Am J Transl Res 2025;17(10):8336-8346
www.ajtr.org /ISSN:1943-8141/AJTR0164655

Original Article

Escin displays neuroprotective effects in mice with intracerebral hemorrhage through ameliorating intestinal injury

Xue Jia[1*], Xiaohan Zhang[2*], Huiwen Li[1], Huijin Chen[1], Bing Han[2], Fenghua Fu[1], Tian Wang[1]

[1]*School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation, Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai, Shandong, China;* [2]*Center for Mitochondria and Healthy Aging, College of Life Science, Yantai University, Yantai, Shandong, China.* [*]*Equal contributors.*

Received March 20, 2025; Accepted October 16, 2025; Epub October 25, 2025; Published October 30, 2025

Abstract: Objective: To investigate the relationship between secondary brain injury and intestinal injury in intracerebral hemorrhage (ICH) and whether escin can protect the function of the intestine and inhibit lipopolysaccharide (LPS) entry into the blood, thereby attenuating brain injury after ICH. Methods: Mice were injected stereotactically with collagenase to establish a model of ICH. The Garcia test, forelimb placement test, brain water content, blood-brain barrier (BBB) permeability, intestinal injury, intestinal permeability, and serum LPS levels were evaluated. The ICH mice were treated with escin to investigate the effect of escin on intestinal injury and neurological function. Results: The severity of secondary brain injury was highly correlated with the degree of intestinal injury. After administering escin, intestinal injury was significantly alleviated, intestinal permeability was markedly reduced, and LPS blood levels were significantly decreased. Additionally, results from the Garcia and forelimb placement tests showed significant improvement. However, intraperitoneal injection of LPS, simulating the entry of LPS into the bloodstream due to intestinal injury, weakened the neuroprotective effects of escin without affecting its intestinal protective effects. Conclusions: The ICH-induced brain injury caused intestinal barrier damage, resulting in LPS in the gut to enter blood circulation, which subsequently disrupted the BBB. Therefore, LPS plays an important role in ICH-induced secondary brain injury. Escin exerts its neuroprotective effect by attenuating gut injury following ICH.

Keywords: Intracerebral hemorrhage, escin, inflammation, intestinal barrier

Introduction

Stroke is a leading cause of death worldwide. It can be either hemorrhagic (about 15%) or ischemic (about 85%) [1]. Intracerebral hemorrhage (ICH) is the most devastating type of stroke. The 30-day mortality of ICH is 55%, with half of these deaths occurring in the first 2 days [2]. Much of the early mortality of hemorrhagic stroke is due to the increase of intracranial pressure that leads to herniation and death. ICH leads to brain injury through two pathways. The presence of blood in the parenchyma causes damage to the surrounding brain tissues through a mechanical effect. Secondary injury from ICH may result from the blood components and their degradation products [3, 4]. Inflammation, oxidative stress, and excitotoxicity are associated with secondary brain injury from ICH. Increasing evidence suggests that inflammation plays a key factor in ICH-induced secondary brain injury [5].

In patients with acute central nervous system (CNS) injuries, abnormalities in gut microbiota metabolism, intestinal mucosal morphological damage, and gastrointestinal inflammatory responses may occur [6]. Specifically, CNS diseases such as stroke can lead to dysbiosis of the gut microbiota, which further results in the disruption of tight junctions and a decrease in the levels of occludin/ZO-1 [7]. Additionally, gut microbiota dysbiosis can alter dendritic cell activity, thereby changing immune homeostasis in the small intestine [8]. These pathologic changes lead to intestinal barrier dysfunction and an increase in intestinal mucosal permeability [9]. Therefore, the toxic substances in the

https://doi.org/10.62347/BQWN8476

intestine, such as lipopolysaccharide (LPS), will leak into the peripheral blood, leading to systemic inflammation. More importantly, LPS in the circulation may penetrate the damaged blood-brain barrier (BBB), exacerbating brain injury. The BBB is a part of the neurovascular unit (NVU). The cell-to-cell and cell-to-matrix communications maintain the physiological structure and function of the brain [10]. Disruption of the BBB leads to the loss of NVU integrity and results in injury to the CNS. BBB impairment-induces microvascular leakage as early as six hours after stroke. Moreover, BBB disruption also occurs in the peri-stroke region, such as the contralateral hemisphere [11, 12]. Stroke causes pathophysiological changes in the gut, allowing LPS to enter the blood circulation. LPS will penetrate the damaged BBB and trigger an inflammatory response, exacerbating brain injury [13].

Escin, a natural mixture of triterpene saponins, is extracted from the horse chestnut tree and has anti-inflammatory and anti-edematous properties [14]. A study from our laboratory also demonstrated that escin enhanced neurological function and mitigated BBB damage in mice with ICH by suppressing systemic inflammation [15]. Furthermore, escin exerts a protective effect on indomethacin-induced gastric ulcers in mice through its antioxidant and anti-inflammatory properties [16]. In the animal model of intestinal damage produced by cecal ligation and puncture (CLP), escin inhibits acute inflammation and reduces mucosal damage [17]. Additionally, escin improves inflammatory bowel disease by repairing the intestinal barrier and promoting intestinal motility [18]. Mice with ICH were administered escin, which was then detected in their serum. However, no escin was detected in hemorrhagic cerebral hemispheres or non-hemorrhagic cerebral hemispheres. These findings suggest that escin does not cross the BBB in mice with ICH [15]. The present study aimed to investigate the relationship between secondary brain injury and intestinal injury in ICH and whether escin protects the function of the intestine and inhibits LPS from entering the blood, thereby attenuating brain injury following ICH.

Materials and methods

Animals

Male CD1 mice (Conformity certificate: SCXK20220006), weighing 26 to 30 g, were obtained from Jinan Pengyue Experimental Animal Breeding Co. (Jinan, China). The mice were housed in an environment with a 12-hour light/12-hour dark cycle (light period starting at 8 AM), with free access to standard laboratory feed and water. All experiments were conducted in accordance with the contemporary revision of the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health (US NIH publication No. 8023, 8th edition published in 2011) and were approved by the Ethics Committee of Yantai University (YTDX20230306).

