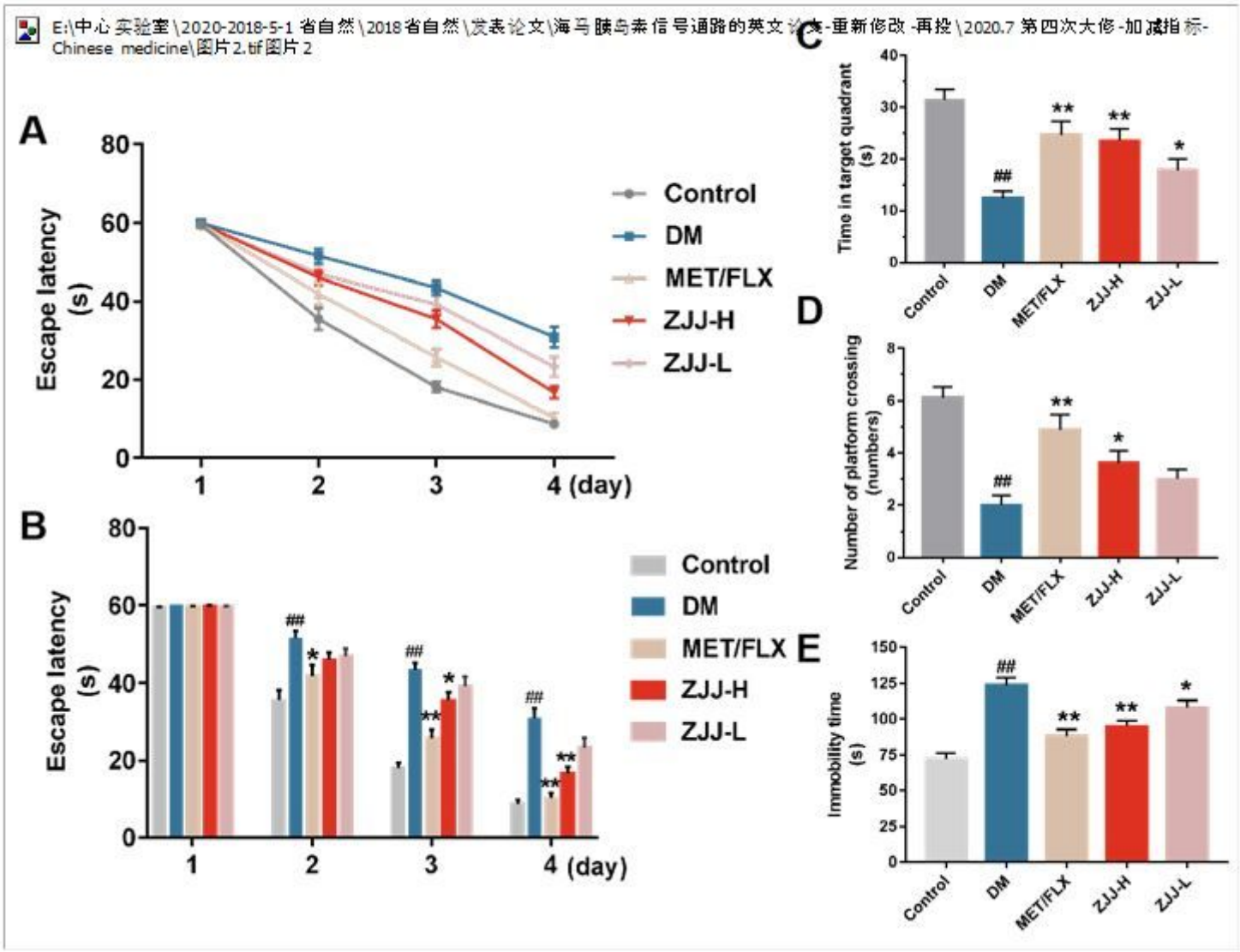

Figure 2

Zuogui Jiangtang Jieyu decoction (ZJJ) ameliorated the depression-like behavior and cognitive function in diabetic rats. (A) The learning curve of rats in all groups in place navigation. (B) The escape latency time of rats during four training days of place navigation. (C) ZJJ increased the swimming time of diabetic rats in target quadrant. (D) ZJJ increased the times of rats crossing the platform in spacial probe test. (E) ZJJ reduced the immobility time of diabetic rats in forced swimming test. n= 8 in each group. ##P<0.01 vs the Control group. **P<0.01 and *P<0.05 vs the DM group. Data are presented as the mean ± S.E.M.

