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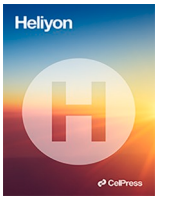

Research article

Genistein-3′-sodium sulfonate enhances neurological function in neonatal rats with hypoxia-ischemia during the recovery period

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ABSTRACT

Hypoxic-ischemic (HI) can cause neonatal brain damage leading to disability. Patients with HI experience long-term neurological issues impacting quality of life. Limited clinical treatments are available despite extensive research on HI's molecular mechanisms. Genistein-3′-sodium sulfonate (GSS), a phytoestrogen, has been found to improve acute brain injury in neonatal rats caused by hypoxic-ischemia, but its potential for chronic stage neurological recovery in HI is unknown. HI neonatal rats were treated with 1 mg/kg GSS once a day for 21 days. Then, a series of behavioral experiments was performed to evaluate the learning, memory, cognition, anxiety level and depression-like behaviors of the rats. GSS treatment reduced neuronal loss, enhanced learning, memory and cognitive function while also alleviated anxiety and depression-like behaviors in HI rats during the recovery period. These findings indicated that GSS exerted enhance neurological function in HI rats during the chronic stage, prompting further research on how it works to potentially develop new therapies.

1. Introduction

Hypoxic-ischemic encephalopathy (HIE) is a prominent etiology of neonatal cerebral injury, impacting neural development and manifesting in a spectrum of neurological sequelae including cognitive deficits, anxiety, depression, and potential chronic stage disability or mortality [1–3]. Therapeutic hypothermia has been established as the sole effective method for mitigating neurological damage in cases of HI, but most newborn children will still suffer from chronic brain damage after hypothermia treatment [2,4]. Multiple clinical investigations have identified a heightened likelihood of chronic stage neurological dysfunction in individuals with HI [5]. Most research conducted thus far has centered on the mechanisms and treatments of HI in the context of acute neurological events, with comparatively limited attention given to the long-term consequences. Consequently, there is a pressing necessity to investigate interventions aimed at mitigating the persistent negative outcomes of HI.

Estrogen exerts a significant influence on both behavioral and physiological processes, including glucose homeostasis, immunity, and fertility [6,7]. Recent research has increasingly highlighted the profound impact of estrogen on cognitive functions, emotional regulation, and neurodevelopmental and neurodegenerative processes, underscoring its critical role in brain function [8–10].

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Abbreviations

GSS	Genistein-3′-sodium sulfonate
NeuN	neurnal nuclear antigen
NS	normal saline
PBS	phosphate-buffered saline
HIE	hypoxic-ischemic encephalopathy
SD	Sprague-Dawley
MWM	Morris water maze test

Moreover, emerging studies have suggested that estrogen may confer neuroprotective effects against ischemic stroke and hippocampal ischemia-reperfusion injury both in vitro and in vivo [11]. These studies illustrate the neuroprotective impact of estrogen following ischemic brain injury. Phytoestrogens, derived from plants, particularly legumes, possess molecular structures and biological properties akin to those of estrogen, enabling them to mimic estrogen's functions without inducing its adverse effects [12].

Genistein, a phytoestrogen derived from leguminous plants, is known to have significant implications in the pathogenesis of cardiovascular diseases and breast cancer, as well as exhibiting neuroprotective properties against cerebral ischemia-induced nerve injury [13]. Despite these beneficial effects, genistein is hindered by its limited water solubility and bioavailability. In response to this limitation, genistein-3′-sodium sulfonate (GSS), a novel compound, was synthesized, and its effectiveness was evaluated.

In earlier studies, we demonstrated that GSS exhibits antioxidant properties, suppresses neuroinflammation, and mitigates cerebral ischemia-reperfusion injury [14–16]. More recently, we also discovered that GSS protects the brain against chronic cerebral ischemia by inhibiting neuronal apoptosis and also ameliorates hypoxic-ischemic acute brain injury in neonatal rats [17]. Nonetheless, whether GSS has the same protective effect during chronic HI encephalopathy and facilitates recovery of its neurological function has not been determined and deserves to be explored. Nevertheless, the potential protective effects of GSS on secondary brain injury and neurological function during the recovery period remain unknown.

This study primarily investigated the neuroprotective impact of GSS in neonatal HI rats, with a specific emphasis on its potential role in promoting chronic stage neurological function recovery. The findings of this study aim to lay the groundwork for further mechanistic research and the development of novel and effective pharmaceutical interventions for clinical applications.

2. Experimental methods

2.1. Experimental animals

Healthy specific pathogen-free male adult Sprague-Dawley (SD) rats were purchased from Hunan SJA Laboratory Animal Co., LTD. (License No. SCXK 2019-0004). The breeding ratio of male to female is equal to 1:2. Newborn rats were cared for by their maternal rats and kept together in 20–25 °C, 50–60 % relative humidity and 12 h dark/light cycle with adequate food and water. 7-day-old newborn rats of either sex, weighing 12–18 g, were selected for ischemic and hypoxia. Before the experimental tests, rats were allowed to adapt to the environment which were kept quiet. Each experiment was taken to reduce the discomfort of rats as much as possible. All animal experiments were approved by the biomedical research ethics committee of Gannan Medical University.

