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RESEARCH ARTICLE

Acteoside-improved streptozotocin-induced learning and memory impairment by upregulating hippocampal insulin, glucose transport, and energy metabolism

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Alzheimer's disease (AD), a neurodegenerative disease, has been, by and large, correlated to insulin pathway, glucose level, and energy metabolism in the brain. Intracerebroventricular administration of streptozotocin (ICV-STZ) leads to glucose and energy metabolism dysfunction, cognitive impairment, and increased oxidative stress in the brain. Acteoside has a myriad of pharmacological effects on the brain, namely, neuroprotection and recuperation of cognitive functions. The primary focus of the current study was to examine the effect of acteoside on insulin, glucose transport, and energy metabolism in the hippocampal area of the brain. The behavioral experiments such as spatial memory, active learning, and passive memory suggested that acetoside ameliorated the ICV-STZ-induced learning and cognitive impairment. The acteoside induced increase in the protein expression of glucose transporters (Glu T1, Glu T3, and Glu T4), glucose, and insulin levels in the hippocampus for maintaining normal learning and memory function were demonstrated by Western blot. In addition, acteoside's long-term oral administration increased the the ratio of ATP content divided by ADP content (ATP/ADP) ratio, which, in turn, reduced the reactiveoxygen species (ROS) level and improved the cellular oxidative stress response. Compared with the model group, the above results show significant differences in different degrees ($p < .05$ or $p < .01$). This study suggests that acteoside can ameliorate the ICV-STZ-induced learning and memory impairment caused due to insulin receptor, insulin receptor substrate 1, Glu T1, Glu T3, and Glu T4 pathways by triggering intracerebral metabolism.

KEYWORDS
acteoside, Alzheimer's disease, behavior, energy metabolism, glucose transport, insulin transport

1 | INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder, cardinal features of which includes heavy deposition of senile plaques in neuronal fiber entanglement and neurons of specific brain regions, accompanied by the synaptic loss (Huang & Mucke, 2012; Mattson, 2004). The insulin signaling pathway and glucose metabolism process are speculated to play a significant role in the pathological manifestation of AD as these processes are substantially altered in the brain of AD subjects (de la Monte, 2014). Intracerebroventricular administration of

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streptozotocin- (ICV-STZ)-hampered phosphorylation of central insulin receptors (IRs; Grünblatt, Salkovic-Petrisic, Osmanovic, Riederer, & Hoyer, 2007; Ma, 2017) impairs the functionality of insulin signaling pathway, which culminates in metabolic disorders associated with sugar utilization and energy metabolism (Steen et al., 2005), together with learning and memory impairment, and cholinergic neuronal loss in rats (Agrawal, Tyagi, Shukla, & Nath, 2009; Kamat et al., 2015; Plaschke & Hoyer, 1993; Sharma & Gupta, 2001; Wesson & Wilson, 2011).

Acteoside is a well-known phenyl ethanol glycosides (Yin, Gong, Liu, & Yao, 2013). Its pharmacological effects include protection against oxidative damage, neuroprotection, liver protection, kidney tonification, enhanced yang (male sexual function), and anti-AD activity, such as reduce accumulation of amyloid β peptide (Zhang, Li, & Song, 2011). Acteoside oral administration can not only prevent the ICV-STZ induced neurodegeneration in mice, but also it can mitigate the oxidative damage, with the concurrent improvement of cognitive functions (Q. Liu, Wang, & Ding, 2017; Ma, 2017; Yin et al., 2013). However, the acteoside effect on glucose and energy metabolism dysfunction in AD remains unexplored.

In this study, we induced AD in the experimental animals by ICV-STZ administration. The changes associated with the brain insulin signaling and glucose transport pathway, energy metabolism, and oxidative stress in the brain were investigated as the potential underlying mechanism of acteoside action in AD. The outcome of this analysis will provide a platform for the further investigation of acteoside as a potential therapeutic agent for the AD treatment.

2 | MATERIALS AND METHODS

2.1 | Animals

SPF-grade adult male Sprague–Dawley rats, weighing 240 ± 20 g, were procured from the Xinjiang Experimental Animal Center. Experimental rats were housed at the Xinjiang Uygur Autonomous Region Uygur Institute of Medicine SPF class animal room (license number: 2018-0001) in the controlled environmental condition (22°C–23°C temperature, 45%–55% humidity, 12-hr day/light cycle); food and water were provided ad libitum. All animal studies were conducted as per the Chinese legislation on the use and care of experimental animals and guidelines developed by the Xinjiang Uygur Medical Institute. This study was ethically approved by the Xingjiang approving committee (Reference number: XJ/23/80/CPCSEA/IAEC/2019/33).

2.2 | Animal modeling

The chloral hydrate (350 mg/kg, Kelun, Tianjin, China) was used to anesthetize rats, which were placed on a stereotactic device (Kiel, WI) for the administration of unilateral ventricular injection of chloral hydrate (5 μL per side), the dose was repeated after 48-hr interval. The sham group of rats was injected with artificial cerebrospinal fluid, and rats in the other group were injected with 60 μg/μl STZ (A. Akhtar et al., 2020) (STZ was purchased from Sigma, purity≥98%). Injection site coordinates: anterior fontanelle as origin, backward 0.8–1.0 mm; lateral, 1.2–1.5 mm from left and right side of the sagittal seam; depth, 3.1–3.5 mm from the skull. The injection was performed for 5 min, 1 μL/min, and the needle was left for 5 min after injection, the mortality rate of modeling was 7.5%.

2.3 | Groups and drug treatment

Post-modeling rats were randomly divided into six groups (n = 8–10 per group): the sham group, the model group (STZ injection group), the ICV-STZ plus Donepezil (1 mg/kg) treatment group, the ICV-STZ plus acteoside 15, 30, and 60 mg/kg treatment groups. Acteoside was manufactured by Uighur Pharmaceutical Research Institute of Xinjiang Uygur Autonomous Region, extracted from *Cistanche deserticola* Ma, a Chinese herbal medicine (purity ≥ 98%, Figure 1A), and donepezil hydrochloride tablets were purchased from sanitary Pharmaceutical Co., Ltd. (China). Acteoside (15, 30, and 60 mg/kg) and donepezil (1 mg/kg) were suspended in 0.2% tween 20 solution for intragastric administration (0.1 mL/100 g, Figure 1B). The sham group and the ICV-STZ model rats were fed with 0.2% tween 20 solution (0.1 mL/100 g).

