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Research Article

Glibenclamide improves learning and memory in streptozotocin-induced diabetic rats

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ABSTRACT

Alzheimer's disease (AD) is closely associated with impaired insulin signaling in brain. It has been shown that diabetic mice had increased tau phosphorylated proteins and Aβ levels in their brains and treatment with Metformin attenuates the increase of tau phosphorylated proteins. Peripheral and central injections of glucose enhance learning and memory in rats, and block memory impairments produced by morphine. One mechanism by which glucose might act on memory is by regulating the ATP-sensitive potassium channels (K-ATP channels). The aim of present study, was to investigate the effects ofthe K-ATP channel blocker Glibenclamide (Gli) on learning and memory, in streptozotocin (STZ)-induced diabetic rats. The diabetic model was induced by intraperitoneal injection ofa single dose of 65 mg/kg for three successive days to induce hyperglycaemia.Animals were divided into 4 experimental groups:(1) Control group (n = 10), which was thenormal rats and received saline intraperitoneally(0.1 ml/100 g), (2) Vehicle group (DM), which was the diabetic rats and received DMSO intraperitoneally(0.1 ml/100 g), (3) DM+ Gligroups, which were diabetic rats and treated with Gli (3, and 6 mg/kg per d) for 20 days. All rates were trained in the Morris water maze (MWM) and in shuttle-box apparatus respectively. **Results :** our results show that pre-training injection of Gli improves spatial learning and memory in STZ-induced diabetic ratsin a dose dependent manner, so that rats of Gligroups found platform in less time and with less distance traveled, in comparison with DM group. Gli also increased the percentage of time elapsed and the distance swum in the target quadrant in STZ-induced diabetic rats, in probe test. In the Passive avoidance test, Gli also dose-dependently increased the step-through latency and total time spent in thelightarea in STZ-induced diabetic rats. Conclusion: An ip injection of STZ resulted in a significant decline in spatial learning and memory and treatment with Gli can enhance learning and memory. Gli dose-dependently improved spatial learning and memory and also enhanced retention performance in STZ-induced diabetic rats. The results show that Gli as an antidiabetic drug and K-ATP channel blocker through, blocking of K-ATP channels or by sensitizing insulin in the brain improves learning and memory storageinadose-dependent manner and so isuseful for AD treatment.

Keywords:;Streptozotocin; Glibenclamide,; Morris Water Maze; diabetic rats.

INTRODUCTION

Many Studies have indicated that Diabetes Mellitus (DM) and hyperinsulinemia, increases the risk for dementia and Alzheimer's disease (AD) (1, 2). Both forms of type I and II diabetes are associated with cognitive function impairment (3).AD is characterized by the accumulation of extracellular amyloid-β (Aβ) plaques, and intracellular hyper phosphorylated Tau protein (4,

5). It was shown that animals with DM have increased hyper phosphorylated Tau protein and Aβ expression in their brains. (6, 7).Findings that in AD brains the function of multiple players in the insulin signaling are changed, has led to use the term “Type 3 diabetes” for AD (8). Therefore investigating the role of pharmacological agents that could improve neuronal insulin resistance merit attention in AD therapeutics.

Li et al (2012) study show that obese, leptin-resistant mice (diabetic mice) had increased tau phosphorylated proteins and Aβ levels in their brains and treatment with Metformin attenuates the increase of tau phosphorylated proteins (9). On the other hand it was shown that Peripheral and central injections of glucose enhance learning and memory in rats, and block memory impairments produced by morphine. The mechanisms for these effects are as yet unknown. One mechanism by which glucose might act on memory and other brain functions is by regulating the ATP-sensitive potassium channel. This channel may couple glucose metabolism and neuronal excitability, with channel blockade increasing the likelihood of stimulus-evoked neurotransmitter release. Glucose, and sulfonylurea drugs such as Gli decrease K-ATP channel conductance (10). Decreased K-ATP channel conductance renders the cell more sensitive to depolarizing stimuli, and increases neurotransmitter release. Glihas been reportedto affect neurotransmitter release both in vitro and in vivo and toenhance hippocampal acetylcholine output (11). It has been reportedthat intra-septal injections of glucose and the direct K-ATP channel blocker Gli attenuate spatial memory impairments induced by intra-septal injection of the neuroactive peptide galanin (12). Also it has been reportedthat intra-septal injections of the direct ATP-sensitive potassium channel blocker Gliboth alone and in conjunction with a sub-effective dose of glucose, enhance spontaneous alternation performance and attenuate the performance-impairing effects of morphine(13).Finally it has been reportedthat intracerebroventricularinjection of Aβ impaired spatial cognition and also increase in hippocampal hyperphosphorylated tau protein as well as galanin. On the other hand Administration of Gli, resulted in significant improvement in spatial cognition and in learning and memory performance, as well as significant decrease in hippocampal hyperphosphorylated tau protein and hippocampal galanin (14). Thesestudies suggested that Gli can improve symptoms in AD and so is useful for AD treatment. The experiments described in this report tested the hypotheses that: peripheral injections of the direct K-ATP channel blocker Gli would attenuate STZ -induced spatial cognition and learning and memory performance, deficits. The aim of present study, was to investigate the therapeutic efficacy of the K-ATP channel blocker Gli on learning and memory in STZ-induced diabetic rats