2.2. Drugs and reagents

GSS (molecular formula $C_{15}H_{10}O_8SNa$), a white crystalline powder with purity ≥99 %, was provided from Shanghai Tianxi Chemical Co., Ltd. Its synthesis process is described briefly: 40 mL of concentrated sulfuric acid was added to 10 g of 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one. The mixture was heated for 4 h at 80 °C, and TLC showed that the reaction was complete. The pH of the cold reaction mixture was adjusted to 3 with 20 % aqueous NaOH, and then 50 mL of saturated aqueous NaCl was added. The precipitated yellow solid was separated and dried under vacuum at 40 °C for 18 h. GSS was dissolved and diluted with normal saline (NS) to the required concentration before use. In our previous research, the optimal dosage of GSS has been determined to be 1.0 mg/kg [17]. The NeuN primary anti-body (item No. ab104224) was purchased from Abcam company; Isoflurane was purchased from Shenzhen RWD Life Science and Technology Co., Ltd.

2.3. Experimental design and HI model construction

The experimental protocol utilized to assess the potential of GSS in facilitating chronic stage neurological recovery in HI model rats

is detailed in Fig. 1A. In order to comprehensively evaluate alterations in behavior, two series of experimental tests were conducted. In the initial experiment, fear memory, cognitive function, anxiety, and depression-like behaviors were evaluated through fear conditioning, novel object recognition, tail suspension, and open field tests, respectively. The subsequent experiment employed the water maze test to explore the potential of GSS in enhancing chronic stage learning memory in HI model rats.

Eighty-seven seven-day-old neonatal SD rats for both sexes were divided into were divided into two experiments, which were distributed among four groups using random number method: the sham group, GSS group, HI group, and HI + GSS group (Fig. 1A). The HI model was constructed by the Rice-Vannucci method as described in our previous study [18,19]. Briefly, rats were anesthetized with isoflurane (the time from induction to awakening did not exceed 10 min), and the right common carotid artery was quickly severed with single electrocoagulation. After the rats awoke, they were returned to their mothers for 90 min and then subjected to hypoxia for 1 h in a chamber containing 7.0 % O_2 and 93 % N_2 at a stable temperature of 37 °C. Thereafter, they were returned to their mothers for feeding. In the sham group, the right common carotid artery was exposed but not severed. Rats in the GSS and HI + GSS groups were administered GSS (1.0 mg/kg) by intraperitoneal injection (i.p.) once daily for 21 consecutive days. Rats in both sham and HI groups received equal volumes of normal saline.

2.4. Fear conditioning test

The equipment utilized in the study consisted of a white polymer box containing hardware modules for generating sound, electric shocks, and light stimuli. Video tracking software (EthoVision XT) was employed to analyze various parameters, such as freezing behavior. The experimental procedure involved three consecutive phases (Fig. 2F). During phase 1, the conditioned training phase, rats were exposed to a series of stimuli including light, acoustic stimulation, and electrical shocks, repeated five times with intervals of 2 min. During phase 2, the environment-related training phase, rats were placed in a chamber without stimulation and observed for 6 min by the same operator as before. During phase 3, the environmental change phase, modifications were made to the chamber environment. These alterations included the application of paper of various colors on the walls, smearing of lemon juice, placement of cardboard on the chamber floor, adjustment of light color, alteration of rat placement sequence, substitution of the experimenter, and change in the color of the experimenter's gloves. The freezing response of rats was evaluated over a 3-min period under conditions of both no stimulation and with a uniform acoustic stimulus.

2.5. Morris water maze test(MWM)

The setup included a 120 cm diameter circular pool, camera system, and behavior analysis software (Ethovision XT). The pool is partitioned into four quadrants, with a platform situated in one quadrant, and adorned with images of varying colors and shapes to facilitate clear identification. A camera above the pool connected to a computer tracked and analyzed rat behavior. Creating a controlled environment is crucial in experiments to minimize the effects of light or shadows. During the training experiment, rats underwent a five-day training regimen in which water was placed in the pool and an appropriate quantity of ink was added. The horizontal surface was elevated 2 cm above the platform to render it imperceptible. Each day, the rats were trained for 1 min in each of the four quadrants. During each training session, the rats were positioned in the water facing the pool wall from a predetermined entry point. If a rat failed to locate the platform within 1 min, it was gently guided to the platform and permitted to remain there for 15 s. Rats in the sham group consistently located the platform within 15 s, indicating successful training. The duration from immersion in the water to the successful location of the platform was documented as the escape latency. In the spatial exploration experiment, the platform was removed and rats were placed in the water from the opposite quadrant. The number of times the rats crossed the platform, the time spent in the destination quadrant and the trajectory of their movements were recorded. Finally, rats were dried and returned to their cages after each experiment.