2.4 | Nest construction experiment

Nest construction experiments were carried out on days 0, 14, and 26 of drug treatment (Figure 1B). The bedding was replaced 2 hr before the onset of the night rhythm, and the napkins were placed in the cage before the test. The nests were scored on a scale of 5 as per the night rhythm (Wesson & Wilson, 2011). Nesting reflects the social behavior and daily life ability of rats. Besides, the nest construction experiment is simple, easy to operate, and noninvasive.

2.5 | Open field test

Open field test (OFT) was performed on day 20 of drug treatment (Figure 1B). A rectangular box (60 cm × 40 cm × 50 cm) and a computer-operated animal tracking video system were used to test the spontaneous activity of rats (Stanford, 2007). In this experiment, rats were gently put into the activity box; the spontaneous activities of the rats were recorded starting 2 min until 5 min. The activity time and distance of the central region were analyzed from spontaneous sites.

2.6 | Novel object recognition task

Novel object recognition (NOR) task was used to evaluate rodents' instinct to explore new objects, which determined their recognition

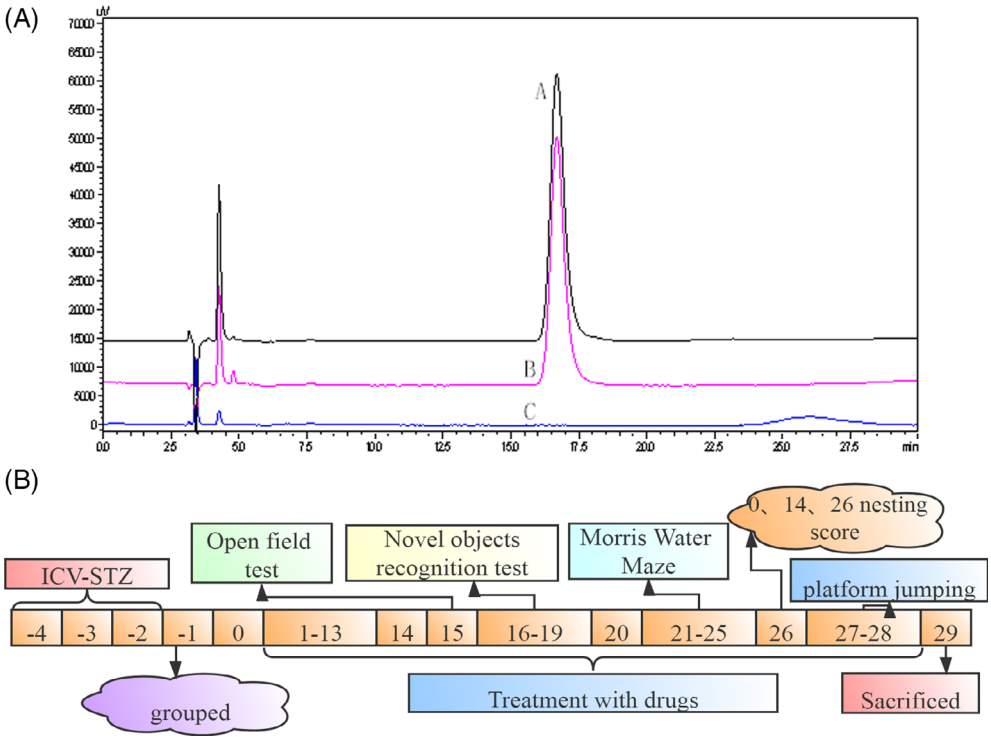

FIGURE 1 (A) HPLC chromatograms. (A) Acteoside test samples, (B) reference substances, (C) blank sham. (B) Experimental workflow [Colour figure can be viewed at wileyonlinelibrary.com]

ability and memory (A. Jackson, 2010). 16 and17 days postdrug treatment, rats were placed into a rectangular box for environmental acclimatization. On day 18, animals were subjected to a NOR experiment (Figure 1B). Firstly, two identical objects, A and B, were placed at one side of a rectangular box, and an animal's response was recorded computationally. The number and time of exploration of objects A, B, by experimental animals were determined within a period of 5 min. One hour later, in the open field, B object was replaced by C object, and time as well as the incubation period for the two objects, were observed and recorded within 5 min. The discrimination index (DI) and preferential index (PI) were calculated to evaluate the experimental animal's exploration of novel objects. Subsequently, the experimental data were compared and screened, and special, abnormal data were eliminated (inactivity or excessive anxiety). The DI and PI were calculated using the following formulae: DI = time to explore new objects/sum of time to explore two objects in rats*100%; PI = times to explore new objects/sum of times to explore two objects in rats*100%.

2.7 | Morris water maze test

Morris water maze (MWM) experiment was divided into two stages: positioning navigation stage and space exploration stage (Nuñez, 2008). The positioning navigation stage evaluated the learning ability of rats by avoiding the incubation period, while the space exploration stage evaluated the memory ability of rats by allowing them to cross the virtual platform. Rats were trained for locating the four water-entry points of different quadrants every day in the positioning navigation stage. In front of each water entry point, the rats were placed on the platform for 10 s in advance, and each time they were trained for 60 s. The escape latency of each animal was recorded for 4 days as a spatial memory learning achievement, and the exploration strategies were analyzed. The incubation period of rats without a platform was calculated and found to be 60 s. The space exploration test phase was performed on the 25th day (Figure 1B). Before the experiment, the platform was removed, and the animals were put into the water from the diagonal quadrant of the platform, the swimming track of the animals was recorded within 60 s, and the number of times of the rats' passing through the ring was analyzed and recorded as the memory score.

