Available online on 15.11.2018 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Protective effect of ethanolic extract from the root of *Argyreia speciosa* against global cerebral ischemic reperfusion injury in rats

Jaiswal Bhagat Singh*, Tailang Mukul

SOS in Pharmaceutical Sciences, Jiwaji University, Gwalior, M.P., India

ABSTRACT

Cerebral stroke is the principal reason of death without effective treatment in the world and recognized as the common cause of disability. *Argyreia speciosa* (Linn.f.) (Convolvulaceae, Synonyms: *Argyreia nervosa*) is widely distributed plant species in India. It is commonly known as Elephant creeper and Vryddhadaru. *A. speciosa* is a very valuable plant in the Ayurvedic system. In 'Rasayan' drug it has been used for the treatment of various neurological diseases. Its root taste is bitter and having the multiple uses like as a brain tonic, nootropic, anti-anxiety and anticonvulsant activity. The current study, plan to investigate the neuroprotective effect of ethanolic extract of *A. speciosa* root (ASEE) in a validate rat model of stroke known as global cerebral ischemic reperfusion injury (GCIRI). We divided 36 male Wistar rats to six experimental groups (n= 6). The group-I considered as sham control (no GCIRI), Group-II saline treated GCIRI, Group-III, IV, and V received ASEE (100, 200 and 400 mg/kg, p.o.) for 7 days prior to the induction of GCIRI while Group-VI termed as standard and it received quercetin (20 mg/kg, i.p.) 30 min prior induction of GCIRI. GCIRI produced the significant neurological deficit, sensorimotor dysfunction, decrease neurobehavioral parameters, increased cerebral infarction area and brain edema as compared with sham control rats. Seven days of pretreatment with ASEE markedly attenuates all the changes caused by GCIRI to the normal level. Our results proved that ASEE possess the protective effect on GCIRI induced stroke and aforementioned neuroprotection may be due to its antioxidant and anti-inflammatory property.

Keywords: Brain stroke, BCCAO, Antioxidants, Neuroprotection, Argyreia speciosa

Article Info: Received 18 Sep, 2018; Review Completed 21 Oct 2018; Accepted 22 Oct 2018; Available online 15 Nov 2018

Cite this article as:

Jaiswal BS, Tailang M, Protective effect of ethanolic extract from the root of Argyreia speciosa against global cerebral ischemic reperfusion injury in rats, Journal of Drug Delivery and Therapeutics. 2018; 8(6):8-15

DOI: http://dx.doi.org/10.22270/jddt.v8i6.1990

***Address for Correspondence:**

Bhagat Singh Jaiswal, SOS in Pharmaceutical Sciences, Jiwaji University, Gwalior, M.P.

INTRODUCTION

Stroke is listed in the second position between all the life-threatening diseases and estimates for almost 11% of total deaths globally. It severely impairs the condition of life of the patient and further makes the enormous financial burden on the family and community[1, 2]. Stroke holds the fifth leading factor in mortality amongst people aged the periods of 15-59 years and the second important reason for death more than 60 years[3]. As the focus of brain tissue experiences necrotic cell destruction within a few minutes of the incipience of ischemic stroke, initial restoration of blood reanalyzed with thrombolytic minimizes morbidity and fatality in stroke subjects[4]. Nevertheless, that can be practiced hardly in maximum 5% of the stroke cases due to its short therapeutic time window and critical difficulties for therapy[5].

The etiology of ischemic-reperfusion injury (IRI) remains complicated, furthermore implicated in the interaction of many pathophysiological fashions. Oxidative stress performs a significant role in the overall pathogenesis of IRI[6]. Extreme reactive oxygen species (ROS) may upset the equilibrium between their generation and scavenge in the neuronal tissues, and they can offer quickly initiation of apoptotic and necrotic processes through a number of ion channels in ischemic-reperfused organ/tissue[7].

In addition cerebral ischemia triggers multiple cascades of pathophysiological effects including excitotoxicity, oxidative stress, leukocytes infiltration, calcium overload, inflammation, and apoptosis[8].

According to WHO and the results of various clinical investigation reveals that synthesized drugs remains usually challenging to attain desirable results because of severe adverse effects and single therapeutic target[9,10]. Hence, it is required to investigate more efficient drug with minimal adverse effects to treat the cerebral ischemia-reperfusion, induced injury.

A. speciosa is widely distributed plant species in India. It is commonly known as Elephant creeper, Samundar ka pat

and Vryddhadaru. It is found throughout in India up to altitude of 300-500 m. It is great climber with big ovate-cordate leaves found growing native in north-eastern Himalaya, Dehradun, Konkan, Rajasthan, Mysore, and Bengal[11].

A. speciosa is a very valuable plant in the Ayurvedic system. In 'Rasayan' drug it has been used for the treatment of various neurological diseases.

Its root taste is bitter and having the multiple uses like as a brain tonic, diuretic, aphrodisiac, rheumatism. In other hands for persistent cold & cough, and in resulting fever, root paste with *Grewia hirsute, Asparagus racemosus* and *Hemidesmus indicus* prescribed for immediate relief.

Its seven times root powder is macerated throughout 7 days with tubers juice of *Asparagus racemosus* as nervine tonic. It promotes intellect, strengthens body and counteracts influences of age. In addition one of its preparation known as Ajmodadi Churna used for unilateral paralysis, dysentry and rheumatic ailments[12,13].

A. speciosa contains various chemical constituents such as alkaloids (mainly ergoline), flavonoids, lipids, triterpenoids, saponin and steroids. The seeds mainly consisted of various fatty oils such as palmitic glycosides, stearic, oleic, linoleic and linolenic acid[14].

Principally roots contain the tetradecanyl palmitate, stigma steryl p-hydroxy cinnamate, hexadecanyl p-hydroxy cinnamate, quercetin and caffeic acid. Recently 6-methoxy coumarin-7-O-α-D-glucopyranoside (coumarin glucoside) also isolated by the researchers[15].

The phytochemical investigation revealed the presence of kaempferol, quercetin, kaempferol

3-O-L-rhamnopyranoside, 7, 8, 3′, 4′, 5′-penta hydroxyl flavone and 5-O-β-D-glucopyranoside in *A. speciosa* leaves[16].

A. speciosa possess various pharmacological activity such as wound healing activity[17], aphrodisiac activity[18], hepatoprotective activity[19] analgesic and anti-inflammatory activity[20,21], antidiabetic activity[22], antiviral activity[23], antiulcer activity[24].