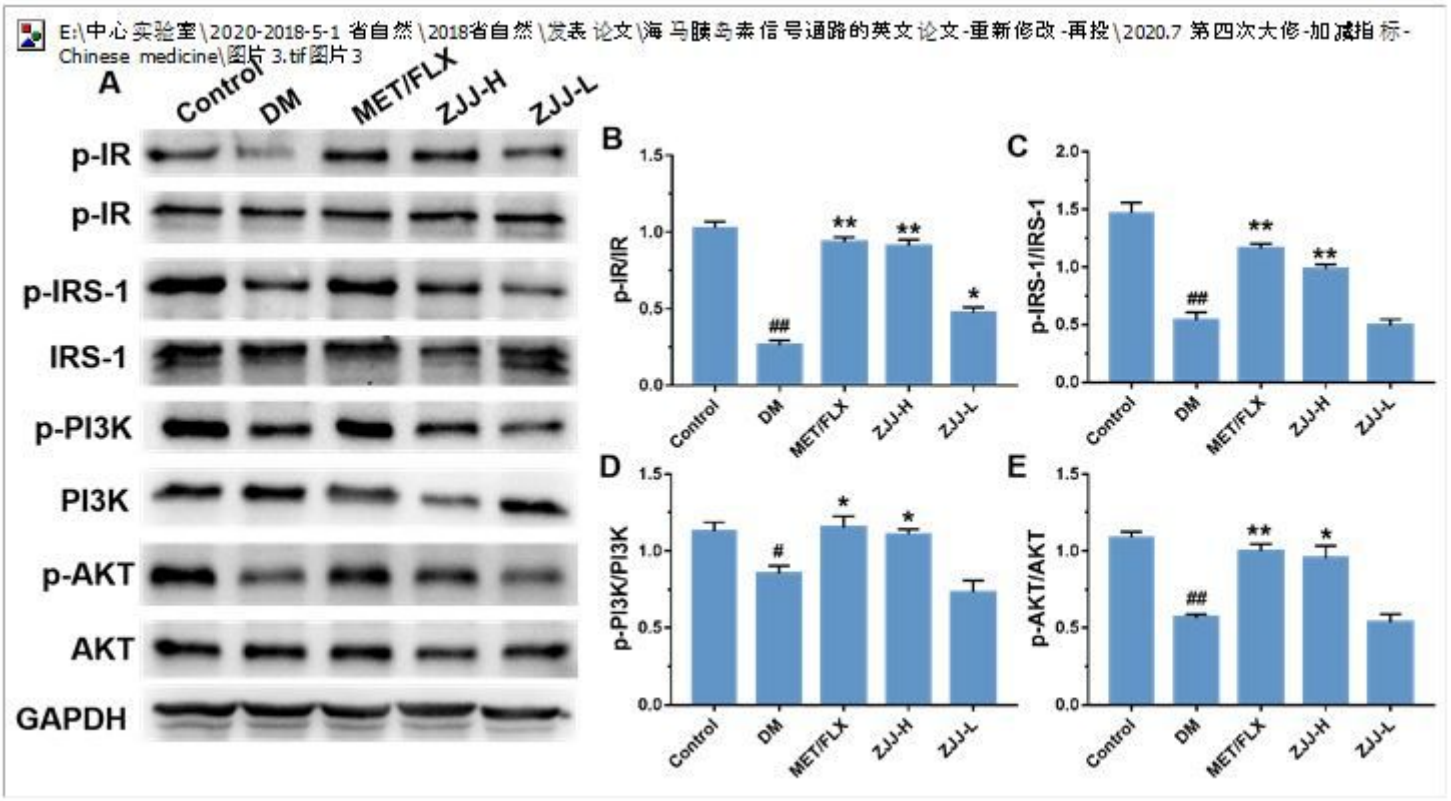

Figure 3

Zuogui Jiangtang Jieyu decoction activated hippocampal insulin signaling pathway by increasing the expression of p-IR (B), p-IRS-1(C), p-PI3K(D), and p-AKT(E) in the diabetic rats. n= 6 in each group. ##P<0.01 and ##P<0.05 vs the Control group. **P<0.01 and *P<0.05 vs the DM group. Data are presented as the mean ± S.E.M.