2.8 | Step-down latency test

The passive escape experiment was performed on day 27 of drug treatment (Figures 1 and 2) to assess the passive learning ability of rats (L. Gao, Peng, Huo, Liu, & Yan, 2015). The platform jumping experiment was divided into two stages: training period and testing period. In the training period, the rats were placed in the box of the diving platform to let them adapt to the environment for a period of 3 min. Subsequently, rats were placed on the platform connected to the power supply, and the electric current of 36 V was delivered to the grids. In this process, rats learned to find the platform as a response for avoiding electric shock. After training the rats for 5 min, Step-down latency (SDL) was recorded as the time when the rat jumped off the platform for the first time due to electric shock, the number of times rats stepped down the platform

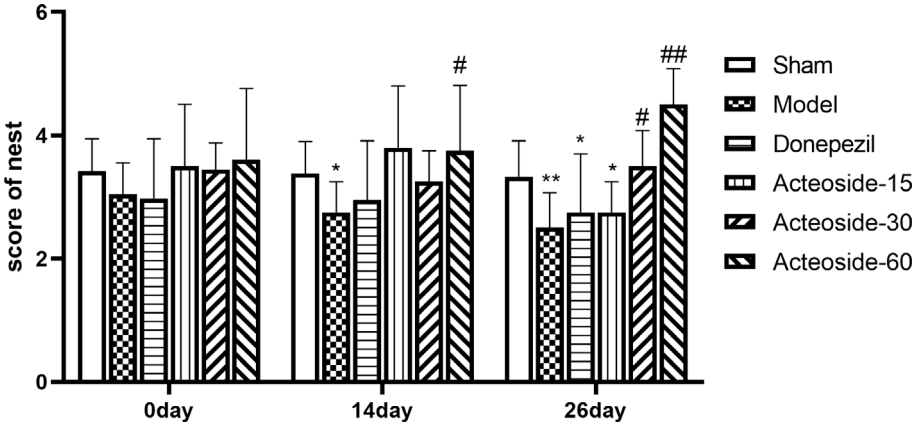

FIGURE 2 Effect of acteoside on the nesting score. Results are expressed as mean ± *SD*, 6–7 per group, $^{*}p < .05$, $^{**}p < .01$ versus sham group; $^{\#}p < .05$, $^{\#\#}p < .01$ versus model group

within 5 min of receiving the electric shock was recorded as the number of mistakes. After 24 hr, the test was repeated to examine the incubation period and the number of mistakes during the testing period.

2.9 | ELISA analysis

The cortex and hippocampal area of the rat brain were isolated on ice put into the preweighed EP tube. The weight of cortex and hippocampus in the EP tube was calculated by the subtraction method. PBS was added to the hippocampus and cortex (9:1), and these tissues were homogenized in the PBS by the ultrasonic cell grinder (XinZhi Biotechnology Co., Ltd, Ningbo, China). The resulting homogenized solution was centrifuged at 4°C and 3,500 rpm for 10 min (Allegra 64R, BECKMAN, China). ELISA kit (Jianglai Biotechnology Co., Ltd, Shanghai, China) was used to determine the insulin and glucose levels in the hippocampus and cortex, whereas adenosine triphosphate (ATP), adenosine diphosphate (ADP), the ratio of ATP content divided by ADP content (ATP/ADP) ratio, and reactiveoxygen species (ROS) levels were determined in the hippocampus.

2.10 | Western blot analysis

The western blot analysis was carried out for the rat hippocampus homogenate. The primary antibodies used for WB were anti-IR, anti-IRS1, anti-Glu T1, anti-Glu T3, anti-Glu T4, anti-β-actin. The secondary antibody for the WB analysis was HRP labeled (BOSTER, Wuhan, China). The visualization was made through chemiluminescence reagents (SAIGUO, BL520A, ECL), and the gel imaging analysis system was used to capture the WB images (Bole, Shanghai, China).

2.11 | Statistical analysis

The experimental results were statistically analyzed by using the SPSS version 17.0 software. All data were analyzed by one-way analysis of variance. Post hoc tests were conducted by employing the Tukey's test, and the significance was set at $p < .05$; all group data were presented as ($x \pm s$).

3 | EXPERIMENTAL RESULTS

3.1 | Effect of acteoside on the social capacity

As shown in Figure 2, the nesting score of the model group of rats was found to be significantly lower than the sham and acteoside-30 group of rats ($p < .05$); however, the acteoside-60 group of rats showed a significantly higher nesting score than the model group of rats ($p < .05$).

3.2 | Impact of acteoside on autonomous activities

In the current study, the impact of acteoside on the autonomous activity of rats, that is, speed, distance, and the number of standing were assessed (Figure 3). We observed that rats in the model group had a significantly high distance (Figure 3C) and average speed activity (Figure 3B) but a lower upright inquiry (Figure 3A) as compared to the sham group of rats ($p < .05$), which signifies that the ICV-TZ rat model had comparatively high anxiety but low exploring behavior. Furthermore, the rats in the acteoside group exhibited a decreased distance and average speed activity and an insignificantly increased upright inquiry than the model group of rats.

3.3 | Effect of acteoside on new object recognition and active learning

The new object recognition (NOR) test evaluates the memory by assessing the propensity of object recognition. As shown in Figure 4, we observed a significantly lower DI and PI ratios in the model group of rats than the sham group of rats ($p < .05$). However, acteoside-15, -30, -60 groups of rats showed a considerably higher DI and PI ratios than the model group of rats ($p < .01$ or $p < .05$).

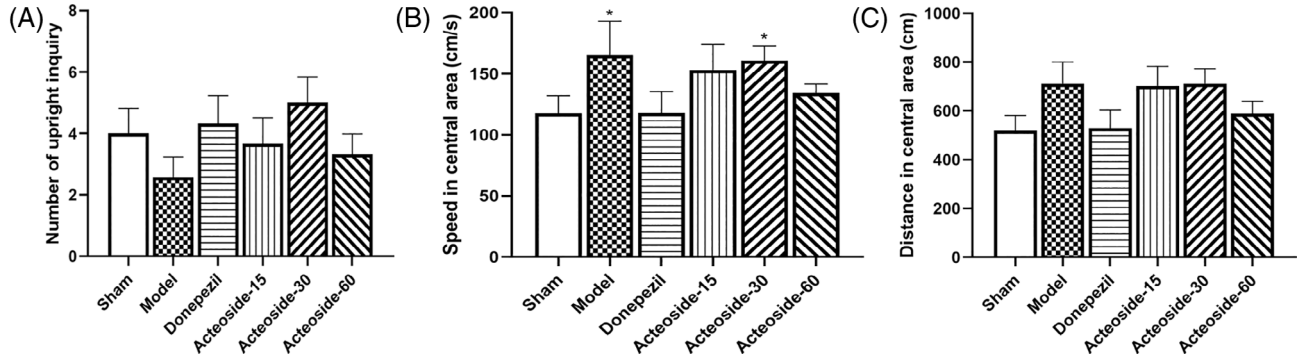

FIGURE 3 Effects of acteoside on the autonomous activities of rats: (A) number of upright inquiry, (B) speed in central, (C) distance in central. Results are expressed as mean ± *SD*, 8–10 per group, $^{*}p < .05$ versus sham group