Intracerebral hemorrhage model

A model of ICH was prepared according to a previous report [19]. In brief, mice were anesthetized with an intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). A longitudinal incision was made in the scalp to expose the skull surface. The mice were fixed in a stereotaxic apparatus (Model 902-A, Kopf Instruments, Tujunga, CA, USA). A burr hole was drilled at the right coronal suture (0.2 mm posterior to the bregma and 2.2 mm lateral to the midline). A 26-gauge needle (Hamilton Company, Reno, NV, USA) was stereotactically inserted into the right basal ganglia (3.5 mm depth below the skull surface). A microinfusion pump was used to inject 0.5 μl of 0.15 U/μl type VII bacterial collagenase (Sigma-Aldrich, St. Louis, Missouri, USA, Lot: 128M4107v). The needle was maintained in the basal ganglia for 5 min after collagenase injection. Animals in the sham group were injected with an equivalent volume of phosphate-buffered saline (PBS). The skull was sealed with bone wax, and the skin incision was sutured. The mice were subsequently placed on a heating pad at 37°C to maintain their body temperature near physiological levels, and upon awakening, they were returned to their cages.

Experimental design

The experiments were conducted as follows (**Figure 1**).

Experiment 1

Mice were allocated into 8 groups without special selection: sham, ICH at 3 h, 6 h, 12 h, 1 d, 2 d, 3 d, 7 d groups. Except for the sham group, mice in the other groups were injected with collagenase to prepare the model of ICH, whereas the sham group received an equivalent volume

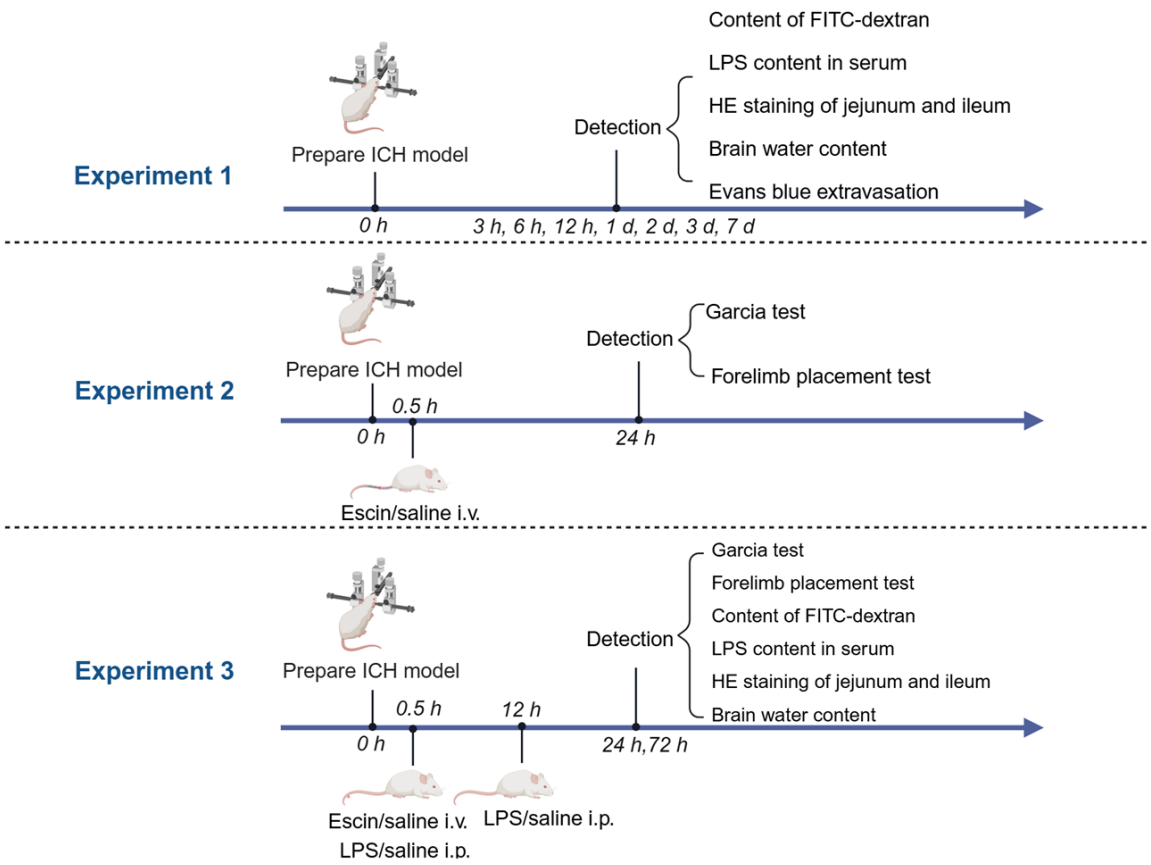

Figure 1. Experimental design. Experiment 1. Mice were subjected to the stereotactic injection of collagenase to induce the intracerebral hemorrhage (ICH) model. The parameters were evaluated at 3 h, 6 h, 12 h, 1 d, 2 d, 3 d and 7 d. Experiment 2. ICH mice model was treated with escin and then neurological functions of mice were investigated. Experiment 3. ICH mice model was treated with escin. At 0.5, 12 h after ICH, mice in escin group were exposed to lipopolysaccharide (LPS). The parameters were evaluated at 24 h and 72 h after ICH.

of PBS in the same procedure. At the corresponding time points after modeling, blood samples were collected, mice were euthanized, and tissue samples were collected. The levels of fluorescein isothiocyanate (FITC)-dextran and lipopolysaccharides in the serum were assayed. The jejunum and ileum were stained with hematoxylin and eosin (H&E). Brain water content and BBB permeability were also measured.

Experiment 2

Male CD-1 mice were allocated into 5 groups without special selection: sham, model, and escin at 0.225, 0.45, and 0.9 mg/kg groups. Mice in the model group and the escin groups (0.225, 0.45, and 0.9 mg/kg) underwent ICH modeling, whereas the mice in the sham group were injected with an equivalent volume of PBS. At 0.5 h after ICH, mice in the escin groups were administered with the corresponding doses of escin (Shandong Luye Pharmaceutical Co., Yantai, China, Lot: 2021020111) intravenously via the tail vein. The mice in the sham and model groups were similarly injected with an equivalent volume of PBS. The Garcia and forelimb placement tests were performed 24 h after the ICH model or sham creation.