MATERIALS AND METHODS

80Adult male Wistar rats (Razi Institute, Karaj, Iran), weighing 200–300 g were used for Morris Water Maze apparatus and Passive avoidance test respectively. Animals were kept in an animal house with a 12/12-h light–dark cycle and controlled temperature (22 ± 2 °C). Animals had free access to food and tap water except during the limited periods of experiments. Eight animals were used in each group; each animal was used once only and killed immediately after the experiment. Behavioral experiments were done during the light phase of the light/dark cycle (light on 07:00). The diabetic model was induced by intraperitoneal injection ofa single dose of 65 mg/kg STZ which was freshly dissolved incitrate buffer (pH 4. 4, 0. 1 M) for three successive days toinduce hyperglycaemia. The control animals were injectedwith citrate buffer. Seven days after STZ injection, fastingblood glucose levels were determined. Animals were considereddiabetic if plasma glucose levels exceeded 7.8 mmol/L(30). Animals were divided into 4 experimental groups:(1) Control group (n = 10), which was thenormal rats and received saline intraperitoneally (physiological saline 0.1 ml/100 g), (2) Vehicle group (DM) (n = 10),which was the diabetic rats and received DMSOasvehicle of Gli, intraperitoneally(0.1 ml/100 g), (3) DM+ Gligroups (n = 20), which were diabetic rats and

treated withGli (3, and 6 mg/kg per d) for 20 days. All drugs were prepared immediately prior to use and given intraperitoneally (i.p.) in a volume of 0.1 ml per 100 g body weight of rats(15). Learning performance of the rats was evaluated in the MWM and shuttle -box starting 24 h after the last (24^{th} day), Glior DMO injection. All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Qazvin University of Medical Sciences. Gli was a gift from Chemidaru (Tehran, Iran).and DMSO and STZ were purchased from SIGMA-ALDRICH Company Gli was dissolved in water/dimethylsulfoxide (9:1) solvent, STZ, was dissolved in 0.9% saline.

Morris water maze test

After Gli treatment for 20days, the morris water mazetests were conducted to assess the learning and memory performance.The escape latency (s) and path length (cm) wereanalyzed in each trial and averaged over four trials for eachrat. The frequency the rat reached the former placement of theplatform as well as the time spent in the former platform quadrantwere detected within 60 s (30).

Passive avoidance performance (shuttle box):

To assess memory retention of animals, Passive avoidance test were performed according to(15). In this task, the animal learns that a specific place should be avoided since it is associated with an aversive event. Decrease in step through latency (STL) indicates an impairment in memory in the PA task. The passive avoidance apparatus consisted of two light (Plexiglas) and dark (Black) compartments of the same size (20×20×30 cm3) separated by a door. The floor of the dark compartment (i.e. conditioning chamber) was made of stainless-steel bars (0.5 cm diameter) separated by a distance of 1 cm. Intermittent electric shocks (50 Hz, 3 s), 1 mA intensity, were delivered to the floor of the dark compartment by an isolated stimulator.

Inhibitory-avoidance training.