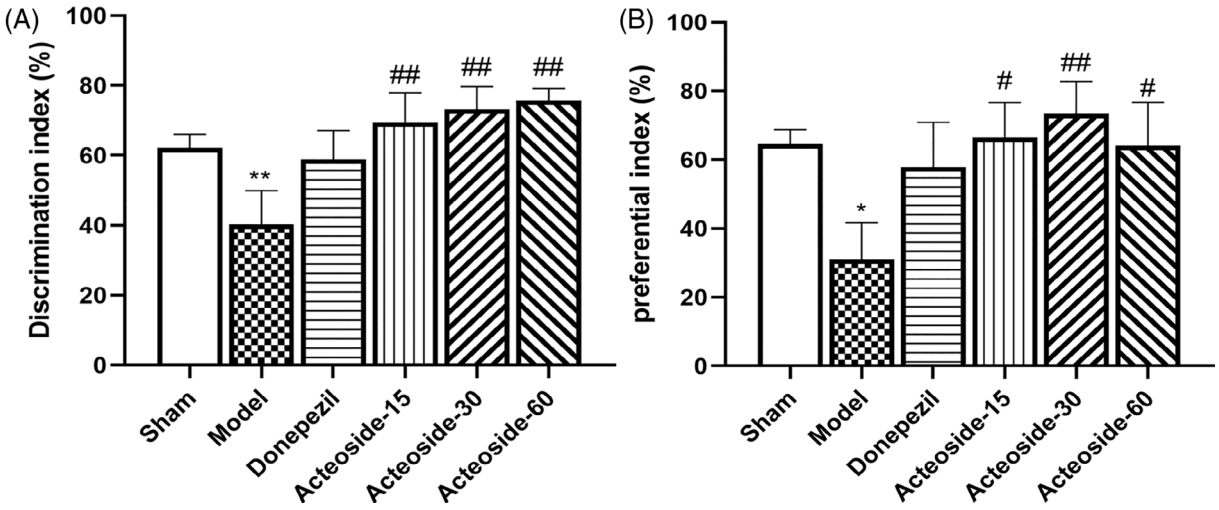

FIGURE 4 Effect of acteoside on the novel object recognition (NOR) test: (A) discrimination index, (B) preferential index. Results are expressed as mean ± *SD*, 8–10 per group, $^{*}p < .05$, $^{**}p < .01$ versus sham group; $^{\#}p < .05$, $^{\#\#}p < .01$ versus model group

3.4 | Effect of acteoside on the spatial learning ability

MWM evaluates the learning, memory ability, and spatial position cognitive ability of rats. As shown in Figure 5A, the outcome of the MWM test indicates that training time is inversely correlated to the stage incubation period in rats. We found that 3–4 days of learning period led to a significant increase in the stage incubation period in a sham group of rats as compared to the model group of rats ($p < .05$). However, the acteoside group of rats exhibited a significantly shortened incubation period than the model group of rats ($p < .05$). These findings corroborate the fact that acteoside positively affects learning and memory. Moreover, the spatial exploration of the water experiment indicated that the incubation period of the model group of rats was significantly higher than the sham group of rats ($p < .01$), and the incubation period of acteoside group of rats was significantly lower than the model group of rats ($p < .05$) (Figure 5B). Besides, the number of piercing loops in the model group of rats was significantly lower than the sham group of rats ($p < .05$) but significantly higher than the acteoside-15, -60 groups of rats ($p < .05$). It suggests that the acteoside ameliorates the STZ-induced impairment of spatial exploration ability. Additionally, we recorded four exploration strategies by using MWM software, that is, random, marginal, tendency, and directly, which recorded the swimming trajectories of rats. Analysis of the exploration model demonstrated that the composition of the rat strategy and straight trend in the model group of rats was less than the sham group and the acteoside group of rats (Figure 5C,D).

3.5 | Effect of acteoside on passive learning ability

During the training of rats, the model group of rats made significantly more mistakes than the sham group of rats ($p < .01$). However, as compared to the model group of rats, the number of mistakes in the acteoside-60 group of rats was significantly lower ($p < .05$, Figure 6B).

During the testing period, a significantly higher number of errors were made by the model groups of rats ($p < .05$, Figure 6B) also, the incubation period of stepping down was significantly lower ($p < .05$,