Experiment 3

Male CD-1 mice were allocated into 4 groups without special selection: sham, model, escin, and escin + LPS groups. The mouse model of ICH was prepared according to the previous procedure. Thirty minutes later, the mice in the escin and escin + LPS groups were administered escin (0.45 mg/kg), and the mice in the escin + LPS group were injected intraperitoneally with LPS (0.5 mg/kg, Sigma-Aldrich, St. Louis, Missouri, USA, Lot: 0000155609). Twelve hours later, the mice were given the same dose of LPS one more time. At 24 h and

72 h after inducing ICH or the sham treatment, the Garcia and forelimb placement tests were conducted. Blood samples were collected to measure the levels of FITC-dextran and LPS in the serum; the mice were then euthanized, and tissue samples were collected. The jejunum and ileum were stained with H&E, and brain water content was also assessed.

Behavioral tests

The Garcia and forelimb placement tests are commonly used to assess neurological function. At 24 or 72 h after inducing the model of ICH or sham treatment, the modified Garcia and forelimb placement tests were performed by an investigator who was blind to the grouping [20, 21]. The modified Garcia test consists of seven independent tests: spontaneous activity, side stroking, vibrissae touch, limb symmetry, lateral turning, forelimb walking, and climbing. The performance in each test is scored on a scale from 0 (worst performance) to 3 (best performance). The total score (max = 21) indicates the best neurological function of mice.

BBB permeability evaluation

The Evans blue extravasation assay was employed to evaluate BBB permeability [22]. Briefly, mice were intraperitoneally injected with a 2% Evans blue solution (4 mL/kg; Sigma-Aldrich, St. Louis, Missouri, USA, Lot: MKCG7507). Three hours post-injection, transcardial perfusion with cold PBS was performed, and the right hemisphere of the brain was subsequently harvested. The tissues were homogenized with 50% trichloroacetic acid to brain tissue (1:3, v:w) to obtain the homogenate. The homogenate was then centrifuged at 15,000×*g* at 4°C for 20 min. The supernatant was combined with 70% ethanol at a 1:1 (v/v) ratio and incubated overnight at 4°C in the dark. Evans blue extravasation was quantified spectrophotometrically at 610 nm using a standard calibration curve. The results were expressed as ng/mg brain tissue.

Brain water content

The dry-wet weight method was used to assess brain edema [23]. In brief, mice were euthanized with an overdose of ketamine and xylazine. The right hemisphere was rapidly collected and weighed on an analytical balance to obtain its wet weight. The samples were dried at 100°C for 24 hours, and then the dry weight was measured. Brain water content = (wet weight - dry weight)/wet weight × 100%.

Intestinal injury

Intestinal tissues were fixed with 4% paraformaldehyde and then embedded in paraffin. The embedded tissue was sectioned and stained with H&E using standard procedures. The stained sections were photographed, and the histological changes of intestinal tissues were graded according to previously described methods [24]: 0 = normal intestinal mucosa structure; 1 = degeneration of the intestinal mucosa, with widened subepithelial space; 2 = lifting of the epithelium from the small intestinal villi, with further widening of the subepithelial space; 3 = loss of epithelium on the small intestinal villi; 4 = loss of epithelium on the small intestinal villi, with only the lamina propria remaining.

Intestinal permeability

The FITC-dextran method was employed to evaluate the intestinal permeability [25]. In brief, FITC-dextran (Sigma-Aldrich, St. Louis, Missouri, USA, Lot: 46944) was administered intragastrically to mice at a dose of 300 mg/kg. Three hours later, the mice were anesthetized, and blood was collected from the retro-orbital venous plexus. The blood was centrifuged (4°C, 11,000×g, 5 min) and serum was collected to measure levels of FITC-dextran content (excitation wavelength: 485 nm, emission wavelength: 525 nm).

LPS assay

Serum was harvested according to the same procedure mentioned above. LPS content was assayed according to the instructions of the ELISA kit (Cusabio Biotech Co., Wuhan, China, catalog No: M25015903).

Euthanasia

Euthanasia of mice was performed in accordance with the AVMA Guidelines for the Euthanasia of Animals (2020 edition) [26]. The mouse was placed in a transparent, sealed

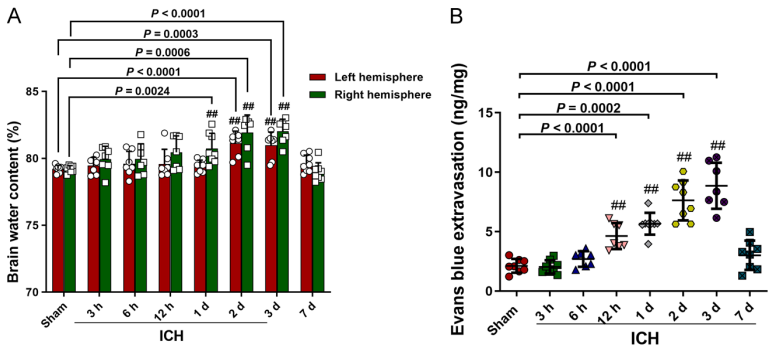

Figure 2. Temporal pattern of brain water content and blood-brain barrier (BBB) permeability evaluation after ICH. A. Brain water content. B. Evans blue extravasation. The data are expressed as mean ± SD (n = 7-8 animals for each group). $^{\#\#}P < 0.01$ compared with the sham group.

chamber connected to a gas anesthesia machine (Model R580S, RWD Life Science, Shenzhen, China). Isoflurane (Catalog No: 20220101, RWD Life Science, Shenzhen, China) was continuously delivered via the anesthesia machine to induce deep anesthesia, with a constant 5% isoflurane concentration in the chamber. Mice lost consciousness within 60 s, followed by cessation of respiration within 3 min.

Statistical analysis

Data were analyzed using GraphPad Prism 8.0 software. The data are expressed as the means ± SD. Spearman's test was used for correlation analysis. A Shapiro-Wilk test was used to assess whether the data met the standard of normal distribution. For data with a normal distribution, differences between the two groups were analyzed using a *t*-test. Differences between multiple groups were analyzed using one-way ANOVA followed by a Dunnett multiple comparisons test. Non-normally distributed data were analyzed using a Mann-Whitney *U* test (two groups) or a Kruskal-Wallis test followed by a Dunnett multiple comparisons test (more than two groups). Significant differences were defined as those with $P < 0.05$.

Results

Temporal pattern of brain water content and Evans blue extravasation after ICH

Compared with the sham group, the water content in the right hemisphere was significantly increased at 12 h, 1 d, 2 d, and 3 d after the ICH. Interestingly, the water content in the left hemisphere was also significantly increased at 2 d and 3 d after the ICH (**Figure 2A**). The Evans blue extravasation in the right hemisphere was significantly increased at 12 h, 1 d, 2 d, and 3 d after the ICH (**Figure 2B**).