The rats were allowed to become familiar with the laboratory environment 1 h before each of the training. Each animal was placed in the light compartment for 20 s, after which the door was opened and the time the animal waited before crossing to the dark (shock) compartment was recorded as the latency. The animal was removed from the experiment when it waited for more than 180 s to cross to the other side. Once the animal completely crossed to the dark compartment, the door was closed and a 1 mA foot shock was delivered for 3 s. The rat was then removed from the apparatus and 2 min later, the procedure was repeated. Training was terminated when the rat remained in the light compartment for 120 consecutive seconds. All the animals were trained with a maximum of two trials.

Retention test.

24h after training, each animal was placed in the light compartment for 20 s, the door was opened, and the latency for entering into the shock compartment was measured as STL. During these sessions, no foot shock was applied and the test session ended when the animal entered the shock compartment or remained in the light compartment for 600 s (criterion for retention) (15).

STATISTICAL ANALYSES:

Data are expressed as mean ± SEM (standard error of mean). In order to compare the latency time, the number of quadrants that the animals crossed and path length to reach the platform (distance) and values for the probe trial in MWM and values for the shuttle box separately were assessed by one-way ANOVA followed by Tukey's test to detect statistical differences between the groups. P values less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Place learning

Figure 1 display place learning of different experimental groups in the MWM. As expected, the average escape latency (The latency time to find the hidden platform), escape distance (the path length to find the platform) in searching for the hidden platform decreased with the increase in training days. In the control group there was

shorter average escape latency and a shorter escape distance. It wasnotable that diabetic rats exhibited longer transfer latency($p<0.01$), than the control group rats, while Glitreatment remarkablydecreased the mean escapes latency in a dose dependent manner ($p<0.01$), as illustrated inFig. 1A. Furthermore, diabetic rats exhibited longer escape distance (swimming distance)($p<0.01$), than the control group, as illustrated inFig. 1B. while Gli(3, and 6 mg/kg per d) for 20 daysremarkablydecreased both of them (the mean escapes latency andthe mean escape distance($p<0.01$).In the probe test, control group rats spent most time and swum most in the target quadrant indicating memory consolidation were took place well in this group. However, in the diabetic rats, time spent and swimming distance in the target quadrant were significantly less than those in control group. .Moreover there were obvious reductions of timeshe animals crossed the former platform location and the percentages of the total time elapsed in the target quadrant in diabetic rats($p <0.01$), in comparison with control groups illustrated inFig. 1C,D. However, thevalues during the probe trial were obviously reversed after administrationwith Gli(3, and 6 mg/kg),($p<0.01$), as displayed in Fig. 1C, D.Also, our results show that swimming speed of all groups of rats increased in the consecutive training days. But there was no significant difference between experimental groups indicating both STZ and Gli treatment had no effect on the motor activity of rats.

Passive avoidance:

Figure 2 shows the effects of Gli treatment on memory retention of passive avoidance learning. The data showed that the STL of STZ+ DMSO group rats were significantly reduced compared to control group rats. The STL in the STZ + Gli (3, 6mg/kg) groups rats similar to control group were significantly higher than those in the STZ+ DMSO group rats. So Gli could improve memory retention in STZ -induced diabetic rats