2.6. Novel object recognition test

The experimental apparatus consisted of an 80 cm × 80 cm × 30 cm black plastic box, two identical cylinders and a cube-shaped white plastic object. A video camera was installed above the plastic box and connected to a computer with EthoVision XT video tracking software, which was used to monitor and analyze the movement and activity of the rats. The experiment was performed in three phases, i.e., the adaptation phase, the familiarization phase and the testing phase, and each phase lasted one day (Fig. 3A). The rats were placed in the testing room to allow adaptation to the test environment 1 h prior to the experiment. Then, they were put in the identical location for a 10 min adaptation period. After each experiment, any feces were promptly cleaned up. The next day, two identical cylindrical objects were placed in the chambers. The rats were allowed to explore the objects, and their movements were recorded. On the last day, the object on the right was replaced with a novel cubic object, and then the familiarization phase was repeated. Exploration time for left object (TL), right object (TR) and fresh object (TF) of rats were counted. The recognition index for the novel object were calculated as TF/(TL + TF).

2.7. Tail suspension test

The experiment was carried out in an undisturbed environment employing full-shape recognition technology. Each rat was suspended from a hook on a box by the tail with tape (Fig. 4A). A camera connected to a computer with animal behavior analysis software (EthoVision XT) was placed immediately in front on the box to record and analyze the activity of the rats within 6 min. The number of immobility bouts and time spent immobile within the final 4 min were analyzed.

2.8. Open field test

The experiment was performed in a black 80 × 80 × 30 cm chamber under appropriate red light conditions. A camera was mounted directly above the box and connected to a computer with animal video tracking software (EthoVision XT). Prior to testing, the rats were placed into the testing room in advance to allow adaptation to the experimental environment. All experimental rats were individually placed in the chamber from the same starting position, and they were video recorded for 10 min. The number of entries into and time spent in the central zone were recorded, and the total distance traveled and average velocity were analyzed.

2.9. Assessment of right/left brain weight ratio

Right/left brain weight ratio was referenced the method of brain edema analysis [20].Rats were anesthetized with 1 % pentobarbital sodium (50 mg/kg, i.p.) and subsequently sacrificed. The brain was removed, photographed, and dissected symmetrically along the sagittal axis. The weights of the left and right brains were measured separately, and the right/left brain weight ratio was calculated using the formula: (right brain weight/left brain weight) × 100 %.

2.10. Immunofluorescence staining

Rats were anesthetized and subsequently fixed via cardiac perfusion with a 4 % paraformaldehyde solution. Their brains were then sliced into 30 μm sections and stored in PBS with 0.3 % sodium azide after dehydration. Brain slices were washed with PBS, blocked with 3 % BSA for 60 min, and incubated with anti-NeuN primary antibody (1:1000) overnight at 4 °C. The next day, slices were treated with Alexa Fluor488 mouse secondary antibody (1:500) for and 60 min and sealed with an anti-fluorescence blocker containing 4′, 6′-diamidino-2-phenylindole (DAPI, 32670, Sigma, USA). A confocal fluorescence microscope (Carl Zeiss Lsm880, Germany) was used to acquire immunofluorescence images. Three distinct sections of each rat and three non-overlapping area of the peri-infarct region were randomly chosen. The images were examined for the average fluorescence intensity of NeuN and DAPI positive cells using ZEN software (Carl Zeiss Lsm880, Germany).

2.11. Data analysis

GraphPad Prism 8.0 statistical analysis software was used for data processing and statistical analysis of the experimental results, all of which were expressed as mean ± standard deviation. The one-way analysis of variance (ANOVA) was carried out on pairwise difference analysis among multiple groups, followed by Tukey's test. The two-way ANOVA followed by Bonferroni post-hoc was used for multiple comparisons. $p < 0.05$ was considered statistically significant.

3. Results

3.1. GSS treatment reduced HI-induced neuronal loss and promoted recovery

On the 21st day post-HI induction, brains of HI model rats displayed notable atrophy on the ischemic side compared to sham rats, but administration of GSS resulted in partial restoration of brain size (Fig. 1B). Similar findings were corroborated by analysis of the right/left brain weight ratio (Fig. 1C). Additionally, a substantial loss of neurons was observed on the injured side following HI, with GSS treatment mitigating this neuronal loss (Fig. 1D–F). These findings suggest the successful establishment of a HI model and the significant neuroprotective effects of GSS against HI-induced brain injury.