In addition *A. speciosa* also posses various neurological property such as nootropic activity, anticonvulsion activity[25,26], central nervous system depressant activity[27].

The abovementioned literature survey exhibits that *A. speciosa* possesses many pharmacological properties including nootropic, anti-anxiety and anticonvulsant activity but no work has ever been carried-out to evaluate the protective effect of *A. speciosa* on the stroke or stroke-related disorder. Hence, in the present study, we investigated the neuroprotective effect of root extract of *A. speciosa* on global cerebral ischemic reperfusion injury in rats.

MATERIALS AND METHODS

Chemicals

Thiobarbituric acid (TBA), trichloroacetic acid (TCA), triphenyltetrazolium chloride (TTC), nitroblue tetrazolium (NBT), gallic acid and quercetin were purchased from Sigma Chemicals USA. All other solvents and chemicals used in study were analytical grade.

Plant materials

The roots of *A. speciosa* were obtained from local market of Gwalior. The roots were authenticated by research officer of the Regional Ayurveda Research Institute for Drug Development, Gwalior, Madhya Pradesh. A voucher specimen No.5-4/17-18/RARIDD-Gwl/Tech/Survey/999 was submitted to institute.

Preparation of ethanolic extract of *A. speciosa*

The roots were clean with water, cut in small parts and air dried then with the help of an electrical grinding machine to get coarsely powdered. For removing the various fatty materials, powdered root was extracted with petroleum ether in Soxhlet extractor at least for 24 h. The marc was subsequently macerated with ethanol to obtain an ethanolic extract. Extract was concentrated in a rotating evaporator and stored (airtight container) in the refrigerator for experimental used. The yield of the obtained extract was 11% (w/w) and termed as ASEE.

Phytochemical analysis of ASEE

Phytochemical screening was done to assess the presence of alkaloids, phenolic compounds, flavonoids, triterpenoids, coumarin, caffeic acid, carbohydrates, tannins, saponin, proteins, and steroids in ASEE as per approved methods[28].

Determination of total phenolic and total flavonoid content

Total phenolic content of the ASEE was assessed using the spectrometric method given by Slinkard and Singleton[29] and expressed in terms of gallic acid equivalent (mg/g of extract) while total flavonoid content was estimated through aluminum chloride[30] (colorimetric method) and expressed in term of quercetin equivalents (mg/g of extract).

Animals

Male (adult & Healthy) Wistar rats (220 g to 250 g) obtained from the animal house of the Jiwaji University, Gwalior. Rats remained transparent cage in the adequately vented room, maintained in 12-12 h (light/dark cycle) and were housed at 25°C ± 2°C temperature. All rats had easy entrance to a standard nutritional diet and water *ad libitum*. The experimental protocol was approved by IAEC (IAEC/JU/16). All the experimental surgery was conducted in accordance with the CPCSEA.

Preliminary acute oral toxicity study

Healthy Wistar rats (adult male, 220 to 250 g) were taken to acute oral toxicity studies and done as per the 423 guidelines recommended by the Organization for Economic Co-operation and Development (OECD: 2001). Firstly we have taken overnight fasted 3 rats then the single oral dose (2000mg/kg) of ASEE given for the limit test, and carefully observed over 14 days for any death, behavioral and neurological alterations.

Induction of global cerebral ischemic reperfusion injury (GCIRI)

GCIRI was caused by occlusion of the bilateral common carotid artery (BCCA) as the surgical technique described earlier[31]. Overnight fasted rats were anesthetized under pentobarbital sodium (30 mg/kg, i.p.). To induce the cerebral ischemia, the individual common carotid artery was temporarily blocked for the period of 30 min with the help of bulldog clamp. Consequently, bulldog clamps were carefully withdrawn to permit reperfusion for 24 h to produce GCIRI. Sham-operated rats treated as a similar way, without blocking of BCCA.

Following reperfusion period, neurological deficit score, sensorimotor function, behavioral parameters were evaluated and lastly, the animals were ethically sacrificed by decapitation method for the evaluation of cerebral infarction size and brain water content.

Experimental design

Male Wistar rats (220 g-250 g) were separated into six groups (n=6) and administered with ASEE or normal saline for 7 days prior to GCIRI and treated as follows:

Group I Sham control given normal saline orally (10 ml/kg for 7 days)

Group II Ischemic reperfused group, GCIRI induced by blocking of BCCA for 30 min, followed by reperfusion for 24 h

Group III ASEE (100 mg/kg, orally for 7days) followed GCIRI

Group IV ASEE (200 mg/kg, orally for 7days) followed GCIRI

Group V ASEE (400 mg/kg, orally for 7days) followed GCIRI

Group VI Received standard drug (quercetin: 20 mg/kg, i.p. given half hour before induction of GCIRI)