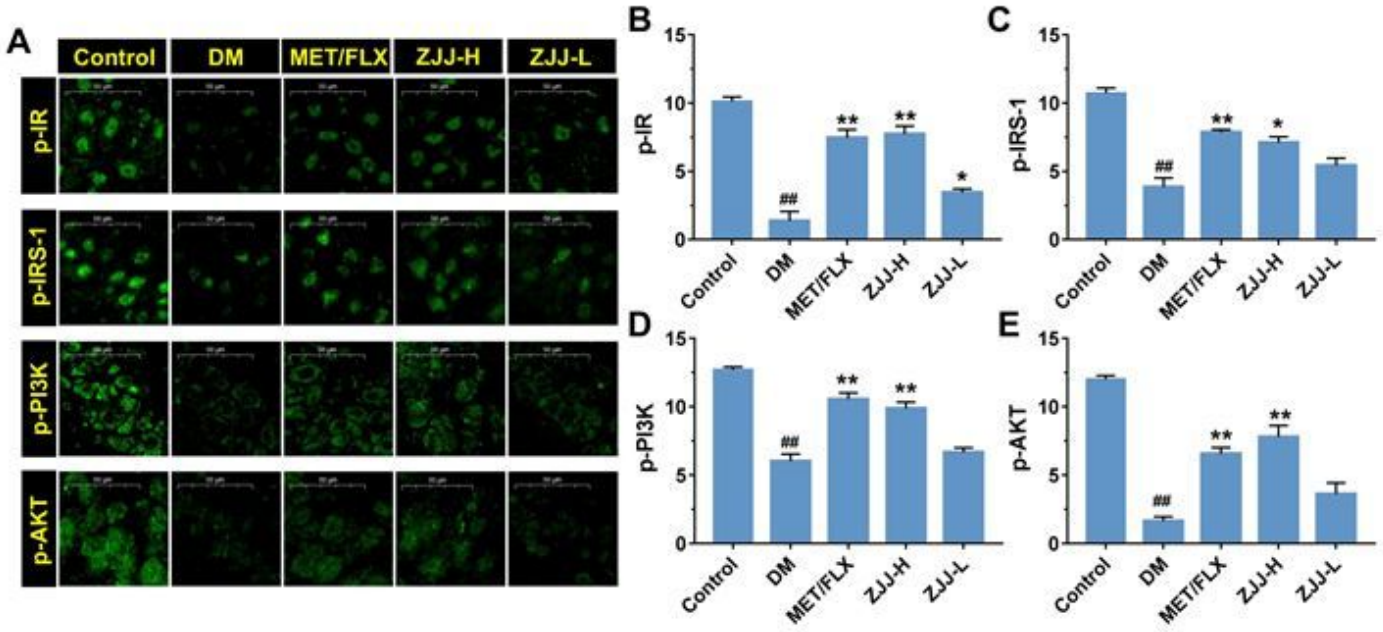

Figure 4

Zuogui Jiangtang Jieyu decoction activated neuronal insulin signaling pathway by increasing the expression of p-IR (B), p-IRS-1(C), p-PI3K(D), and p-AKT(E) in the hippocampus of diabetic rats. n = 5 in each group. Bar = 50μm. ##P<0.01 vs the Control group. **P<0.01 and *P<0.05 vs the DM group. Data are presented as the mean ± S.E.M.