3.2. GSS treatment attenuated learning and memory impairment in HI model rats

To assess the impact of GSS on the spatial learning and memory of neonatal rats, we conducted the water maze test at 16–21 days following HI induction. The results of the training experiment showed that compared with sham group rats, the HI group rats took more time to find the platform, indicating a decrease in learning ability, while the HI + GSS group rats took less time to find the

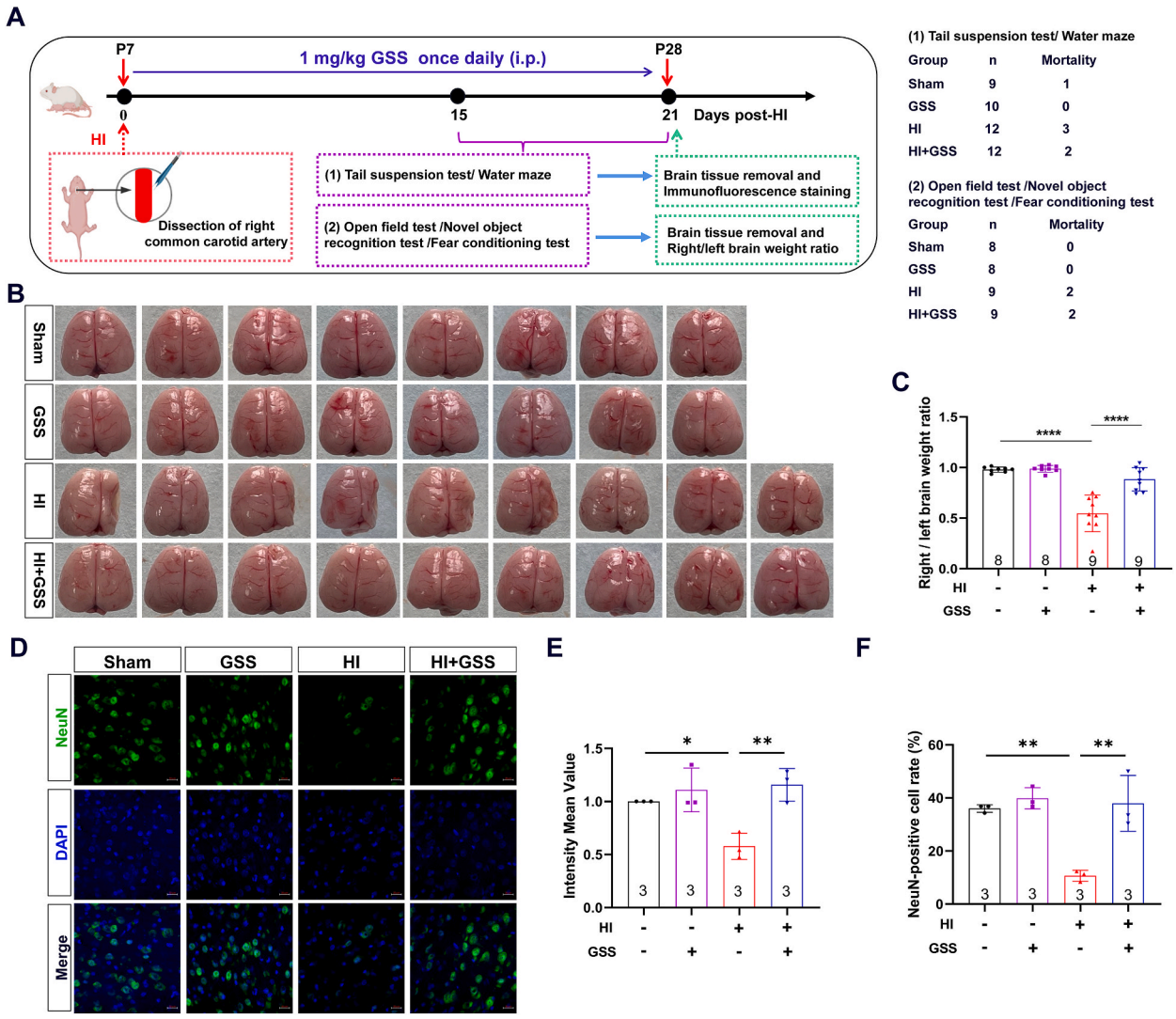

Fig. 1. GSS reduced brain injury and neuronal loss in newborn rat. **(A)** Experimental schematic diagram. **(B)** The anatomical appearance of whole brain. **(C)** Weight ratio of right brain to left brain. **(D)** Representative images of immunofluorescent staining from cortex (scale bar = 20 μm, n = 3). **(E)** Fluorescence intensity of NeuN. **(F)** NeuN positive cell rate. All data were expressed as the mean ± SD, one-way ANOVA followed by Tukey's test, $^{**}p < 0.01$, $^{****}p < 0.0001$.

platform, showing a significant improvement in learning ability (Fig. 2C). In addition, there was no difference in average swimming speed of the rats among the groups (Fig. 2A). The results of the spatial exploration experiment revealed that the number of platform crossings and time spent in the platform quadrant were significantly reduced in the HI group compared with the sham group rats, suggesting the impairment of spatial memory, while GSS treatment significantly improved the ability of spatial memory (Fig. 2B–D and E). These findings demonstrated that rats subjected to the HI exhibited impairments in learning and memory, which were notably ameliorated by treatment with GSS.