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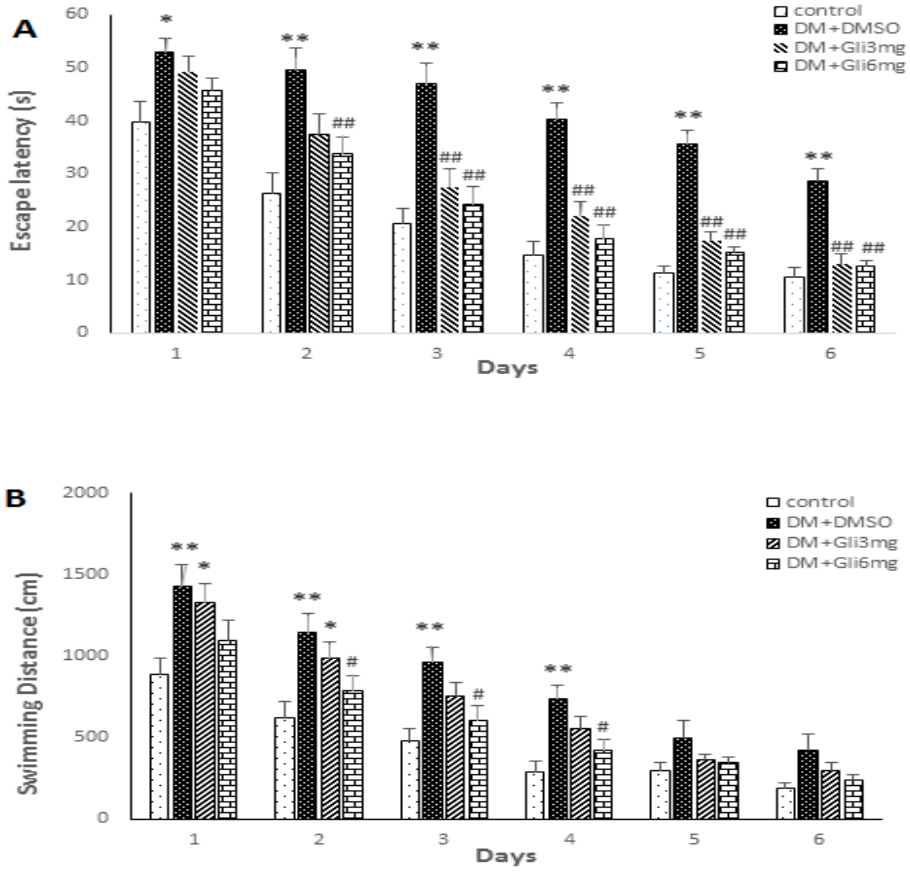

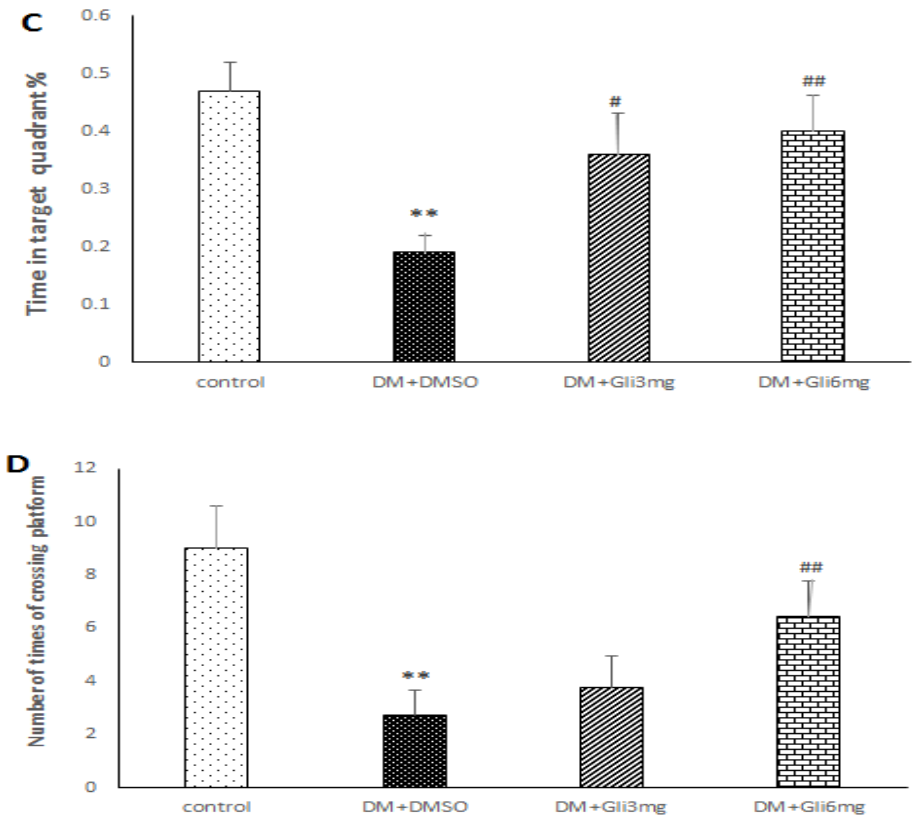

Fig. 1 Effects of Gli on the escapelatency (A) and the swimming distance (B) and thepercentage of the total time elapsed in the target quadrant (C)and the number of times of crossing platform(D) in control anddiabetic rats (n = 10, mean ± SEM).*p < 0.05, ** p < 0.01 compared with Control group; #p < 0.05, ## p < 0.01 compared with DM + DMSO group. DM +DMSO,diabetic rats and treated with DMSO (0.1 ml/100 gip); DM+ Gli, diabetic rats and treated with Gli (3and 6mg/kg,i.p. ,per day for 10 day).