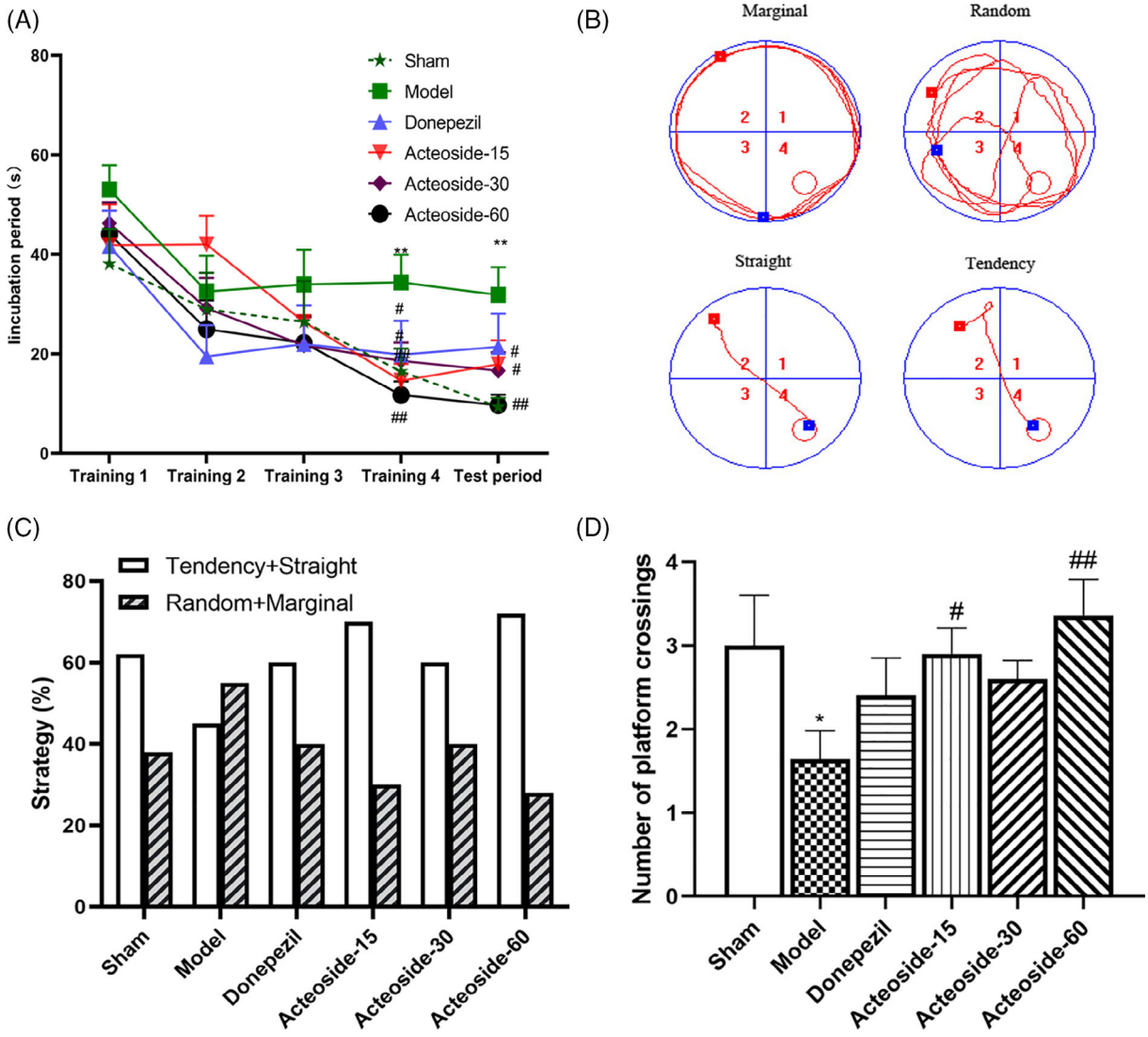

FIGURE 5 Effect of acteoside on the Morris water maze (MWM) test. (A) Changes in the latency to reach the platform during the training and testing period. (B) The number of platform crossings. (C) The percentage of strategies. (D) Representative track chart. Results are expressed as mean ± *SD*, 8–10 per group, $^{*}p < .05$, $^{**}p < .01$ versus sham group; $^{\#}p < .05$, $^{\#\#}p < .01$ versus model group [Colour figure can be viewed at wileyonlinelibrary.com]

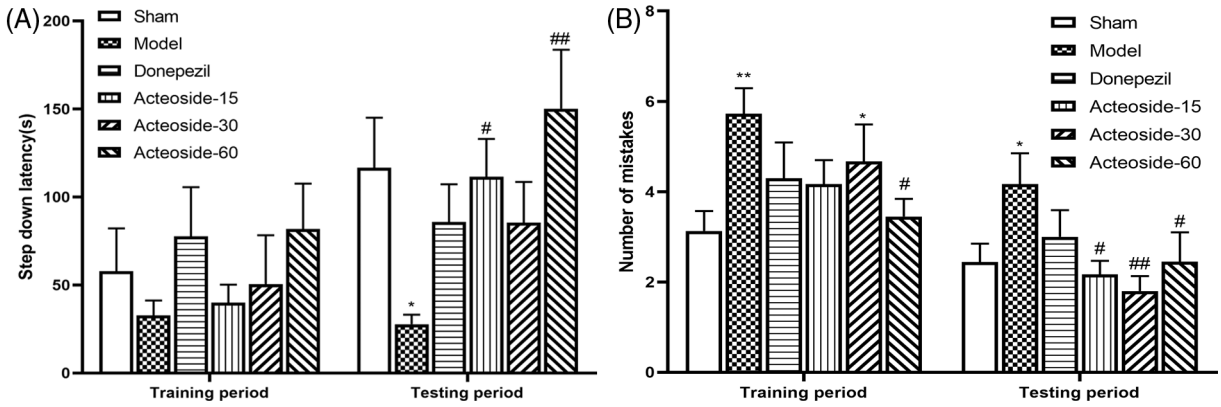

FIGURE 6 Effect of acteoside on the step-down latency (SDL) test. (A) Off-stage latency in the training and testing period. (B) The number of errors in the training and testing period. Results are expressed as mean ± *SD*, 7–9 per group, $^{*}p < .05$, $^{**}p < .01$ versus sham group; $^{\#}p < .05$, $^{\#\#}p < .01$ versus model group

Figure 6A) than the sham group of rats. As compared to the model group, the number of mistakes in the acteoside-15, -30, -60 groups of rats was significantly lower ($p < .05$ or $p < .01$, Figure 6B), and the incubation period in the acteoside-15, 60 groups of rats was significantly higher ($p < .05$ or $p < .01$, Figure 6A).

3.6 | Effect of acteoside on insulin and glucose levels in the brain

Metabolism led energy generation in the brain primarily relies on glucose uptake. Moreover, insulin plays a crucial role in the physiological activity of the brain. The hippocampus regulated processes, such as spatial localization, learning, and memory processes, are closely associated with hippocampal glucose supply (Figure 7A). The test results suggest a significant decrease in glucose and insulin level in the hippocampus of the model group of rats than the sham group of rats ($p < .05$). A significant increase in glucose and insulin level was exhibited in the acteoside-30 and -60 groups of rats than the model group of rats. A significant increase in glucose and insulin level was exhibited by the acteoside-30 and -60 group of rats than the model group of rats (Figure 7B).

3.7 | Effect of acteoside on IR, IRS1 signaling pathway in the hippocampus

The expression of insulin-related signaling pathway proteins in the hippocampus is shown in Figure 8. The expression of IR and IRS1 expression in the model group of rats was significantly depleted, but it was increased in donepezil and acteoside-15, -30, -60 groups of rats ($p < .01$) as compared to the sham ($p < .01$) group of rats and the model group. These outcomes illustrate that the acteoside increases the IR/IRS1 expression in the hippocampus of rats in order to promote insulin transport, and the associated learning and memory processes in rats.

3.8 | Effect of acteoside on Glu T1, Glu T3, Glu T4 signaling pathways in the rat hippocampus

The expression of Glu T1, Glu T3, Glu T4 in the model group of rats was significantly decreased than the sham group of rats ($p < .01$) (Figure 9). Moreover, Glu T4 expression in the hippocampus of the acteoside-15 group of rats ($p < .05$), and Glu T3, Glu T4 expression in the acteoside-30 group of rats was significantly increased ($p < .05$) as compared to the model group of rats. These outcomes suggest that acteoside increased the Glu T3 and Glu T4 expression in the hippocampus in order to increase glucose transport, and enhance the process of learning and memory.