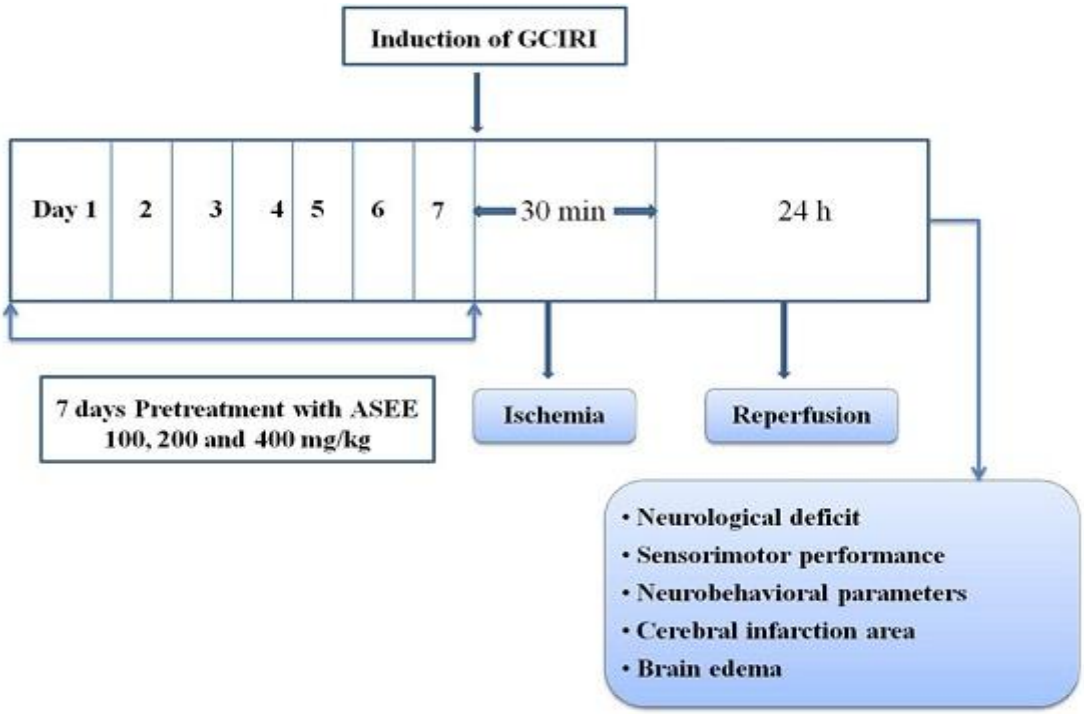

Figure 1: Schematic representation of experimental design.

Neurological deficit scoring

For the assessment of neurological deficit scoring, we adopted a four-point scale method, which was reported earlier[32]. As per our study design after causing GCIRI we determined the neurological deficit scoring through an evaluator who was masked to experimental plan.

The neurological results were scored and recorded as followed:

0: normal movement and absent of neurological deficit

1: the obstacle to fully expanding of right paw

2: circling movement toward the right side

3: falling toward the right side

4: problem to walk spontaneously and reduced the consciousness

Evaluation of sensorimotor performance

Hanging wire test

After completion of the treatment plan, the rats were evaluated for their sensorimotor function and grip strength. For that, rats were suspended through its forelimbs on a thin metal wire, which pulled between two pillars, 60 cm over foam bedding. To stop the rat from using their hind limbs both are comfortably covered by adhering band. The duration of the period in seconds (s), until the rat fell down on the foam bedding, was recorded[33].

Rotarod test

The sensorimotor performance of rats was assessed using a rotarod experiment. In this test firstly we have taken the pre-training trials of rats for reducing the dominance influence of learning ability. The pre-training assured the steady baseline performance on rotarod. All rats from different groups were examined for their ability to endure on the rotating metal rod at a accelerating speed of 10-30 rpm. At last, Latency to fall off (s) from the rotating rod was noted with a 3 min cut off time[34].

Assessment of neurobehavioral parameters

Open-field test

The locomotor and anxiety activity of rat was assessed through an open field apparatus. It made up from a wooden pli-board, in the square shape box (96 × 96 × 50 cm). In addition, the basement of the box was separated into equal 16 squares. The whole experimental room beside the open

field apparatus was retained dark throughout the test. While apparatus was lighted by a low 40 W bulb suspended 100 cm over. Individually rat from different groups was put in any one corner of the open field. After it, we carefully observed rats for 5 min for ambulation (number of squares crossed), number of rearing, grooming, and fecal pellets[35].

Elevated plus maze

Anxiety behavior in rats was evaluated by Elevated plus-maze apparatus. It consisted of the central platform (5 cm x 5 cm) that attached to two open arms (25 cm x 5 cm) intersect two enclosed arms (25 cm x 5 cm x 20 cm). The entire maze was elevated 50 cm above from the room floor. At the starting of the experiment, rat stayed put on the central platform seeing towards open arms. During 5 min of the observation period, we counted the total time spent, number of entries in the open arms and also evaluated the risk assessment behavior[36].

Evaluation of cerebral infarction area

After the evaluation of sensorimotor function and neurobehavioral parameters, animals were subjected to the measurement of cerebral infarction (ischemic) area. The ischemic area was identified and measured by the TTC staining method[37]. Rats were sacrificed by decapitation method and very delicately brains were removed. Suddenly the brains were fixed in ice-cold saline and coronal brain sections of nearly 2 mm thickness were obtained. After that, the brain sections were incubated in normal saline containing 2% TTC for 30 min. The cerebral infarction area was noted and analyzed within sham-operated and treated rats. Cerebral infarction area was determined to apply the following formula:

Cerebral infarction % = unstained (white) infarct area / section area × 100

Estimation of brain edema (water content)

Following decapitation, the rat's brains were quickly removed from the skull[38]. The brain, wet weights were estimated through an electrical balance, thereafter brains were dried oven at 100 °C for 24 h. Brain edema (water content) was computed using the following formula:

Water content in the brain (%) = (wet weight – dry weight) / wet weight× 100

Statistical analysis

All results were expressed as mean **±** standard error of the mean (SEM). Statistical analyses were done using the statistical software Graph Pad Prism package, version 4.0. Differences between the sham-operated group and other treated groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's comparison test. In addition, the scoring of neurological deficit was analyzed by employing a Kruskal-Wallis test followed by Dunn's multiple test. After analysis, if we obtained the $P < 0.05$ (probability value) than it was considered statistically significant.

RESULTS

Phytochemical analysis

Phytochemical analysis of ASEE showed the occurrence of alkaloids, phenolic compounds, flavonoids, triterpenoids, coumarin, caffeic acid, carbohydrates, tannins, saponin, proteins, and steroids.

Determination of total phenolic and total flavonoids content in ASEE

The total phenolic and total flavonoid content was estimated to be 102 mg gallic acid equivalents/g of the extract, and 65.4 mg quercetin equivalents/g of the extract respectively.

Present doses of ASEE holds no adverse effects to rats

After evaluating the acute oral toxicity test we found that tested animals exhibited an absence of any toxic response, no abnormal change in body weight, behavior or death/moribund state up to the endpoint of the study. ASEE was seen to be safe up to 2000 mg/kg of dose (limit test) and approx LD50 was more than 2500 mg/kg.

ASEE improves the neurological deficit score in GCIRI rats

The statistical differences were found in neurological deficit scoring between the sham-operated group, GCIRI group and ASEE/standard treated groups, as depicted in Fig.2. GCIRI caused the rise in the neurological scoring ($p < 0.001$) compared to sham rats. The neurological deficit had low scores in ASEE pretreatment animals at the dose of 100, 200 and 400 mg/kg and quercetin-treated groups at the dose of 20 mg/kg ($p < 0.05$), compared with GCIRI.