In order to examine the effect of GSS on fear memory in neonatal rats, we analyzed variations in the freezing duration by using the contextual fear conditioning test conducted 19–21 days post HI induction (Fig. 2F). The experiment revealed that the activity of the animals did not decrease after the environment was changed (Fig. 2G). However, following delivery of auditory stimuli, a significant conditioned freezing response was observed in the sham group (Fig. 2H). The freezing time of the HI group rats was obviously shorter than that of the sham group rats, while the freezing time of the HI + GSS group rats increased markedly and approached that of the sham group rats (Fig. 2H). The findings indicated a reduction in fear memory in rats with HI and a noticeable restoration following the administration of GSS.

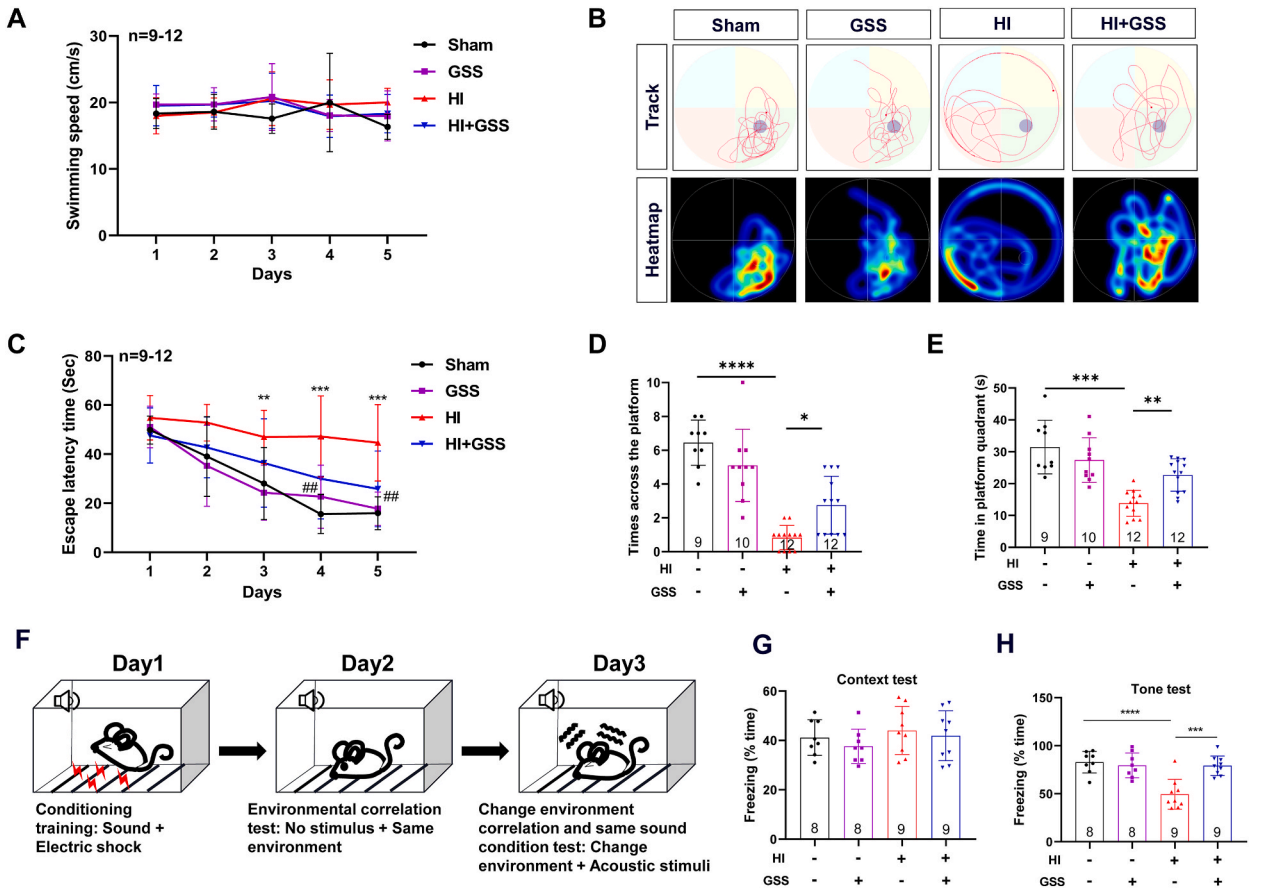

Fig. 2. GSS improved spatial memory and fear memory of neonatal HI rat. **(A)** Swimming speed. **(B)** The swimming track and heatmap. **(C)** Escape latency time. **(D)** Times across the platform. **(E)** Time spent in the platform quadrant. **(F)** Schematic diagram of fear box experiment. **(G)** Percentage of the freezing time without any stimulus during the first 3 min of the test period. **(H)** Percentage of freezing time given the same acoustic stimulation 3 min after the test period. All data were expressed as the mean ± SD. Two-way ANOVA followed by Bonferroni post-hoc was used for multiple comparisons. One-way ANOVA followed by Tukey's test $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

3.3. GSS treatment mitigated cognitive impairment in HI model rats

To assess the effects of GSS on cognitive impairment in neonatal rats, we conducted the novel object recognition test at 15–17 days following HI induction (Fig. 3A). There was no difference in the time spent exploring the two identical objects (Fig. 3B). However, when one of the objects was replaced with a novel object, the HI group rats explored that novel object for a shorter amount of time than the old object and had a lower recognition index for the novel object than the sham group rats. However, these changes were reversed by GSS treatment (Fig. 3C and D). These results illustrated that HI model rats exhibit cognitive impairment, and that the administration of GSS can effectively enhance their recognition capabilities.