3.9 | Effect of acteoside on ATP/ADP and ROS in the hippocampus

ATP plays a crucial role in the cognitive and learning process as it is the direct energy source for the cells. ROS is a natural by-product of aerobic metabolism and plays a vital role in cellular signal-transduction and in vivo metabolize balance. ATP to ADP ratio and ROS level is shown in Figure 10. The ratio of ATP/ADP decreased, and the ROS level increased significantly in the model group of rats as compared to the sham group of rats ($p < .01$). However, the ratio of ATP/ADP increased, and the ROS level decreased significantly in the acteoside-15, -30, -60 groups of rats ($p < .05$ or $p < .01$) as compared to the model group of rats. It indicates that acteoside can improve learning and memory ability by increasing the ATP/ADP ratio during metabolism and by inhibiting the ROS level.

4 | DISCUSSION

In the current study, we investigated the effect of acteoside on STZ-induced behavioral and cognitive impairment through multiple behavioral experiments. The outcome of nest construction experiment

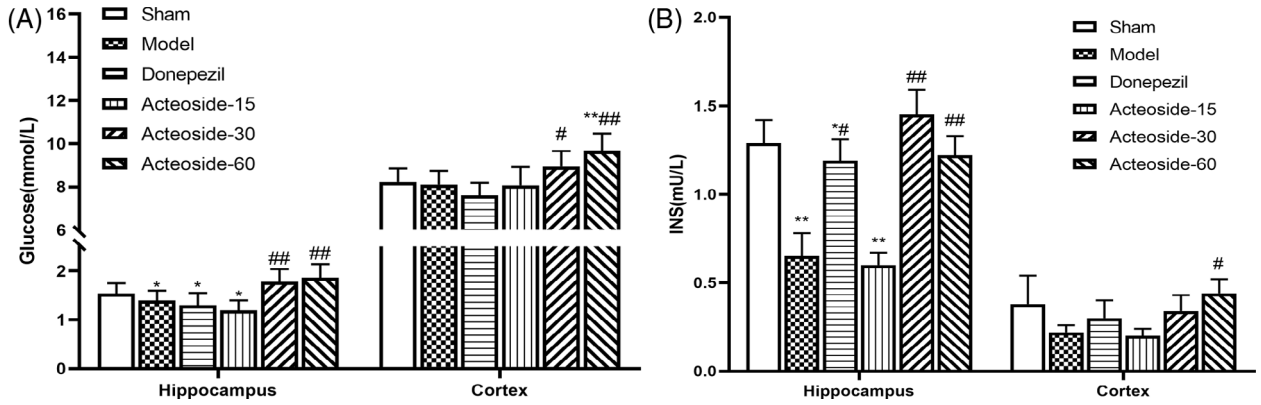

FIGURE 7 Effect of acteoside on the rat brain: (A) glucose level, (B) insulin level. Results are expressed as mean ± *SD*, 7–9 per group, $^{*}p < .05$, $^{**}p < .01$ versus sham group; $^{\#}p < .05$, $^{\#\#}p < .01$ versus model group

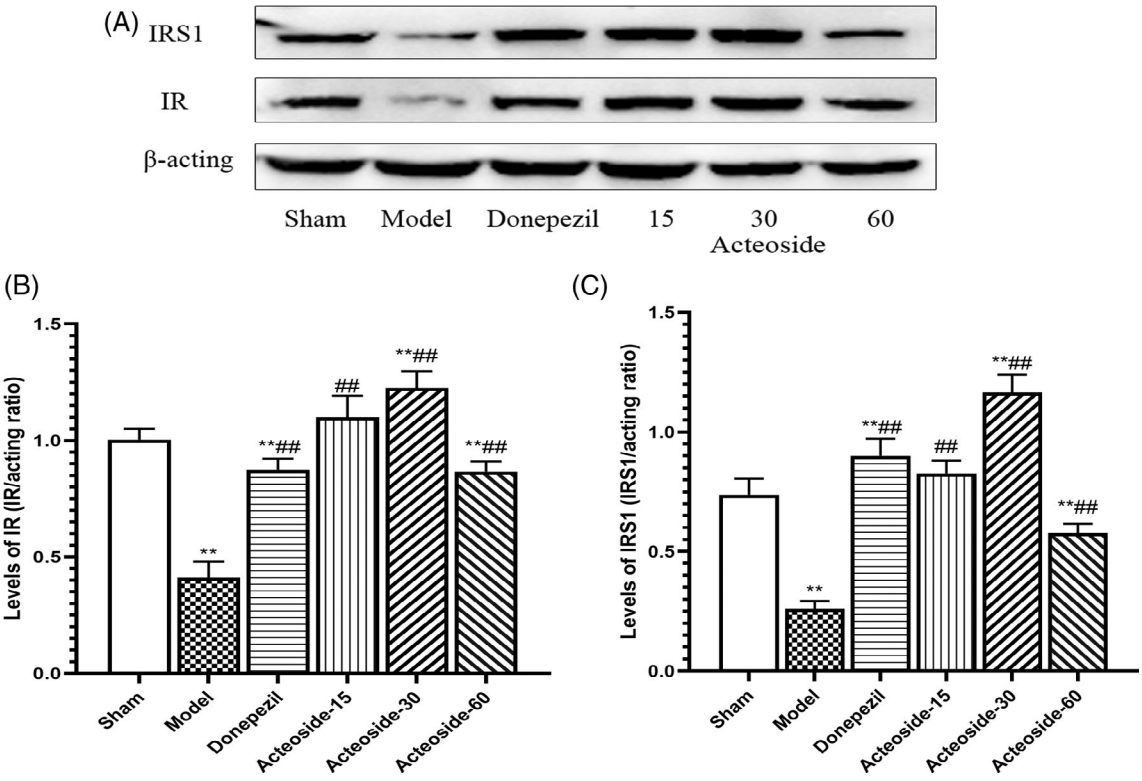

FIGURE 8 (A) Effect of acteoside on IR, IRS1 in the hippocampus. Quantification of (B) insulin receptor, (C) insulin receptor substrate 1, protein expression was analyzed by Image J. Results were expressed as mean ± *SD*, 3 per group, $^{**}p < .01$ versus sham group; $^{\#}p < .05$, $^{\#\#}p < .01$ versus model group

showed that acteoside substantially improved the social behavior and daily living ability of rats. Open field test outcomes suggested that acteoside could alleviate anxiety and improve the autonomic activity in rats, however, this effect was not significant. Acteoside's ability to improve memory and enhance the active learning ability in rats was demonstrated by the NOR task experiment. Besides, acteoside led improvement of spatial cognitive ability was demonstrated by the MWM test. The SDL test results showed that acteoside can also improve the passive learning and memory impairment in the ICV-STZ model of rats.