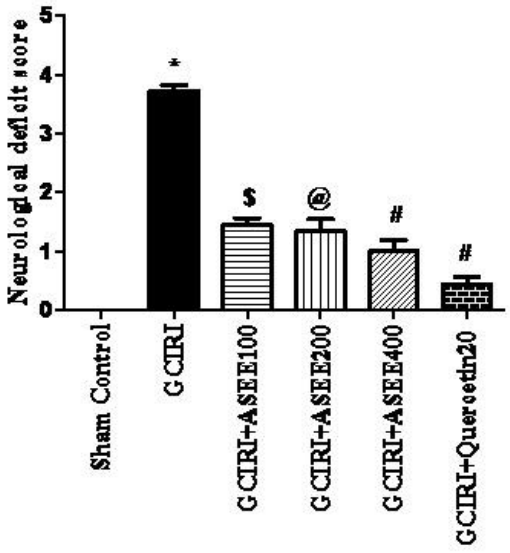

Figure 2: Effect of ASEE on neurological deficits. Results are expressed as mean ± SEM, n = 6. *p < 0.001 when compared to sham control group; $p < 0.05, @p < 0.01 and #p < 0.001 when compared to GCIRI group.

ASEE promotes the sensorimotor function and rotarod performance of GCIRI rats

Fig. 3 shows the results of the effect of ASEE on sensorimotor performance. The grip strength on the hanging wire test was significantly lower ($p < 0.001$) in GCIRI rats when compared with the sham control rats. Pretreatment with ASEE (200, 400 mg/kg) and quercetin 20 mg/kg statistically ($p < 0.05$- $p < 0.001$) increased the time spent while the lower dose (100 mg/kg) of ASEE failed to show significant response when compared with GCIRI rats. Pretreatment with ASEE (100, 200 and 400 mg/kg) and quercetin dose-dependently improved their rotarod performance compared to GCIRI rats (Fig. 4).

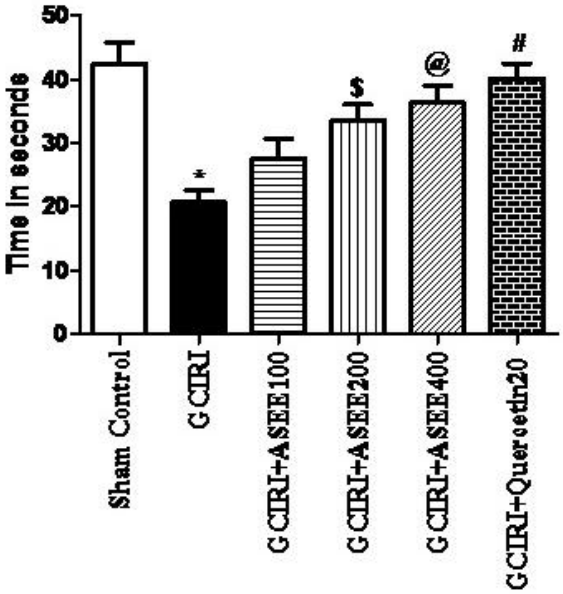

Figure 3: Effect of ASEE on sensorimotor function (hanging wire test). Results are expressed as mean ± SEM, n = 6. *p < 0.001 when compared to sham control group; $p < 0.05, @p < 0.01 and #p < 0.001 when compared to GCIRI group.

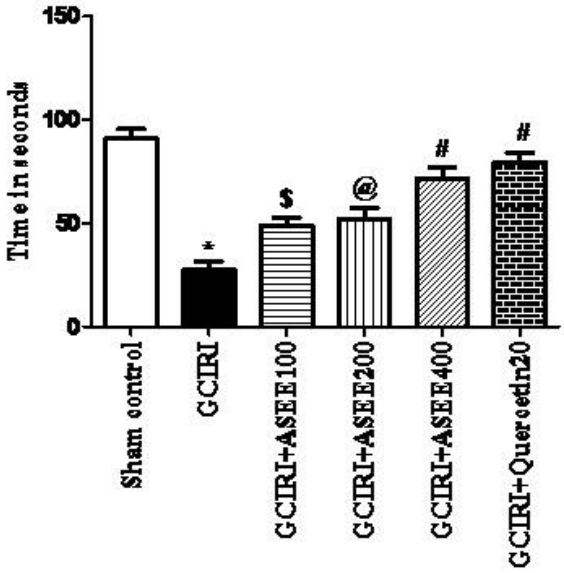

Figure 4: Effect of ASEE on sensorimotor performance (rotarod test). Results are expressed as mean ± SEM, n = 6. *p < 0.001 when compared to sham control group; $p < 0.05, @p < 0.01 and #p < 0.001 when compared to GCIRI group.

ASEE improves the neurobehavioral parameters

Open-field test

GCIRI rats showed significant changes in locomotor activities in open field test (Table 1). GCIRI reduced the number of ambulation (p <0.001) and an increase in the period of immobility (P <0.001) when compared to control rats. In addition, GCIRI showed no changes in the value of rearing, grooming and fecal pellets. ASEE pretreatment significantly abolished the above alterations at dose level of 200 and 400 mg/kg (p < 0.05 - p <0.001). Standard drug pretreated rats also reverse the GCIRI induced changes (p <0.001).

Table: 1 Effect of ASEE on open-field behavior in animals subjected to GCIRI

Treatment	Number of squares crossed	Period (s) of immobility	Number of rearing	Number of grooming	Number of fecal pellets
Sham Control	59.41 ± 3.32	98.78 ± 4.67	19.23 ± 1.56	6.26 ± 0.67	5.23 ± 0.52
GCIRI	30.71 ± 3.72*	135.80 ± 6.12*	23.15 ± 2.65	8.53 ± 0.84	4.42 ± 0.44
GCIRI+ ASEE100	46.61 ± 3.86	121.32 ± 6.71	25.54 ± 1.32	7.63 ± 0.74	5.52 ± 0.47
GCIRI + ASEE200	50.24 ± 3.41$	100.66 ± 6.51@	21.52 ± 1.66	7.53 ± 0.56	5.76 ± 0.63
GCIRI + ASEE400	53.84 ± 3.76@	96.50 ± 4.19#	17.1 ± 1.72	7.33 ± 0.42	5.82 ± 0.42
GCIRI + Quercetin	57.68 ± 4.34#	97.43 ± 4.28#	15.57 ± 1.21	7.26 ± 0.72	5.42 ± 0.44

Values are in mean ± SEM, n = 6. *p < 0.001 when compared to sham control group; $p < 0.05, @p < 0.01 and #p < 0.001 when compared to GCIRI group.