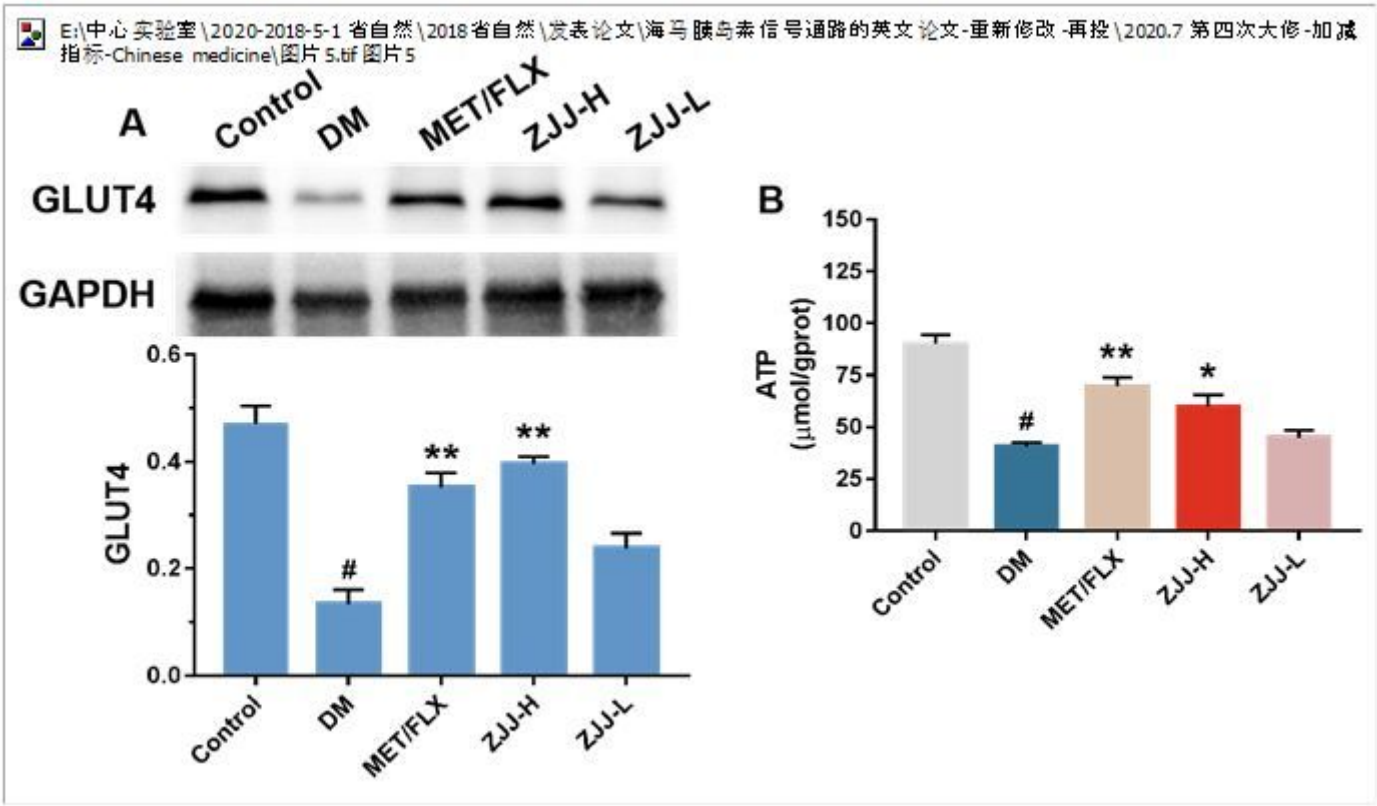

Figure 5

Zuogui Jiangtang Jieyu decoction increased the GLUT4 expression (A) and ATP release (B) in the hippocampus of diabetic rats. n= 6 in each group. #P<0.05 vs the Control group. **P<0.01 and *P<0.05 vs the DM group. Data are presented as the mean ± S.E.M.