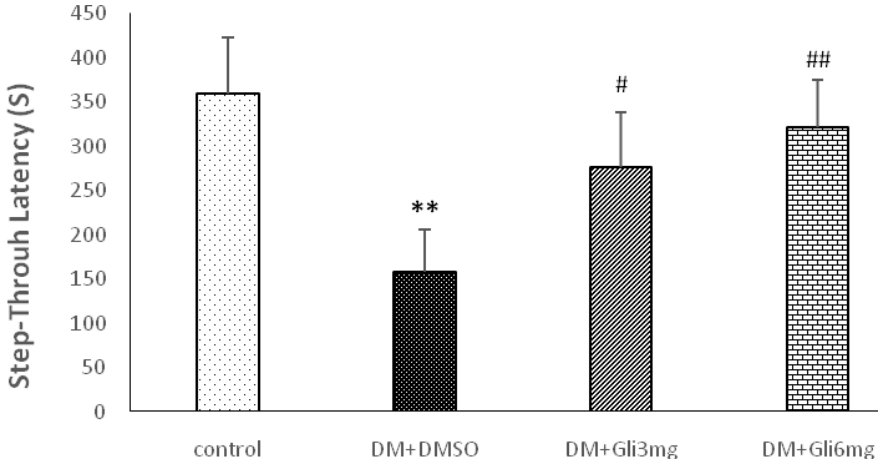

Fig. 2 Comparison of STL during the Passive Avoidance test. Each value represents the mean ± SEM of the latency before entering the dark compartment. **p<0.001 relative to the control group, #p < 0.05, ## p<0.01 relative to the DM +DMSO group.

DISCUSTION

Present results indicate that pre-training i.p. administration of STZ impaired learning and memory retrieval in diabetic rats.The present study also showed that the pre-test administrationof the K-ATP channel blocker, Gli, restored the STZ -induced impairment of learning and memory in diabetic rats.The present results are most similar to those of zarindast et al 2006 who found thatPre-test administration of lithium plus lower dose of Gli reversed the amnesia induced by pre-training lithium(15).The cognitive improvement by Gli observed in the current study is in accordance with Slingerland et al., 2008, who reported cognitive improvement with Gli and suggested that Gli is likely to be acting directly on the brain (31). Also our results is in accordance with Baraka et al. who reported i.c.v. injection of Aβ impaired spatial cognition and also increase in hippocampal hyperphosphorylated tau protein as well as galanin. On the other hand administration of Gli, resulted in significant improvement in spatial cognition and in learning and memory performance, as well as significant decrease in hippocampal hyperphosphorylated tau protein and hippocampal galanin and suggested that Gli can improve symptoms in Alzheimer's disease by inhibition of galanin in the brain(32).In accordance with our results, other investigatorshave also reported that the pre-test administration of Gli restored morphine induced impairment of memory acquisition(22). Parsons et al (1992)have shown that intra-ventricular injections of glucose attenuate deficits in spontaneousalternation scores produced by peripheral injectionsof scopolamine(16). Ragozzinoet al (1992, 1995)have shown thatntra-septal injections of glucose attenuate deficits in spontaneousalternation scores produced by morphine injections intothe medial septum and also reverse the effects of intraseptal morphine infusions onhippocampal acetylcholine output(24,25).Stefani et al (1997)have shown that plus-maze spontaneous alternation performancedeficits induced by intra-septal injection of the K-ATP channelopener galanin are antagonized by concurrent administrationof either glucose or Gli (28).Mark et al (1998)have shown thatintraseptaladministration of galanin produces spontaneous alternationdeficits which in turn can be blocked by co-administrationof either glucose or Gli. They have suggested that, in the septal region, galanin and glucose act via K-ATP channels to modulate neural function and behavior(12).Stefani et al (1999)have shown that intra-septal injections of Gli, bothalone and in conjunction with glucose, enhance spontaneous alternation performance and attenuate theperformance-impairing effects of morphine. They have suggested that, glucose may modulate memory-dependentbehavior in the rat by regulating the ATP-sensitive potassium channel (12).Furthermore, it has been shown thatintraseptal morphine administration, at adose that impairs performance of memory tasks, decreasesacetylcholine output in the hippocampal formation (24). Ghelardini et al (1998) have shown that the administrationof potassium channel openers provoke amnesiain the mouse passive avoidance test and thepotassium channel blockers were able to prevent druginducedamnesia(29).Gaineyet al.2016 have shown that a single dose of Gli ameliorates high-fat diet -induced memory impairments. (44) Also our results are in agreement with others that demonstrated that:Antidiabetic drugs such as Gli restore abnormal transport of amyloid-β across the blood-brain barrier and memory impairment in db/db mice reflected by improved performance on MWM and Ymaze tasks. Gli also enhanced hippocampal LTP in diabetic mice(41).Patients with type 2diabetes and acute ischemic stroke takingGli had a better neurological outcome (34). Gli can improve ischemia reperfusion injury via modulating oxidative stress and inflammatory mediators in the rat hippocampus(35). Gli reducescerebral edema and infarct volume, and decreases mortalityin animal stroke models(36,37,38). It has been shown that that Gli is neuroprotective against cerebral ischemia in