3.4. GSS treatment alleviated depressive-like behavior in HI model rats

We tested the impact of GSS on depression in HI model rats using the tail suspension test at 15 days after inducing HI (Fig. 4A). The results showed that compared with sham group rats, HI group rats showed increases in the number of immobility bouts, time spent immobile, while GSS-treated rats presented significant decreases in these parameters (Fig. 4B–D). These data demonstrated that HI model rats showed depressive-like behaviors, which were alleviated by GSS administration.

3.5. GSS treatment decreased anxiety-like behavior in HI model rats

To assess the effect of GSS on anxiety-like behavior in neonatal rats, we used open field test at 15 days following HI induction. The results demonstrated that the HI group exhibited a reduced number of entries into, and decreased time spent in, the central area compared to the sham group. Conversely, GSS treatment showed a tendency to reversed these measures (Fig. 4E–G). The results

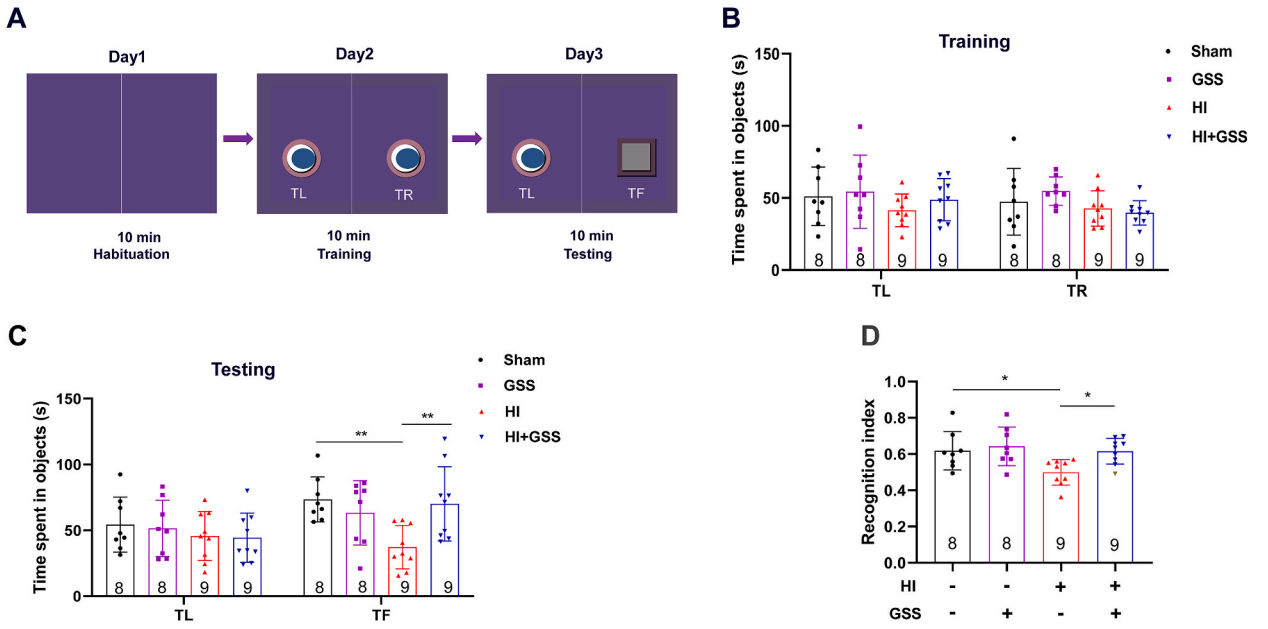

Fig. 3. GSS enhanced the cognitive memory ability of neonatal HI rat. **(A)** New object recognition experiment process. **(B)** The exploring time of rats between two identical objects. **(C)** The exploring time of rats between old and new objects. **(D)** Recognition index. All data were expressed as the mean ± SD. Two-way ANOVA followed by Bonferroni post-hoc was used for multiple comparisons. One-way ANOVA followed by Tukey's test $*p < 0.05$, $**p < 0.01$.