STZ, a nitrosourea derivative, in the central nervous system, blocks the auto-phosphorylation of IR and intrinsic tyrosine kinase activity (Kamat et al., 2015). Disrupted insulin and glucose signaling pathways exacerbate the Aβ aggregation and tau protein phosphorylation in the brain (Plaschke & Hoyer, 1993; Sharma & Gupta, 2001) impairing the cognitive function (Lauretti, Li, Di Meco, & Praticò, 2017). In this study, a series of behavioral tests demonstrated acteoside led amelioration of the ICV-STZ-induced cognitive impairment. According to the previous studies, acteoside substantially improves the spatial memory, active, and passive learning ability in mice (Park, Seeley, Craft, & Woods, 2000; van Praag, Shubert, Zhao, & Gage, 2005; Zhao et al., 1999). In the current study, we have shown the effects of acteoside on glucose and insulin pathway in the cortex and hippocampus of rats by using ELISA. The preliminary focus of this study was on the process of transport and metabolism in the rat hippocampus.

Insulin contributes to the STZ-induced cognitive deficits (Bloch et al., 2018). IR is a transmembrane tetrameric glycoprotein consisting of two extracellular α subunits and two intracellular β subunits (Fulop, Larbi, & Douziech, 2003). Insulin binds to the α subunit of IR, which induces IR autophosphorylation at the tyrosine residue of the β subunit of IR and thus promotes IRS colocalization with IR (Liu et al., 2020; Salkovic-Petrisic & Hoyer, 2007; Matioli & Nitrini, 2015; Moloney et al., 2010; Talbot et al., 2012). In the current study, the expression and functionality of insulin and its receptor were found to be depleted significantly in the ICV-STZ model, which might be the outcome of reduced acetylcholine level in the brain (Rivera et al., 2005). Besides, detection of the insulin-related pathways showed that acteoside significantly increased the expression of IR and IRS1, and promoted binding of insulin to IR. Insulin and its receptors play a crucial role in glucose metabolism and energy homeostasis in brain by regulating the Glu T activity. Alleviation of impaired insulin signaling improves glucose metabolism and energy homeostasis in the brain (Bosco, Fava, Plastino, Montalcini, & Pujia, 2011; Chen, Deng, Zhang, & Gong, 2014; Duarte, Santos, Oliveira, & Moreira, 2018). Also, as demonstrated earlier, the expression of the IR is proximally correlated to the spatial memory ability (Zhao et al., 1999). Insulin enhances the memory of passive avoidance task in the brain (van Praag et al., 2005). Glu T4 is localized in the hippocampal neurons and responds quickly to insulin stimulation. Thus, Glu T4 translocation to the membrane and increased

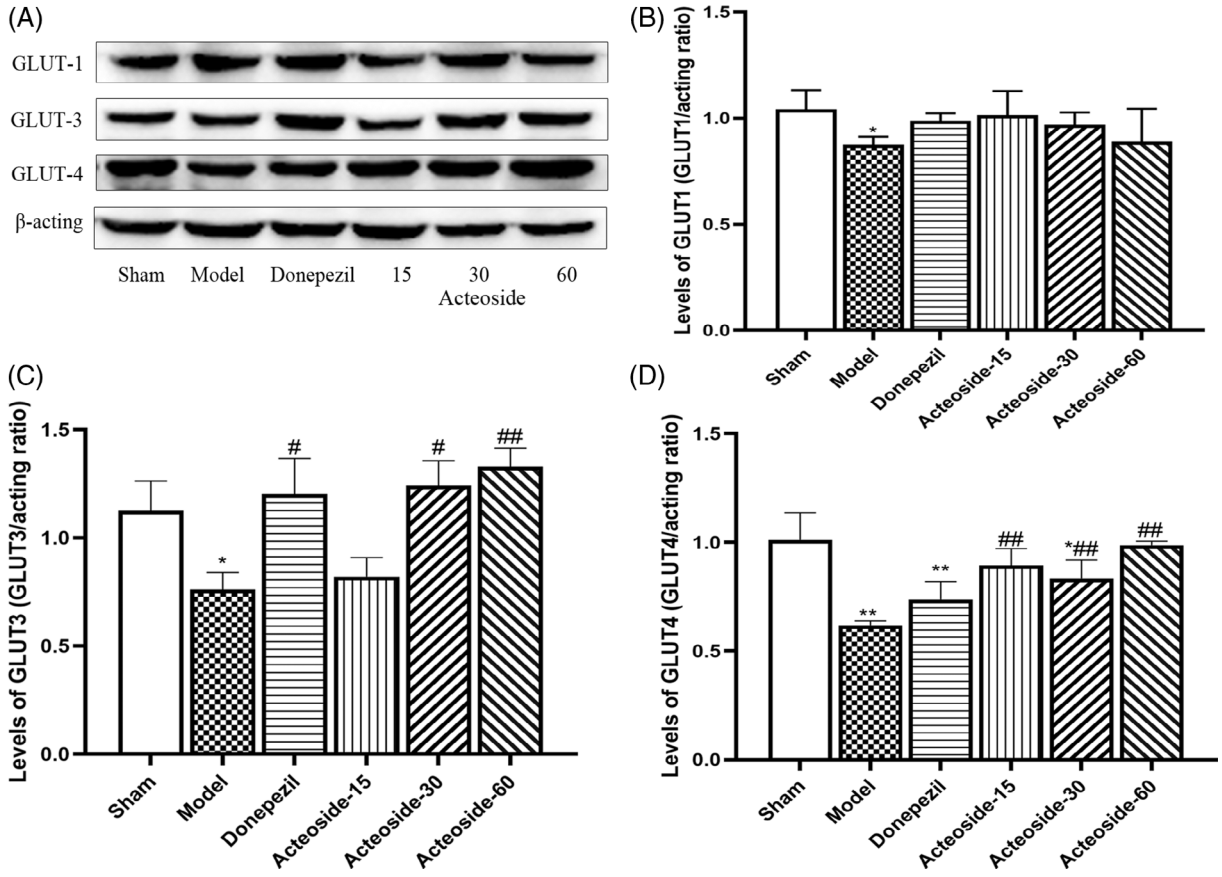

FIGURE 9 (A) Effect of acteoside on Glu T1, Glu T3, and Glu T4 in the hippocampus. (B) Glu T1; (C) Glu T3; (D) Glu T4 protein expressions quantification done by using Image J. Results are expressed as mean ± *SD*, 7–9 per group, $^{*}p < .05$, $^{**}p < .01$ versus sham group; $^{\#}p < .05$, $^{\#\#}p < .01$ versus model group