Temporal pattern of intestinal injury after ICH

The intestinal tissue damage after ICH was investigated using H&E staining. Histological examination revealed there were no abnormalities in the ileum and jejunum of mice in the sham group. Compared with the sham group, there were significant injuries of the jejunum and ileum observed at 6 h, 12 h, 1 d, 2 d, 3 d, and 7 d after ICH. The alterations consisted of shortened intestinal villi, epithelial necrosis, exfoliation, and erosion of the intestinal mucosal layer (**Figure 3A** and **3C**). **Figure 3B** and **3D** present the intestinal mucosal injury scores. Compared with the sham group, the intestinal mucosal injury scores were markedly increased at 6 h, 12 h, 1 d, 2 d, 3 d, and 7 d after ICH.

Temporal pattern of intestine permeability and serum LPS level after ICH

Compared to the sham group, serum FITC-dextran levels were significantly increased at 6 h, 12 h, 1 d, 2 d, 3 d, and 7 d after ICH (**Figure 3E**). Consistent with the findings of the temporal pattern of intestine permeability, the LPS levels in serum were also significantly increased at 6 h, 12 h, 1 d, 2 d, 3 d, and 7 d after ICH (**Figure 3F**).

Correlation between intestinal mucosal injury, intestine permeability, serum level of LPS, and brain edema after ICH

After ICH, the extent of jejunum injury was positively correlated with intestinal permeability, serum LPS level, and brain edema ($r = 0.625$, $P < 0.01$; $r = 0.541$, $P < 0.01$; $r = 0.408$, $P = 0.01$, **Table 1**). The extent of ileal injury was positively correlated with intestinal permeability, serum LPS level, and brain edema ($r = 0.465$, $P < 0.01$; $r = 0.558$, $P < 0.01$; $r = 0.290$, $P = 0.026$,

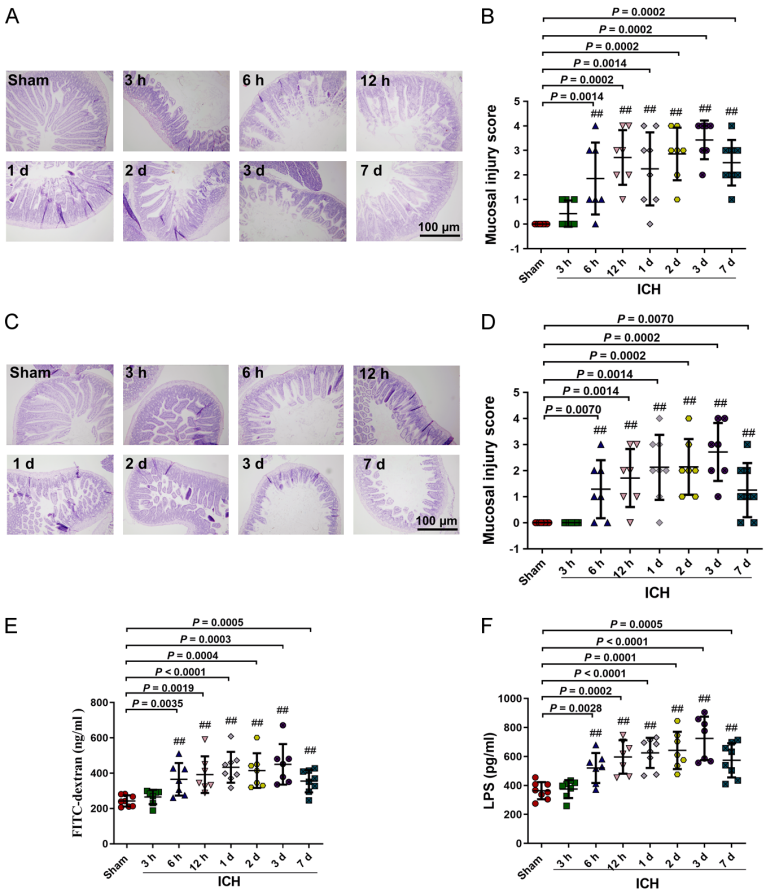

Figure 3. Temporal pattern of intestinal injury, intestine permeability and serum LPS level after ICH. A. Jejunum tissues (Bar = 100 μm). B. Jejunum mucosal injury score. C. Ileum tissues (Bar = 100 μm). D. Ileum mucosal injury score. E. Serum FITC-dextran level. F. Serum LPS level. The data are expressed as mean ± SD (n = 7-8 animals for each group). $^{\#\#}P < 0.01$ compared with the sham group.

Table 1). Intestinal permeability was positively correlated with serum LPS levels and brain edema ($r = 0.585$, $P < 0.01$; $r = 0.483$, $P < 0.01$). Serum LPS levels were positively correlated with brain edema ($r = 0.338$, $P = 0.009$, **Table 1**).

Effects of escin on the neurological outcome in ICH mice

The Garcia test scores ($F_{4,35} = 29.15$) and forelimb placement test ($F_{4,35} = 37.31$) scores exhibited significant differences among groups. Compared with the sham group, mice in the model group exhibited lower scores in the Garcia and forelimb placement tests. When compared with scores in the model group, the administration of escin significantly increased the Garcia and left forelimb placement test scores (**Figure 4A** and **4B**).

LPS did not attenuate the beneficial effects of escin on intestinal injury and intestine permeability in ICH mice

At 24 and 72 h after ICH, escin significantly ameliorated the necrosis and shedding of the intestinal villous epithelium. However, when compared with the escin group, LPS did not attenuate the beneficial effects of escin on intestinal injury (**Figure 5A** and **5B**). At 24 ($F_{3,16} = 11.12$) and 72 h ($F_{3,16} = 21.71$) after ICH, a one-way ANOVA of the serum FITC-dextran levels showed significant differences between groups. Compared with the sham group, the serum FITC-dextran levels in the model group were increased. Compared with the model group, escin significantly inhibited the increase of serum FITC-dextran level. Compared with escin alone, the serum FITC-dextran levels in the escin + LPS group presented no significant change (**Figure 5C** and **5D**).