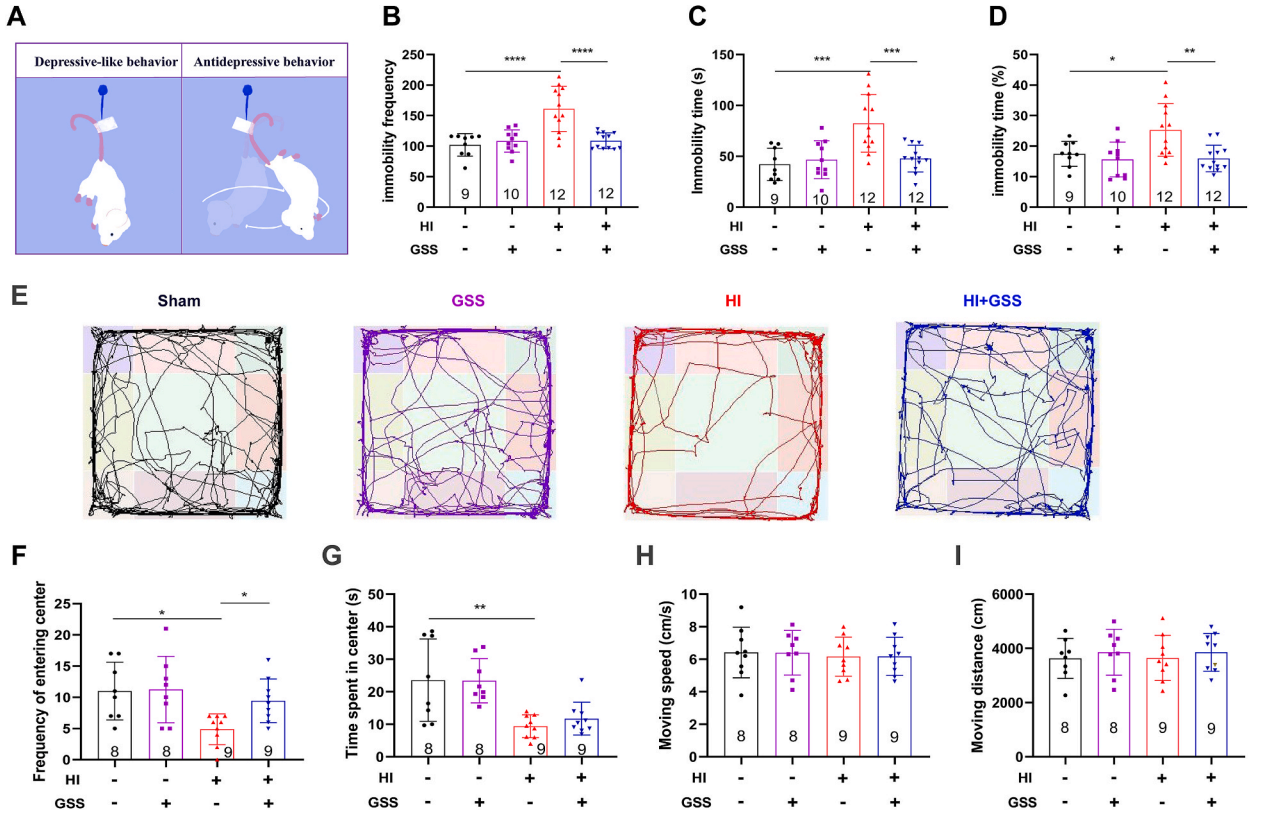

Fig. 4. GSS decreased the depression-like and anxiety behavior of neonatal HI rat. **(A)** Schematic diagram of tail suspension test. **(B)** The frequency of immobility. **(C-D)** Immobility time. **(E)** Representative tracking diagram. **(F–G)** Frequency and accumulation time in the central zone. **(H–I)** Speed and total distance traveled. All data were expressed as the mean ± SD, one-way ANOVA followed by Tukey's test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

suggest that neonatal HI rats exhibit increased anxiety levels and deficits in exploratory behavior and that these changes can be inhibited by GSS administration. To exclude the effects of differences in locomotor activity and measurement time, we analyzed the total distance traveled by and mean velocity of the rats within 10 min. There were no differences in these two parameters among the different groups, indicating that the differences in behavior were not due to differences in the animals' physical activity (Fig. 4H and I).

4. Discussion

This study examined the impact of GSS on brain injury and behavioral abnormalities induced by HI. The findings indicate that sustained GSS treatment mitigated brain injury in HI model rats. Additionally, the behavioral outcomes revealed that GSS enhanced learning memory recovery, ameliorated cognitive impairment, and alleviated anxiety and depression-like behaviors. These results suggest that GSS not only diminishes brain injury but also plays a significant role in promoting chronic stage neurological function recovery in HI model rats.

HI is a significant contributor to neonatal mortality and the development of long-term neurodevelopmental impairments. Numerous clinical and animal studies have demonstrated that infants affected by perinatal ischemia and hypoxia are at increased risk for significant neurological deficits, encompassing impairments in learning, memory, cognition, and mood regulation [20–22]. This study assessed the learning memory and cognitive function of rats through the utilization of water maze, fear conditioning, and novel object recognition tasks, while also evaluating anxiety-like and depressive behavior via open field and tail suspension tests. Consistent with many studies, HI induced chronic stage memory impairment and anxiety-like and depressive behaviors in rats, demonstrating that the animal model of HI employed in this investigation simulates the clinical characteristics of the condition.