Elevated plus maze

The outcomes from the elevated plus maze were also appreciated (Table 2). We found that there was a significant brain injury caused by GCIRI in rats, which confirmed through the significant reduction (p < 0.001) showed in the number of entries, time spent in open arms and the total arm entries when compared to the sham group. Moreover, rats also exhibited the significantly increased risk assessment behavior in GCIRI group (p < 0.001). ASEE (100, 200 and 400 mg/kg) pretreated rats significantly regained their normal behavior with dose-dependently (p < 0.05-p < 0.001). Quercetin also statistically (p < 0.001) reverse the alterations caused by GCIRI.

Table: 2 Effect of ASEE on elevated plus maze test in animals subjected to GCIRI

Treatment	Number of open arms entries	Number of total arms entries	Time (s) spent in open arms	Number of stretch-attend response
Sham Control	15.27 ± 1.24	26.45 ± 2.6	57.19 ± 3.4	4.87 ± 0.51
GCIRI	8.5 ± 0.81*	11.65 ± 1.16*	32.21 ± 2.5*	14.21 ± 1.68*
GCIRI+ ASEE100	12.22 ± 0.74	18.16 ± 1.49	43.76 ± 3.26	11.58 ± 1.21
GCIRI + ASEE200	13.10 ± 0.86$	19.35 ± 1.18$	49.63 ± 2.66@	9.12 ± 0.75@
GCIRI + ASEE400	13.97 ± 0.75@	21.19 ± 1.12@	56.32 ± 3.20#	7.59 ±0.59#
GCIRI + Quercetin	14.45 ± 0.81#	24.13 ± 1.61#	60.72 ± 3.44#	4.63 ± 0.38#

Values are in mean ± SEM, n = 6. *p < 0.001 when compared to sham control group; $p < 0.05, @p < 0.01 and #p < 0.001 when compared to GCIRI group.

ASEE reduces cerebral infarction area and brain edema

GCIRI produced the significant ($p < 0.001$) increases in cerebral infarction (ischemic) size and brain water content when compared with the sham group (Fig. 4 & 5). Seven days pretreatments with ASEE (100, 200 and 400 mg/kg) markedly attenuated the edema in the brain and also diminished the ischemic area by 25.34%, 20.61%, 17.45% and standard drug, quercetin considerably lower infarct area by 17.10% and brain edema ($p < 0.001$) when compared to the GCIRI group.

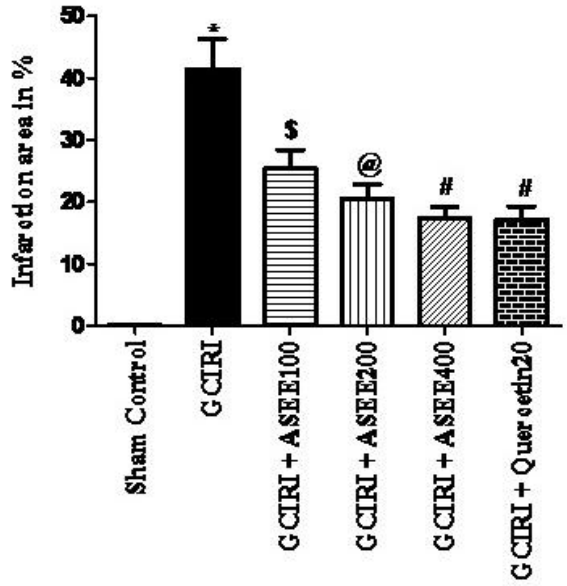

Figure 5: Effect of ASEE on brain infarction (TTC staining). Results are expressed as mean ± SEM, n = 3. $^{*}p < 0.001$ when compared to sham control group; $^{\$}p < 0.05$, $^{@}p < 0.01$ and $^{\#}p < 0.001$ when compared to GCIRI group.

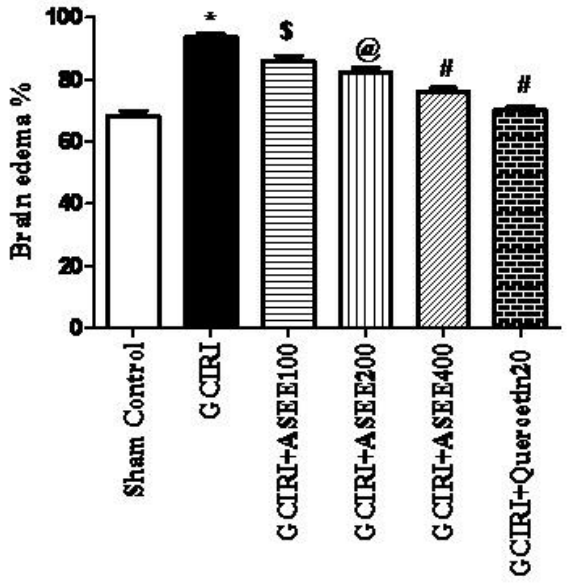

Figure 6: Effect of ASEE on brain edema (water content). Results are expressed as mean ± SEM, n = 3. $^{*}p < 0.001$ when compared to sham control group; $^{\$}p < 0.05$, $^{@}p < 0.01$ and $^{\#}p < 0.001$ when compared to GCIRI group.

DISCUSSION

In the present study, we described the neuroprotective effect of root of *Argyreia speciosa* against oxidative damages following global cerebral ischemic reperfusion injury in rats.

In this current study, results surely indicate that 7 days pretreatment of ASEE and quercetin (standard) treatment shifted the neurological deficit, sensorimotor function, neurobehavioral parameters to the normal level and reduced the cerebral infarct area and brain water content (edema), in GCIRI group. For the induction of stroke, we selected well established global cerebral ischemic reperfusion model of rats. In this model, we blocked both common carotid arteries by bulldog clamp for half an hour followed by 24 h of reperfusion.