The serum LPS level

At 24 h ($F_{3,16} = 20.60$) and 72 h ($F_{3,16} = 20.02$) after ICH, the serum LPS level exhibited significant differences among groups. Compared with the sham group, the serum LPS levels in the model group were elevated. Compared with the model group, administration of escin significantly reduced serum LPS levels. Compared with escin alone, the serum LPS levels in the escin + LPS group were increased (**Figure 5E** and **5F**).

LPS reverses the beneficial effects of escin on neurological outcomes and brain water content in ICH mice

At 24 and 72 h after ICH, the one-way ANOVA of Garcia test scores (24 h, $F_{3,36} = 42.33$; 72 h, $F_{3,36} = 37.41$) and forelimb placement test scores (24 h, $F_{3,36} = 56.67$; 72 h, $F_{3,36} = 30.22$) showed significant differences between groups.

Table 1. Correlation analysis among intestinal mucosal injury, intestine permeability, serum level of lipopolysaccharide (LPS), and brain edema after intracerebral hemorrhage (ICH)

	Jejunum injury	Ileum injury	Intestine permeability	LPS
Jejunum injury				
Ileum injury				
Intestine permeability	0.625 (0.000)	0.465 (0.000)		
LPS	0.541 (0.000)	0.558 (0.000)	0.585 (0.000)	
Brain edema	0.408 (0.001)	0.290 (0.026)	0.483 (0.000)	0.338 (0.009)

The correlation coefficient is indicated outside the parentheses. The *p* value is shown inside the parentheses.

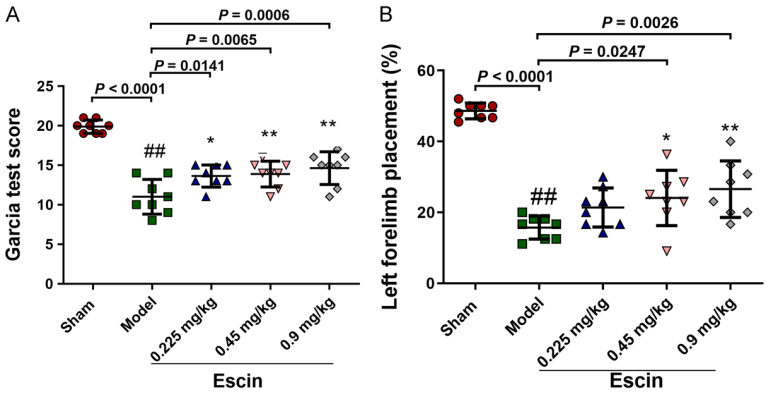

Figure 4. Effects of escin on neurological outcomes in ICH mice. A. Garcia test scores. B. Forelimb placement test. The data are expressed as mean ± SD (n = 8 animals for each group). $^{\#\#}P < 0.01$ compared with the sham group. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the model group.

Compared with the sham group, the model group showed a significant decrease in Garcia and forelimb placement test scores. Compared with the model group, the administration of escin significantly increased the Garcia and left forelimb placement test scores. Compared with escin alone, LPS reversed the improvement in Garcia and left forelimb placement test scores mediated by escin (**Figure 6A**, **6B**, **6D**, and **6E**). At 24 and 72 h after ICH, the brain water content in the basal ganglia (24 h, $F_{3,16}$ = 8.616; 72 h, $F_{3,16}$ = 5.955) and cortex of the right hemisphere (24 h, $F_{3,16}$ = 5.516) exhibited significant differences between the groups. The brain water content in the model group showed an increase in the basal ganglia and cortex of the right hemisphere. Compared with the model group, escin significantly reduced the water content in the basal ganglia and cortex of the right hemisphere. LPS reversed the beneficial escin-mediated effect on reducing the brain water content in the basal ganglia and cortex of the right hemisphere (**Figure 6C** and **6F**).

Discussion

Using a murine model of ICH, the present study investigated the correlation between brain injury and intestinal barrier damage. The findings demonstrated that brain injury caused intestinal barrier damage, allowing LPS from the gut to enter the blood circulation, which in turn disrupts the BBB and further exacerbates brain injury. Therefore, LPS plays an important role in the ICH-induced secondary brain injury. Inhibition of the LPS receptor TLR4 can significantly improve neurological function, reduce neuronal apoptosis, and attenuate neuroinflammation in ICH [27]. TLR4 inhibitors can effectively reduce the degree of brain edema, neurological deficits, and inflammatory cytokine levels in ICH [28].

Escin, has the property of inhibiting inflammation, protecting the gastrointestinal tract, relieving edema, and promoting venous drainage [29]. Our previous studies have demonstrated that escin can alleviate brain injury following ICH and protect the BBB. Its mechanism of action is not exerted through a direct effect on the brain because escin cannot cross BBB [15]. Additionally, escin attenuated ethanol-induced gastric mucosal lesions in rats [30]. In a mouse model, escin showed a gastroprotective effect against indomethacin-induced gastric ulcer [16]. Escin displays a protective effect on the gut by repairing the intestinal barrier and promoting intestinal motility [18]. Based on the evidence presented above, we hypothesize that escin may exert its neuroprotective effect by