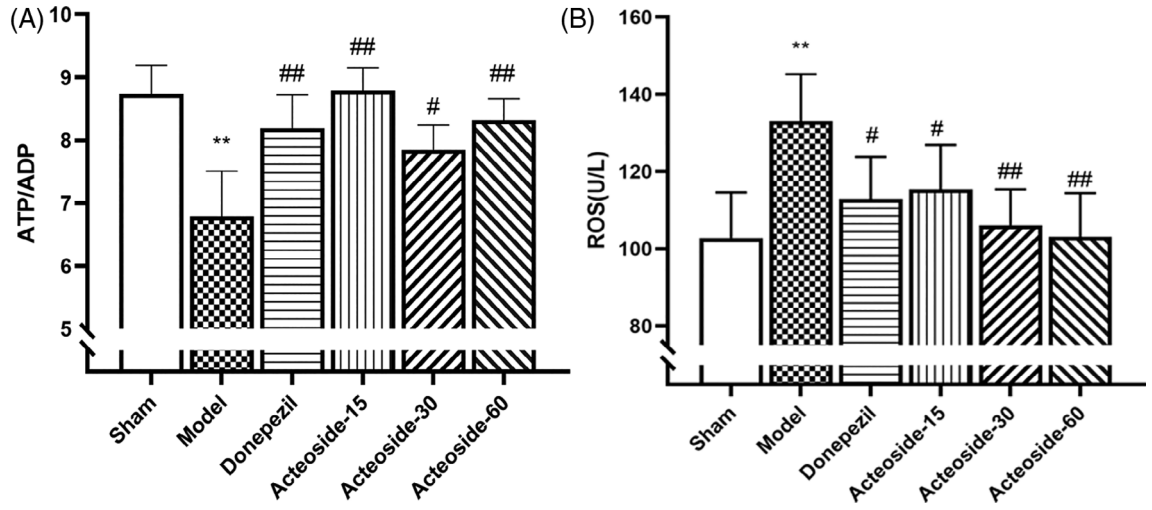

FIGURE 10 Effect of acteoside on the rat hippocampus: (A) ATP/ADP ratio, (B) ROS level. Results are expressed as mean ± *SD*, 7–9 per group, $^{**}p < .01$ versus sham group; $^{\#}p < .05$, $^{\#\#}p < .01$ versus model group

glucose uptake is necessary to meet the energy consumption (Park et al., 2000). Insulin binds to IR and activates the PI3K-AKT signaling pathway (Park et al., 2000; van Praag et al., 2005) and leads to Glu T4 translocation from the intracellular storage vesicles to the cell membrane so as to promote glucose uptake (McNay & Pearson-Leary, 2020).

In the current study, western blot analysis demonstrated that acteoside significantly increased the STZ-induced mitigated expression of Glu T4 in model group of rats. Donepezil augments the IR transduction and expression, and as per previous studies, acteoside affects the Glu T4 pathway more significantly than the donepezil (Deng et al., 2009; Hoyer, 2004). This when integrated with behavioral analysis suggests that acteoside significantly affects the spatial localization and passive learning ability such as water maze and jumping platform. Overall, these findings suggest that acteoside promotes the Glu T4 expression in the hippocampus and plays a vital role in the process of glucose uptake by hippocampal neurons affecting the spatial localization and passive learning ability of the hippocampus in rats.

Although Glu T plays a vital role in regulating glucose transport and maintaining energy homeostasis in the brain, insulin-independent Glu T4, Glu T1, and Glu T3 glucose uptake is absent in the brain (Simpson, Chundu, Davies-Hill, Honer, & Davies, 1994; Szablewski, 2016). The depletion in the Glu T1 and Glu T3 level mitigates glucose basal metabolism in the brain (An et al., 2018; Lannert & Hoyer, 1998; Santos, Mazucanti, Xavier, & Torrão, 2012), which in turn, decreases the O-GlcNAc glycosylation level of tau protein (Liu et al., 2009), and eventually increases the tau protein phosphorylation triggering the onset of AD.

ICV-STZ-induced significant decrease in Glu T1 and Glu T3 protein expression was found in the current study. As shown in a previous study, acteoside administration increased the glucose transport in the hippocampus (Harr, Simonian, & Hyman, 1995) to arbitrate ATP, metabolites, and substrates for biosynthesis of other metabolic pathways, and energy for nerve activity. Previous reports state that (Bosco et al., 2011) depletion in Glu T3 expression in the brain might reduce the activity of proteins involved in the TCA cycle such as ketoglutarate dehydrogenase, pyruvate decarboxylase. It might also hamper deoxyglucose utilization and ATP/ADP ratio. As per our study, acteoside can effectively increase ATP content and ATP/ADP ratio to enhance the basal metabolism in order to improve the learning and memory. Moreover, ATP can inhibit oxidative stress-induced apoptosis (Peters, 2004). The bioenergetic imbalance in the ICV-STZ model aggravates the cellular ROS content culminating in molecular oxidation of tissue and so the tissue damage (Agrawal et al., 2009; Fulop et al., 2003). In this study, we found that the ATP/ADP ratio is inversely correlated to the ROS content. Acteoside treatment led to significant reduction in ROS level in the hippocampus, which in turn increased the metabolic rate. It suggests that acteoside might inhibit the oxidative stress damage through the regulation of energy metabolism.

In the current study, we found that IR and IRS1 expression, autonomic activity, and new object recognition were independent of acteoside dose. The high and medium acteoside dose group decreased the IR, IRS1 protein expression, cognitive performance of the autonomous activity, and NOR, which point toward the sensitivity of IRs to acteoside (Deardorff & Grossberg, 2016). Besides, the decrease of IR protein expression at a high dose of acteoside might be related to the presence of acteoside as a natural drug macromolecular compound after the emergence of insulin-related channel dose window (Banks, Jaspan, Huang, & Kastin, 1997; Patching, 2016). Taken altogether, acteoside can significantly protect the learning ability and memory in STZ-induced dementia. It might also improve the insulin signaling pathway, enhance glucose metabolism, promote energy production, and weaken the oxidative stress response in the brain. These findings indicate that acteoside has a high therapeutic potential to treat Alzheimer's disease by targeting the glucose and insulin pathway, but its underlying mechanism demands further in depth investigation.

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