GCIRI can be distinguished by two-phase: the early, primary brain tissue injury which initiated through ischemia following the subsequent tissue damage caused by reperfusion phase[39]. Presently all WHO approved drugs mainly targeted the initial phase (ischemic) of brain injury and reanalyzed the blocked blood vessels through thrombolytic action. But, they ineffective to cure the damage caused by the consequent reperfusion phase. It was also proved that reperfusion mainly responsible for brain tissue edema and hemorrhagic alteration, which may moreover worsen the cerebral ischemic injury[40]. Hence, GCIRI induced subsequent brain injury may assist as a target for the stroke-related disorder. Excitotoxicity, oxidative stress, excess free radicals, and neuro-inflammation are the leading contributors to the pathogenesis of GCIRI[41]. Brain tissues are highly sensitive to oxidative stress due to the immense consumption of oxygen molecules, the large quantity of polyunsaturated fatty acids and moderate levels of endogenous antioxidants. During GCIRI, the formation of free radicals has been very high which could conquer the antioxidant capability of the ischemic/reperfused brain[42]. During GCIR, lack of cerebral blood flow appears in a deprivation of various nutrients and oxygen supply to brain tissue that immediately begins to neurological dysfunction[43]. It was proved by many researchers that natural antioxidants give promising results in stroke and related disorders. For example, hexahydrocurcumin, *Canna indica*, Danshen, quercetin, and vitamin E attenuate cerebral ischemic reperfusion injury in animals through antioxidants activity[44,45,46].

In addition, antioxidants also give beneficial effects in numerous clinical studies conducted on the human. Hirvonen and colleague proved that diet enriched with antioxidants decreases the risk for brain infarction in male subjects[47]. Likewise, the moderate activity of antioxidant in blood is linked with prominent lesion area followed by neurological dysfunction in stroke patients[48,49]. Brought collectively, these outcomes recommend that antioxidant agents decrease the ischemia-reperfusion mediated injury in the brain.

In the present study 7 days of pretreatment with 100, 200 and 400 mg/kg of ASEE in GCIRI rats improved the neurological deficit score and support the earlier investigations. In addition, ASEE also reduced the infarction area and brain water content in GCIRI group in a dose-dependent way. Stroke model of GCIRI used for the cerebrovascular ischemic condition, grey and white matter damage, loss of sensorimotor function, neurobehavioral dysfunction, optical fiber injury, diminished speech, anxiety and loss of memory[50,51,52]. It is well recorded that cerebral ischemia responsible for the impairment in sensorimotor performance and neurobehavioral function[53,54]. We used hanging wire test and rotarod test in the present study to evaluate the effect of GCIRI on

sensorimotor performance. Furthermore, hippocampal neurons are extremely sensitive to GCIRI and play an important role in anxiety, locomotor and learning process[52]. Hence, in the current study, we employed an open field test and elevated plus maze test to assess impairment in locomotion and anxiety caused by GCIRI. Seven days pretreatment of ASEE in GCIRI group significantly prevents the sensorimotor incoordination, neurobehavioral dysfunction and supports the earlier findings[51,55]. ASEE, standardized on the basis of total phenolic and flavonoid content and it was estimated to be 102 mg gallic acid equivalents/g of the extract, and 65.4 mg quercetin equivalents/g of the extract respectively. *A. speciosa* contains various chemical constituents such as alkaloids (mainly ergoline), flavonoids, lipids, triterpenoids, saponin and steroids. Principally roots contain the tetradecanyl palmitate, stigma steryl p-hydroxy cinnamate, hexadecanyl p-hydroxy cinnamate, quercetin and caffeic acid. Recently 6-methoxy coumarin-7-O-α-D-glucopyranoside (coumarin glucoside) also isolated by the researchers[15].

It's well documented that flavonoids and polyphenolics compounds play the vital role to prevent the stroke and related disorder. Root of *A. speciosa* rich with quercetin, caffeic acid and coumarin. Many investigators claim that quercetin possesses the anti-stroke activity[55,56,57]. Apart from this, coumarin and its derivatives compounds also attenuated the cerebral ischemic/reperfusion injury[58,59,60]. Whereas caffeic acid shown its neuroprotection via LOX and potent antioxidant activity[52,61]. These phytoconstituents have been shown to hold strong free radical scavenging activity, anticoagulants activity, antioxidant and anti-inflammatory activity.

Hence, quercetin, caffeic acid, and coumarin might be accountable for the perceived neuroprotection against the GCIRI. Our findings recommend that ethanolic extract of *A. speciosa* root revealed the statistically significant ameliorative impact on global cerebral ischemic-reperfusion injury.

CONCLUSION

In conclusion, our investigations have shown the neuroprotective effects of ASEE against GCIRI induced brain damage, which was confirmed through turned the neurological deficit, sensorimotor function, neurobehavioral parameters to the normal level and reduced the infarct area and brain water content (edema), in GCIRI group. These results strongly suggest that ASEE is a possible supporting drug for the treatment of stroke or the related disorder.

ACKNOWLEDGEMENT

The authors are grateful to the Dr. Suman Jain, Head, Department of SOS in Pharmaceutical Sciences Jiwaji University Gwalior for being supportive in every aspect.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