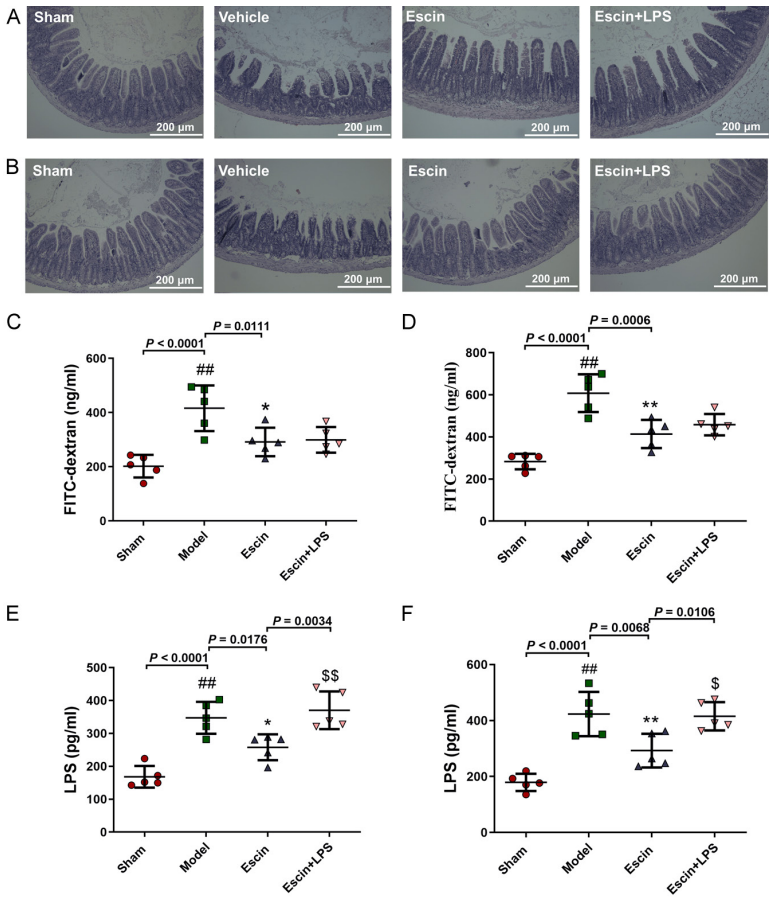

Figure 5. LPS did not attenuate the beneficial effects of escin on intestinal injury and intestine permeability in ICH mice. A. Intestinal tissue at 24 h after ICH (n = 3 animals for each group, Bar = 200 μm). B. Intestinal tissue at 72 h after ICH (n = 3 animals for each group, Bar = 200 μm). C. Serum FITC-dextran level at 24 h after ICH (n = 5 animals for each group). D. Serum FITC-dextran level at 72 h after ICH (n = 5 animals for each group). E. Serum LPS level at 24 h after ICH (n = 5 animals for each group). F. Serum LPS level at 72 h after ICH (n = 5 animals for each group). The data are expressed as mean ± SD. $^{\#\#}P < 0.01$ compared with the sham group. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the model group. $^{\$}P < 0.05$, $^{\$\$}P < 0.01$, compared with the escin group.

attenuating gut injury following ICH. The results demonstrated that administering exogenous LPS could effectively mimic the increase in serum LPS levels caused by intestinal leakage after ICH and reversed the effects of escin on neurological outcomes and the extent of cerebral edema. These results further demonstrate that escin exerts its neuroprotective effect by attenuating gut injury following ICH.

We assessed brain water content, intestinal mucosal injury severity, intestinal permeability, and serum LPS level at different times following ICH and conducted correlation analyses. The results indicated that the severity of both intestinal and cerebral injuries increased with time in the early stages post-ICH, peaking at 3 days and subsequently decreasing. Temporally, the degree of cerebral edema positively correlated with the severity of peripheral intestinal injury and intestinal permeability, as well as with LPS levels in the blood. These findings demonstrated that the brain injury caused intestinal barrier damage, allowing LPS from the gut to enter the bloodstream. Moreover, we also assessed the BBB permeability at various times. The results indicated that the BBB was significantly disrupted following ICH. Elevated levels of LPS in the blood circulation can lead to BBB disruption, which is consistent with the present findings [31]. BBB disruption can lead to vasogenic brain edema and promote the migration of leukocytes from the bloodstream to the brain, allowing an influx of potentially harmful substances from the blood [32]. LPS in the circulation may penetrate the compromised BBB, exacerbating brain injury [13]. Therefore, it is reasonable to speculate that LPS plays an important role in the ICH-induced secondary brain injury.

Subsequently, we evaluated the protective effects and mechanisms of escin on ICH-induced secondary brain injury. First, various doses of escin were administered to the mouse model of ICH. The results showed that escin improved neurological function, with a dose of 0.45 mg/kg being the most effective. Therefore, we chose a dose of 0.45 mg/kg escin for further experiments. In the subsequent experiments, we assessed the extent of intestinal injury, brain injury, and blood LPS level after escin administration. Escin attenuated intestinal injury, improved intestinal permeability, and consequently decreased LPS levels. Consistent with previous findings, escin has been shown to improve neurological function. These results

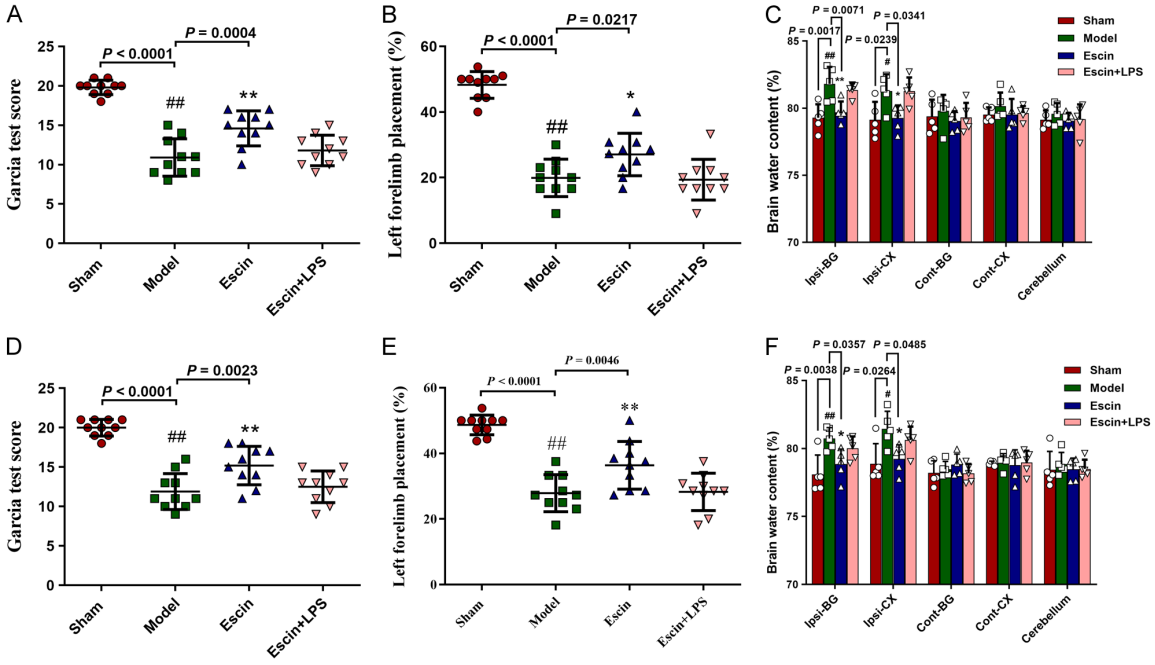

Figure 6. LPS reverses the beneficial effects of escin on neurological outcomes and brain water content in ICH mice. A. Garcia test scores at 24 h after ICH (n = 10 animals for each group). B. Forelimb placement test at 24 h after ICH (n = 10 animals for each group). C. Brain water content at 24 h after ICH (n = 5 animals for each group). D. Garcia test scores at 72 h after ICH (n = 10 animals for each group). E. Forelimb placement test at 72 h after ICH (n = 10 animals for each group). F. Brain water content at 72 h after ICH (n = 5 animals for each group). The data are expressed as mean ± SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the sham group. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the model group.