The fear conditioning test is a relatively well-established test used to assess learning and memory deficits in rodents [23]. In this experiment, it was used to determine whether GSS improved the acquisition and expression of contextual fear memory in HI model rats. The results showed that GSS treatment significantly promoted the recovery of this fear association in HI model rats, indicating that HI results in normal fear memory deficits in early adulthood in rats and that GSS promotes it reinstatement. Research has indicated that the amygdala and hippocampus play critical roles in the consolidation of contextual fear memory [24,25]. This evidence suggests that GSS treatment effectively mitigates HI-induced damage in these brain regions.

In addition to causing memory deficits, HI contributes to learning impairment. MWM is widely used in neurobiological and pharmacological studies of the chronic phase spatial learning and memory and is quite effective in the assessment of hippocampal dysfunction [26,27]. Our results show that the escape latency, number of platform crossings and time spent in the platform quadrant were significantly reduced in HI rats, which were significantly improved after the administration of GSS, indicating that the HI model rats have significant learning and memory deficits, which can be recovered by GSS.

Impairment of neonatal neurodevelopment, especially cognitive impairment, is a predominant concern of parents, clinicians, and society [28]. Here, the novel object recognition test is used to evaluate the cognitive memory of HI model rats [29]. This test is based on the fact that animals tend to approach and explore novel objects, and the time spent exploring the novel object and the recognition indexes for the old and new objects are used as indicators of cognitive memory [30]. In our study, HI model rats had a significantly lower preference for the novel object than the old object, and they were significantly more inclined to explore the novel object after GSS administration, indicating that HI model rats exhibited cognitive memory impairment, which could be alleviated by GSS. Object recognition depends on dissociable memory representations distributed across different brain areas, with perirhinal cortex maintaining long-term memory for what objects had been encountered, and hippocampus supporting memory for where these objects had been placed [31]. The medial prefrontal cortex and lateral entorhinal cortex are a critical part of a neural circuit that underlies episodic-like and associative object-recognition memory [32]. These evidences suggest that GSS treatment may mitigate HI-induced damage in these brain regions.

Normally, depression also results in cognitive changes, and the exploration of novel objects can be used as a measure of missing interest, a depressive behavior [33]. As previously reported, estrogen is essential for mood regulation [34]; this is in line with the results of the tail suspension test in this study, which showed that GSS can ameliorate depressive-like behavior induced by HI.

It is well known that depression and anxiety generally tend to be concomitant [35]. Open field test is the most commonly used behavioral experiment to assess anxiety-like behavior in rodents and overcomes the effect of the animal's locomotor activity on the detection [36,37]. Thus, we used the open field test to assess the anxiety behavior of HI model rats. Increased and decreased animal movement in the central zone is often considered an indicator of anti-anxiety and anxiety [38]. Based on the results, we concluded that locomotor ability was similar among the groups and that HI model rats showed reductions in the number of entries into and time spent in the central area, while GSS reversed these anxiety-like behaviors.

However, our experiment has definite limitations and did not consider the influence of sex. Research in both animals and humans has consistently demonstrated that differences in brain function and behavior, such as memory, cognition, emotion, neurotransmitter levels, and auditory processing, can exist between males and females [39]. Female rats with HI are more likely to have memory deficits, while male rats are more susceptible to oxidative stress [40], so it is important to consider sex as a variable in experiments. Furthermore, this study primarily elucidated the role of GSS from a behavioral perspective. In subsequent research, we will investigate the specific targets of GSS, such as estrogen receptors, as well as the molecular mechanisms through which it regulates learning, memory, and depression-like behaviors.

In conclusion, HI leads to memory and cognitive problems, anxiety and depression-like behaviors in newborn rats, but these can be alleviated with extended GSS treatment. This research indicates that GSS may be a beneficial neuroprotective option, laying the groundwork for potential drug therapies for HI-related brain damage.

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Ethics approval and consent to participate

Animal experimental protocols were approved by the Gannan Medical University Animal Care and Use Committee. All animal experiments were performed following the Guidelines for the Care and Use of Laboratory Animals of China Medical University (No. 2022276, date: 3/5/2022).

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request. All of data were generated in-house, and no paper mill was used.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Liyan Shuang: Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Gaigai Liu:** Writing – review & editing, Visualization, Methodology, Investigation, Data curation. **Yun Huang:** Visualization, Methodology, Investigation, Data curation. **Ting Xie:** Visualization, Methodology, Investigation, Data curation. **Huijie Lin:** Validation, Methodology, Investigation. **Ruizhen Liu:** Supervision, Data curation, Conceptualization. **Jinhua Xue:** Writing – review & editing, Supervision, Software, Resources, Project administration, Funding acquisition, Conceptualization. **Zhihua Huang:** Writing – review & editing, Visualization, Supervision, Software, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lixia Jiang:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

All of authors have read and approved to submit our manuscript "Genistein-3′-Sodium Sulfonate Enhances Neurological Function in Neonatal rats with Hypoxia-Ischemia during the Recovery Period" to ***Heliyon***. All of authors declare that they have no conflict of interest.

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