REFERENCES

1. Banerjee TK, Das SK, Epidemiology of stroke in India, Neurology Asia, 2006; 11:1-4.
2. Pandian JD, Sudhan P, Stroke epidemiology and stroke care services in India, Journal of Stroke, 2013; 15(3):128-134.
3. Byles JE, Francis JL, Chojenta CL, Hubbard IJ, Long-term survival of older Australian women with a history of stroke, Journal of Stroke and Cerebrovascular Diseases, 2015; 24:53-60.
4. Christophe BR, Mehta SH, Garton AL, Sisti J, Connolly ES, Current and future perspectives on the treatment of cerebral ischemia, Expert opinion on pharmacotherapy, 2017; 18:573-580.
5. Sallustio F, Motta C, Pizzuto S, Diomedi M, Giordano A, D'Agostino VC, Sama D, Mangiafico S, Saia V, CT angiography-based collateral flow and time to reperfusion are strong predictors of outcome in endovascular treatment of patients with stroke, Journal of neurointerventional surgery, 2017; 9:940-943.
6. Gong G, Xiang L, Yuan LB, Hu L, Wu W, Cai L, Yin L, Dong HL, Protective effect of glycyrrhizin, a direct hmgb1 inhibitor, on focal cerebral ischemia/reperfusion-induced inflammation, oxidative stress, and apoptosis in rats, PLoS One, 2014; 9(3):89450.
7. Manzanero S, Santro T, Arumugam TV, Neuronal oxidative stress in acute ischemic stroke: sources and contribution to cell injury, Neurochemistry International, 2013; 62:712-718.
8. Coyle JT, Puttfarcken P, Oxidative stress, glutamate, and neurodegenerative disorders, Science, 1993; 262(5134):689-695.
9. Mijajlovic MD, Shulga O, Bloch S, Covickovic-Sternic N, Aleksic V, Bornstein NM, Clinical consequences of aspirin and clopidogrel resistance: an overview, Acta neurologica Scandinavica, 2013; 128:213-219.
10. Zuo FT, Liu H, Wu HJ, Su N, Liu JQ, Dong AQ, The effectiveness and safety of dual antiplatelet therapy in ischemic cerebrovascular disease with intracranial and extracranial arteriostenosis in Chinese patients: A randomized and controlled trail, Medicine, 2017; 96:e5497.
11. Aiyer KN, Kolammal M, Pharamacognosy of ayurvedic drugs (kerala), Journal of pharmacy and pharmacology, 1964; 16(12):836.
12. Nadkarni AK. Indian Materia Medica. Bombay: Popular parkashan private Ltd; 1997. P. 137.
13. Girach RD, Aminuddin, Ahmad M, Medical ethnobotany of sundargarh, Orissa, India, Pharmaceutical Biology, 1998; 36:20-24.
14. Husain OPA, Viramani SP, Popli LN, Misra MM, Gupta GN, Srivastava Z, Singh AK. Dictionary of Indian medicinal plants. Lucknow: Central Institute of Medicinal and Aromatic Plants. 1992. P. 45-46.
15. Joseph A, Mathew S, Skaria BP, Sheeja EC, Medicinal uses and biological activities of *Argyreia speciosa* sweet (Hawaiian baby woodrose) - an overview, Indian Journal of Natural Products and Resources, 2011; 2:286-291.
16. Paulke A, Kremer C, Wunder C, Achenbach J, Djahanschiri B, Argyreia nervosa (Burm. f.): receptor profiling of lysergic acid amide and other potential psychedelic LSD-like compounds by computational and binding assay approaches, Journal of Ethnopharmacology, 2013; 148:492-497.
17. Singhal AK, Gupta H, Bhati VS, Wound healing activity of *Argyreia nervosa* leaves extract, International Journal of Applied and Basic Medical Research, 2011; 1(1):36-39.
18. Mishra SH, Chaturvedi SC, Antibacterial and antifungal activity of the oil and un-saponifiable matter of *Argyreia speciosa* sweet, Indian Drugs and Pharmaceuticals, 1978; 13:29-31.
19. Suvarna CM, Rao YN, Rao MP, Beeravali SR, Ravindranaik R, Hepatoprotective and anxiolytic activity of methanolic extract of *Argyreia nervosa*, International Journal of Universal Pharmacy and Bio Sciences, 2013; 2:56-67.
20. George M, Joseph L, Gupta H, Priya G, Anti-inflammatory and analgesic activity of *Argyreia nervosa* leaves extract, World Journal of Pharmaceutical Research, 2016; 5:2119-2127.
21. Lalan BK, Hiray RS, Ghongane BB, Evaluation of analgesic and anti-inflammatory activity of extract of holoptelea integrifolia and *Argyreia speciosa* in animal models, Journal of Clinical and Diagnostic Research, 2015; 9:01-04.