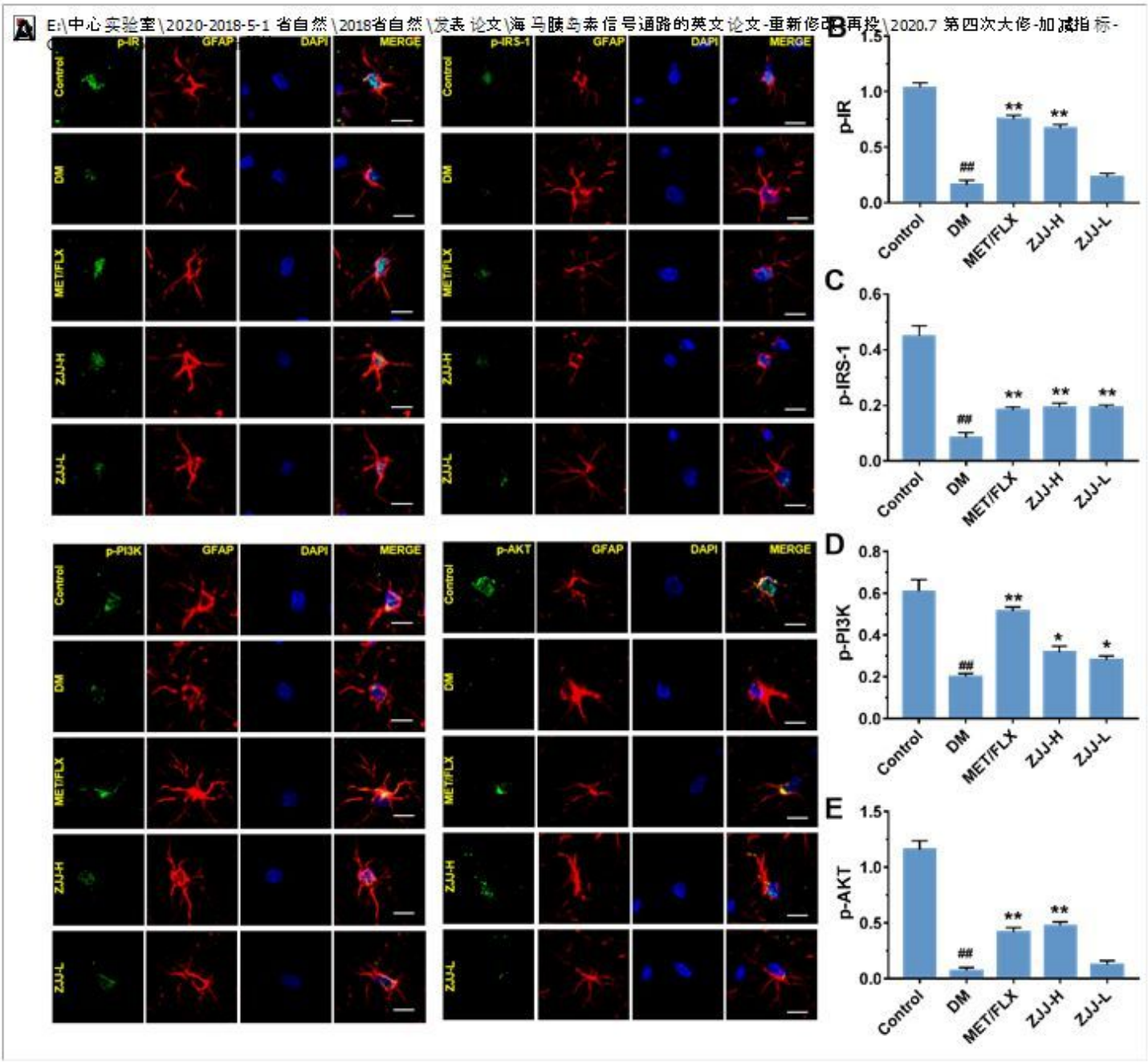

Figure 6

Zuogui Jiangtang Jieyu decoction activated astrocytic insulin signaling pathway by increasing the expression of p-IR (B), p-IRS-1(C), p-PI3K(D), and p-AKT(E) in the hippocampus of diabetic rats. n= 5 in each group. Bar=20μm. ##P<0.01 vs the Control group. **P<0.01 and *P<0.05 vs the DM group. Data are presented as the mean ± S.E.M.