rats. It has been shown thatGli enhances neurogenesis and improves long-term functional recovery after transient focal cerebral ischemia (39).It has been shown that Gli enhanced long-term brain repair and stimulates neuroblast migration toward the striatal lesion, and strengthens neurogenesis in the cortex after cerebral ischemia that was associated with improved long-term behavioral (learning and memory) recovery. Therefor it has been suggested that Gli is a neuroprotective agent against cerebral ischemia that can promotes both longtermneuroprotective processes and brain repair mechanisms(39). The therapeutic potential of the administration of Glihas been confirmed in different brain pathologies (33). These posetive and restorative effects of Gli could be related to the reduction in the neuronal apoptosis and Aβ level and increase neurotransmitter release and modulating oxidative stress and inflammatory mediators in the brain. In this regard it has been reported that Chronic treatment of diabetic mice with Gli decreased hippocampal Aβ1-40 or Aβ1-42 production and inhibited neuronal apoptosis and restored hippocampal synaptic plasticity characterized by enhancing long-term potentiation and also ameliorated memory impairment reflected by improved performance on MWM and Y maze tasks. In addition, Gli significantly improved insulin resistance indicated by decreases in both serum glucose and insulin in diabetic mice (41).Several mechanisms have been suggested for neuroprotective effect of Gli. For example it has been reported that Gli prevents the activation of endothelial caspase-3 through inhibition of the SUR1-regulated NC (Ca-ATP) channels. Caspase-3 activation has been described as a major cause of apoptotic processes (42). Gli also can block the activation of brain caspase-1 triggered by adenosine which is a key biologic in hypoxia-induced anterograde amnesia (44, 45). The other hypothesis explaining how glucose and Glimight enhancememory in STZ -induced diabetic rats, is that they affects neuronal excitability andneurotransmitter release via modulation of K-ATP channels.Decreased K-ATP channel conductance renders the cell more sensitive to depolarizing stimuli, and increases neurotransmitter release. According to this hypothesis, hyperglycemia, throughincreased glucose metabolism, increases intra-neuronal ATPlevels, blocking K-ATP channels. Channel blockade depolarizesthe neuron and increases the probability of stimulusevokedneurotransmission. The hypothesis that Glimodulates behavior via K-ATPchannels is consistent with a substantial amount of other information. K-ATP modulation has been shown to influenceneurotransmitter release both in vitro and in vivo(Amoroso et al 1990, Tanaka et al 1995) (26,27). For example it has be shown that administration of sulfonylureas enhances GABArelease from rat substantianigra, and glutamate release from motorcortex. Stefani and Gold (2001) have demonstrated that K-ATPchannel modulators increase acetylcholine levels in the hippocampus (11). These results suggest that Gli can regulate neurotransmitterrelease.

In general, it seems that Gli as an antidiabetic drug and K-ATP channel blocker through, blocking of K-ATP channels or by sensitizing insulin in the brain improves learning and memory storage in STZ -induced diabetic rats in a dose-dependent manner These actions may contribute to the beneficial effects of Gli on AD treatment and cognitive function improvement in STZ -induced diabetic rats.

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