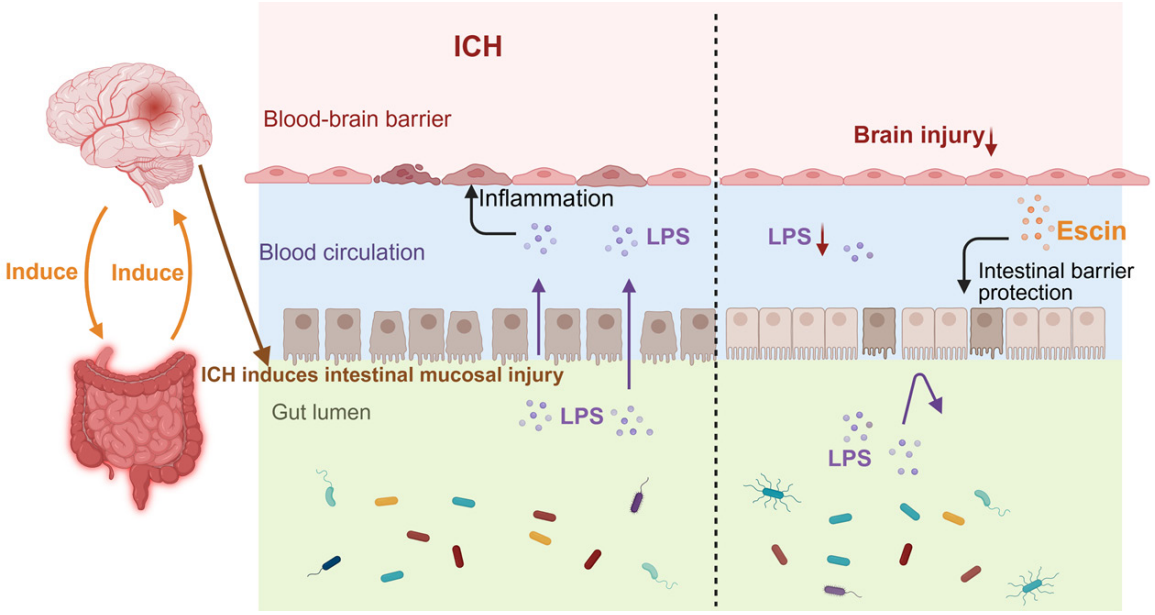

Figure 7. Escin displays neuroprotective effects in mice of intracerebral hemorrhage through ameliorating intestinal injury.

indicate that escin exerts its neuroprotective effect by attenuating gut injury following ICH. Additionally, we simulated the condition of intestinal injury-induced LPS translocation into

the blood by injecting LPS intraperitoneally to determine whether this could diminish the neuroprotective effect of escin. The results showed that LPS could attenuate the neuroprotective effect of escin without affecting its gastroprotective effect. These findings further clarify that the gastroprotective effect of escin plays an important role in the neuroprotective action of escin in ICH. We hypothesize that the specific mechanism by which escin alleviates intestinal injury may be through its anti-inflammatory effects, which directly enhance the connection between intestinal epithelial cells [7].

Conclusions

To our knowledge, we have reported for the first time that brain injury causes intestinal barrier damage allowing LPS from the gut to enter the blood circulation. LPS plays an important role in the ICH-induced secondary brain injury. Escin exerts its neuroprotective effect by attenuating gut injury following ICH. Gastroprotective agents may be a promising approach to attenuating secondary brain injury after stroke (**Figure 7**).

Acknowledgements

This study was supported in part by the Natural Science Foundation of Shandong Province (Grant No. ZR2020MH377). We thank Robin James Storer, PhD, from Liwen Bianji (Edanz) (www.liwenbianji.cn) for editing the English text in a draft of this manuscript.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Tian Wang and Fenghua Fu, School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation, Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai, Shandong, China. E-mail: bluewangtian@ytu.edu.cn (TW); fufh@ytu.edu.cn (FHF)

References

[1] Cordonnier C, Demchuk A, Ziai W and Anderson CS. Intracerebral haemorrhage: current approaches to acute management. Lancet 2018; 392: 1257-1268.

[2] Balami JS and Buchan AM. Complications of intracerebral haemorrhage. Lancet Neurol 2012; 11: 101-118.

[3] Liu M, Wu B, Wang WZ, Lee LM, Zhang SH and Kong LZ. Stroke in China: epidemiology, prevention, and management strategies. Lancet Neurol 2007; 6: 456-464.

[4] Broderick J, Connolly S, Feldmann E, Hanley D, Kase C, Krieger D, Mayberg M, Morgenstern L, Ogilvy CS, Vespa P and Zuccarello M; American Heart Association/American Stroke Association Stroke Council; American Heart Association/American Stroke Association High Blood Pressure Research Council; Quality of Care and Outcomes in Research Interdisciplinary Working Group. Guidelines for the management of spontaneous intracerebral hemorrhage in adults: 2007 update: a guideline from the American heart association/American stroke association stroke council, high blood pressure research council, and the quality of care and outcomes in research interdisciplinary working group. Circulation 2007; 116: e391-413.

[5] Zhou Y, Wang Y, Wang J, Anne Stetler R and Yang QW. Inflammation in intracerebral hemorrhage: from mechanisms to clinical translation. Prog Neurobiol 2014; 115: 25-44.

[6] Niesler B, Kuerten S, Demir IE and Schäfer KH. Disorders of the enteric nervous system - a holistic view. Nat Rev Gastroenterol Hepatol 2021; 18: 393-410.

[7] Li M, Wang S, Zhang C, Chi C, Liu R, Wang T and Fu F. Escin alleviates stress-induced intestinal dysfunction to protect brain injury by regulating the gut-brain axis in ischemic stroke rats. Int Immunopharmacol 2023; 115: 109659.

[8] Benakis C, Brea D, Caballero S, Faraco G, Moore J, Murphy M, Sita G, Racchumi G, Ling L, Pamer EG, Iadecola C and Anrather J. Commensal microbiota affects ischemic stroke outcome by regulating intestinal γδ T cells. Nat Med 2016; 22: 516-523.