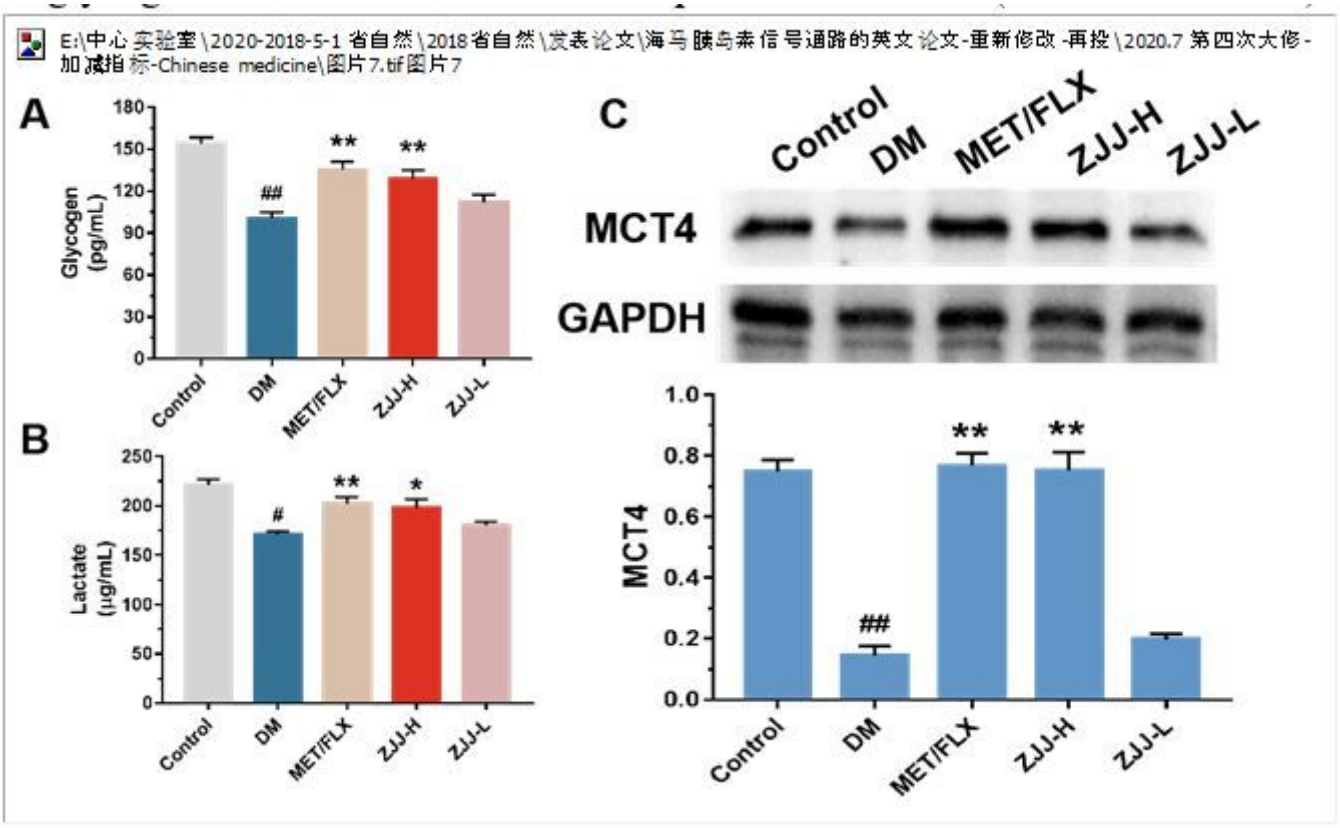

Figure 7

The effect of Zuogui Jiangtang Jieyu decoction on astrocytic glycogen metabolism. Zuogui Jiangtang Jieyu decoction increased the content of glycogen (A) and lactate (B), as well as the expression of MCT4 (C) in the hippocampus of diabetic rats. n= 6 in each group. ##P<0.01 and #P<0.05 vs the Control group. **P<0.01 and *P<0.05 vs the DM group. Data are presented as the mean ± S.E.M.