22. Vivek P, Jayakumari D, Jayasree P, Hypoglycaemic Effect of vriddhadaru *Argyreia nervosa* (Burm. f.) Boj. in alloxan induced diabetic rabbits, International Journal of Advanced Ayurveda, Yoga, Unani, Siddha and Homeopathy, 2016; 5:322-329.
23. Sareedenchai V, Wiwat C, Wongsinkongman P, Soonthornchareonnon N, In vitro testing of anti-HIV and antioxidative activities of *Argyreia nervosa* (Burm.f) Bojor leaves, Pharmaceutical Sciences Asia, 2014; 41:47-53.
24. Thakur J, Sharma S, Mukhija M, Kalia AN, Flavonoid fraction of *Argyreia nervosa* leaves with antiulcer potential in different experimental rat models, International Journal of Pharmaceutical Research and Bio-Science, 2013; 2:557-574.
25. Vyawahare NS, Bodhankar SL, Anticonvulsant activity of *Argyreia speciosa* in mice, Indian Journal of Pharmaceutical Sciences, 2009; 71:131-134.
26. Galani VJ, Patel BG, Central nervous system activity of *Argyreia speciosa* roots in mice, Research Journal of Pharmacy and Technology, 2009; 2:331-334.
27. Vyawahare NS, Pujari R, Kagathara V, Gangurde P, Bodhankar S, Hadambar A, Central nervous system activity of *Argyreia speciosa*, Journal of Pharmacy and Research, 2009; 8:152-158.
28. Harborne JB. Phytochemical methods. London: Chapman and Hall; 1987. P. 286.
29. Slinkard K, Singleton VL, Total phenol analyses: Automation and comparison with manual methods, American Journal of Enology and Viticulture, 1977; 28:49-55.
30. Chang CC, Yang MH, Wen HM, Chern JC, Estimation of total flavonoids content in propolis by two complementary colorimetric methods, Journal of Food and Drug Analysis, 2002; 10:178-182.
31. Farbiszewski R, Bielawski K, Bielawska A, Sobaniec W, Spermine protects *in-vivo* the antioxidant enzymes in transiently hypoperfused rat brain, Acta Neurobiologiae Experimentalis Journal, 1995; 55:253-258.
32. Longa EZ, Weinstein PR, Carlson S, Cummins R, Reversible middle cerebral artery occlusion without craniectomy in rats, Stroke, 1989; 20:84-91.
33. Hunter AJ, Hatcher J, Virley D, Nelson P, Irving E, Handubgham SJ, Parsons AA, Functional assessments in mice and rats after focal stroke, Neuropharmacology, 2000; 39:806-816.
34. Dunham NW, Miya TS, A note on a simple apparatus for detecting neurological deficit in rats and mice, Journal of Pharmaceutical Sciences, 1957; 46:208-210.
35. Lister J, Ethologically based animal models of anxiety disorders, Pharmacology and Therapeutics, 1990; 46:321-340.
36. Lister RG, The use of a plus-maze to measure anxiety in the mouse, Psychopharmacology, 1987; 92:180-185.
37. Bederson JB, Pitts LH, Germano SM, Nishimura MC, Davis RL, Bartkowski HM, Evaluation of 2,3,5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats, Stroke, 1986; 17:1304-1308.
38. Hatashita S, Hoff JT, Salamat SM, Ischemic brain edema and the osmotic gradient between blood and brain, Journal of Cerebral Blood Flow & Metabolism, 1988; 8:552-559.
39. Hossmann KA, The two pathophysiologies of focal brain ischemia: implications for translational stroke research, Journal of cerebral blood flow and metabolism, 2012; 32:1310-1316.
40. Gelderblom M, Leypoldt F, Steinbach K, Behrens D, Choe CU, Siler DA, Arumugam TV, Orthey E, Gerloff C, Tolosa E, Magnus T, Temporal and spatial dynamics of cerebral immune cell accumulation in stroke, Stroke, 1999; 40:1849-1857.
41. Du Y, Zhang X, Ji H, Liu H, Li S, Li L, Probucol and atorvastatin in combination protect rat brains in MCAO model: upregulating Peroxiredoxin2, Foxo3a and Nrf2 expression. Neuroscience letters, 2012; 509:110-115.
42. Madamanchi NR, Vendrov A, Runge MS, Oxidative stress and vascular disease, Arteriosclerosis, Thrombosis and Vascular Biology, 2005; 25:29-38.
43. Drobyshevsky A, Luo K, Derrick M, Yu L, Du H, Prasad PV, Vasquez-Vivar J, Batinic-Haberle I, Tan S, Motor deficits are triggered by reperfusion-reoxygenation injury as diagnosed by MRI and by a mechanism involving oxidants, The Journal of neuroscience, 2012; 32:5500-5509.
44. Wicha P, Tocharus J, Janyou A, Jittiwat J, Changtam C, Suksamrarn A, Hexahydrocurcumin protects against cerebral ischemia/reperfusion injury, attenuates inflammation, and improves antioxidant defenses in a rat stroke model, PLoS ONE, 2017; 12(12):e0189211.
45. Talluri MR, Killari KN, Viswanadh NVS, Manepalli M, Konduri P, Bandaru KK, Protective effect of *Canna indica* on cerebral ischemia-reperfusion injury in rats, Agriculture and Natural Resources, 2017; 51(6):470-477.
46. Qiuyun Tu, Ranran Wang, Binrong Ding, Wei Zhong, Hui Cao, Protective and antioxidant effect of Danshen polysaccharides on cerebral ischemia/reperfusion injury in rats. International Journal of Biological Macromolecules, 2013; 60:268-271.
47. Hirvonen T, Virtamo J, Korhonen P, Albanes DP, Intake of flavonoids, carotenoids, vitamins C and E, and risk of stroke in male smokers, Stroke, 2000; 31:2301-2306.
48. Afshari L, Amani R, Soltani F, Haghighizadeh MH, Afsharmanesh MR, The relation between serum Vitamin D levels and body antioxidant status in ischemic stroke patients: A case-control study, Advanced Biomedical Research, 2015; 16(4):213.
49. Leinonen JS, Ahonen JP, Lonnrot K, Jehkonen M, Dastidar P, Molnar G, Low plasma antioxidant activity is associated with high lesion volume and neurological impairment in stroke, Stroke, 2000; 31:33-39.
50. Kozue K, Shinichi N, Sachiko J, Sachiyo S, Etsuko M, Yan X, Edaravone, a free radical scavenger, mitigates both gray and white matter damages after global cerebral ischemia in rats, Brain Research, 2009; 1279:139-146.
51. Shah ZA, Gilani RA, Sharma P, Vohora SB, Cerebroprotective effect of Korean ginseng tea against global and focal models of ischemia in rats, Journal of Ethnopharmacology, 2005; 101(1-3):299-307.
52. Liang G, Shi B, Luo W, Yang J, The protective effect of caffeic acid on global cerebral ischemia-reperfusion injury in rats, Behavioral and Brain Functions, 2015; 11:1-10.
53. Surapaneni S, Prakash T, Ansari A, Goli D, Neuroprotective effect of *Clerodendron glandulosum* against acute transient ischemia reperfusion cerebral injury in rats, Journal of Neurology and Neurorehabilitation Research, 2017; 2:14-20.
54. Collins RC, Dobkin BH, Choi DW, Selective vulnerability of the brain: New insights into the pathophysiology of stroke, Annals of Internal Medicine, 1989; 110:992-1000.
55. Ghosh A, Sarkar S, Mandal AK, Das N, Neuroprotective role of nano-encapsulated quercetin in combating ischemia-reperfusion induced neuronal damage in young and aged rats, PLoS ONE, 2013; 8(4):e57735.
56. Lei X, Chao H, Zhang Z, Lv J, Li S, Wei H, Xue R, Li F, Li Z, Neuroprotective effects of quercetin in a mouse model of brain ischemic/reperfusion injury via anti-apoptotic mechanisms based on the Akt pathway, Molecular Medicine Report, 2015; 12(3):3688-3696.
57. Dai Y, Zhang H, Zhang J, Yan M, Isoquercetin attenuates oxidative stress and neuronal apoptosis after ischemia/reperfusion injury via Nrf2-mediated inhibition of the NOX4/ROS/NF-κB pathway, Chemico-Biological Interactions, 2018; 25(284):32-40.
58. Liu J, Chen Q, Jian Z, Xiong X, Shao L, Jin T, Zhu X, Wang L, Daphnetin protects against cerebral ischemia/reperfusion injury in mice via inhibition of TLR4/NF-κB signaling pathway, BioMed Research International, 2016; 2016:2816056.
59. Wang C, Pei A, Chen J, Yu H, Sun ML, Liu CF, Xu X, A natural coumarin derivative esculetin offers neuroprotection on cerebral ischemia/reperfusion injury in mice, Journal of Neurochemistry, 2012; 121:1007-1013.
60. Connell BJ, Saleh MC, Rajagopal D, Saleh TM, A conjugate of lipoic acid and scopoletin, mediates neuroprotection in a rat model of ischemia/reperfusion, Food and chemical toxicology, 2017; 100:175-182.
61. Maurya DK, Devasagayam TP, Antioxidant and prooxidant nature of hydroxycinnamic acid derivatives ferulic and caffeic acids, Food and Chemical Toxicology, 2010; 48(12):3369-3373.