[9] Battaglini D, Pimentel-Coelho PM, Robba C, Dos Santos CC, Cruz FF, Pelosi P and Rocco PRM. Gut microbiota in acute ischemic stroke: from pathophysiology to therapeutic implications. Front Neurol 2020; 11: 598.

[10] Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nat Rev Neurosci 2011; 12: 723-738.

[11] Zhang Y, Zhao B, Lai Q, Li Q, Tang X, Zhang Y, Pan Z, Gao Q and Zhong Z. Chronic cerebral hypoperfusion and blood-brain barrier disruption in uninjured brain areas of rhesus monkeys subjected to transient ischemic stroke. J

Cereb Blood Flow Metab 2022; 42: 1335-1346.
[12] Hood RJ, Sanchez-Bezanilla S, Beard DJ, Rust R, Turner RJ, Stuckey SM, Collins-Praino LE, Walker FR, Nilsson M and Ong LK. Leakage beyond the primary lesion: a temporal analysis of cerebrovascular dysregulation at sites of hippocampal secondary neurodegeneration following cortical photothrombotic stroke. J Neurochem 2023; 167: 733-752.
[13] Ferrara F, Zeisig V, Pietsch S, Rütten R, Dreyer AY, Pieper L, Schatzl AK, McLeod DD, Barthel H, Boltze J, Schrödl W and Nitzsche B. Hypothesis and theory: a pathophysiological concept of stroke-induced acute phase response and increased intestinal permeability leading to secondary brain damage. Front Neurosci 2020; 14: 272.
[14] Zhang L, Fu F, Zhang X, Zhu M, Wang T and Fan H. Escin attenuates cognitive deficits and hippocampal injury after transient global cerebral ischemia in mice via regulating certain inflammatory genes. Neurochem Int 2010; 57: 119-127.
[15] Ding YX, Eerduna GW, Duan SJ, Li T, Liu RX, Zhang LM, Wang T and Fu FH. Escin ameliorates the impairments of neurological function and blood brain barrier by inhibiting systemic inflammation in intracerebral hemorrhagic mice. Exp Neurol 2021; 337: 113554.
[16] Wang T, Zhao S, Wang Y, Yang Y, Yao L, Chu L, Du H and Fu F. Protective effects of escin against indomethacin-induced gastric ulcer in mice. Toxicol Mech Methods 2014; 24: 560-566.
[17] Li M, Lu C, Zhang L, Zhang J, Du Y, Duan S, Wang T and Fu F. Oral administration of escin inhibits acute inflammation and reduces intestinal mucosal injury in animal models. Evid Based Complement Alternat Med 2015; 2015: 503617.
[18] Li M, Wang F, Zhang C, Li MA, Wang T, Li YC and Fu FH. Integrated systematic pharmacology analysis and experimental validation to reveal the mechanism of action of Semen aesculi on inflammatory bowel diseases. J Ethnopharmacol 2022; 298: 115627.
[19] Wang T, Nowrangi D, Yu L, Lu T, Tang J, Han B, Ding Y, Fu F and Zhang JH. Activation of dopamine D1 receptor decreased NLRP3-mediated inflammation in intracerebral hemorrhage mice. J Neuroinflammation 2018; 15: 2.
[20] Garcia JH, Wagner S, Liu KF and Hu XJ. Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. Statistical validation. Stroke 1995; 26: 627-634.
[21] Hua Y, Schallert T, Keep RF, Wu J, Hoff JT and Xi G. Behavioral tests after intracerebral hemorrhage in the rat. Stroke 2002; 33: 2478-2484.
[22] Manaenko A, Chen H, Kammer J, Zhang JH and Tang J. Comparison evans blue injection routes: intravenous versus intraperitoneal, for measurement of blood-brain barrier in a mice hemorrhage model. J Neurosci Methods 2011; 195: 206-210.
[23] Song J, Zhang W, Wang J, Yang H, Zhou Q, Wang H, Li L and Du G. Inhibition of FOXO3a/BIM signaling pathway contributes to the protective effect of salvianolic acid A against cerebral ischemia/reperfusion injury. Acta Pharm Sin B 2019; 9: 505-515.
[24] Zhang L, Zhang Z, Liu B, Jin Y, Tian Y, Xin Y and Duan Z. The protective effect of heme oxygenase-1 against intestinal barrier dysfunction in cholestatic liver injury is associated with NF-κB inhibition. Mol Med 2017; 23: 215-224.
[25] Oh NS, Joung JY, Lee JY, Song JG, Oh S, Kim Y, Kim HW and Kim SH. Glycated milk protein fermented with Lactobacillus rhamnosus ameliorates the cognitive health of mice under mild-stress condition. Gut Microbes 2020; 11: 1643-1661.
[26] Leary SL, Underwood W, Anthony R, Cartner S, Grandin T, Greenacre C, Gwaltney-Brant, S, McCrackin MA, Meyer R, Miller D, Shearer J, Turner T and Yanong R. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. AVMA 2020.
[27] Wang S, Cui Y, Xu J and Gao H. miR-140-5p attenuates neuroinflammation and brain injury in rats following intracerebral hemorrhage by targeting TLR4. Inflammation 2019; 42: 1869-1877.
[28] Wang YC, Wang PF, Fang H, Chen J, Xiong XY and Yang QW. Toll-like receptor 4 antagonist attenuates intracerebral hemorrhage-induced brain injury. Stroke 2013; 44: 2545-2552.
[29] Gallelli L, Cione E, Wang T and Zhang L. Glucocorticoid-like activity of Escin: a new mechanism for an old drug. Drug Des Devel Ther 2021; 15: 699-704.
[30] Matsuda H, Li Y and Yoshikawa M. Gastroprotections of escins Ia, Ib, IIa, and IIb on ethanol-induced gastric mucosal lesions in rats. Eur J Pharmacol 1999; 373: 63-70.
[31] Barton SM, Janve VA, McClure R, Anderson A, Matsubara JA, Gore JC and Pham W. Lipopolysaccharide induced opening of the blood brain barrier on aging 5XFAD mouse model. J Alzheimers Dis 2019; 67: 503-513.
[32] Persidsky Y, Ramirez SH, Haorah J and Kanmogne GD. Blood-brain barrier: structural components and function under physiologic and pathologic conditions. J Neuroimmune Pharmacol 2006; 1: 223-236.