

Original Article

Protective effect of *Spatholobus suberectus* on brain tissues in cerebral ischemia

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Abstract: Cerebral ischemia is the major causes the neuronal damages throughout the world. Present investigation evaluates the neuroprotective effect of (SS) in cerebral ischemic rat. All the rats were separated in to four group such as control group, ischemia/reperfusion (I/R) group and *Spatholobus suberectus* (100 and 200 mg/kg, p.o.) treated group which receives extract for 15 days prior to I/R. At the end of protocol all the rats were sacrificed and brain was isolated for the biochemical estimation. Further, oxidative stress was estimated by measuring the level of malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD) and glutathione peroxidase (GPX) in the brain tissue. Moreover other parameters like cytokine (IL-10 and TNF- α), nuclear factor kappa B p65 (NF- κ B), caspase 3, brain ATP level and DNA damage by comet assay was estimated in the brain tissues of cerebral ischemic rats. Result of the study suggested that treatment with *Spatholobus suberectus* significantly ($P < 0.01$) decreases the MDA and NO level and increases in the activity of SOD and GPX in the brain tissues of cerebral ischemic rats compared to I/R rats. Moreover, treatment with SS significantly increases the expressions of IL-10 and brain ATP and decreases the expressions of TNF- α , caspase 3 and NF- κ B in the brain tissues of cerebral ischemic rats compared to I/R rats. Comet assay also postulates that SS treated rats brain shows less DNA damage than ischemic rats. Present study concludes the neuroproreective effect of *Spatholobus suberectus* in cerebral ischemic rats by its antioxidant, anti apoptotic and anti-inflammatory activity.

Keywords: *Spatholobus suberectus*, cerebral ischemia, neuroprotection, antioxidant, apoptosis

Introduction

Cerebral stroke is the major causes of death and physical disability across the world [1]. Brain is a highly perused organ which requires 25% of blood pump by heart and thus any hindrance in the flow of blood may produce cerebral ischemia and neurological deficits [2]. Reported studies suggested that in cerebral ischemia oxidative stress and inflammation induces apoptosis and there by causes neurological defects [3]. Increased oxidative stress is related to depletion of ATP, alteration in iron homeostasis, dysfunction of mitochondria, neurotoxicity and changes in intracellular calcium in brain tissue which results in neuronal damage [4]. Moreover, all these condition increases the production of reactive oxygen species (ROS) and thus damages the brain tissues through oxidative damage [5].

Drugs were used for the management of cerebral ischemia; in long term therapy causes cerebral hemorrhages and bleeding [6, 7]. Thus in recent years more stress is given on use of alternative and natural medicine for the management of cerebral ischemia. There are various herbal plants that show promising effect in the management of cerebral infraction/ischemia and protect the neuronal function.

Spatholobus suberectus is a medicinal plant (Leguminosae) traditionally used in China for the management of anemia, rheumatism, abnormality in menstruation and other disorders [8-10]. Stem of *Spatholobus suberectus* contain several chemical consistent like eriodictyol, dihydroquercetin, butin, neoisoliquiritigenin, plathymenin, dihydrokaempferol and liquiritigenin [11]. *Spatholobus suberectus* stem extract reported to posses various medici-

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nal activities such as anti-inflammatory, anti platelet, anti rheumatic and antioxidant activity.

Material and methods

Plant extract

Spatholobus suberectus was collected from the local vendor and authenticated by Institute of Medicinal Plant Development, Beijing. Stem of *S. suberectus* was dried and boiled in distilled water for a specific duration of time. Thereafter extract was filtered by passing it through 150 μ m and lyophilized. This powder of extract was reused by dissolving it in water and centrifuged at 10000 RPM for the duration of 5 min. This solution was filter and used for the study.

Animals

Healthy male wistar rats (250-300 g) at about 8 weeks of age were used for the pharmacological screening in the present study. The animals were housed at $25\pm 2^\circ\text{C}$ temperature, 12 h light/dark cycle and $60\pm 5\%$ of relative humidity. Rats were feed with standard diet and water ad libitum. Protocols of the present investigation for all the animal studies were approved by the Institutional Animal Ethical Committee (39/2015).

Experimental protocol

All the rats were separated into four different groups each group carries 10 rats such as sham operated group (control), ischemia/reperfusion group which receives saline solution for 15 days (I/R group) and *S. suberectus* (SS) treated group which receives extract of SS at a dose of 100 and 200 mg/kg, p.o. for the duration of 15 days I/R rats. At the end of protocol all the rats were exposed to cerebral ischemia for 1 hr and thereafter for 1 hr for reperfusion [12].

Cerebral ischemia/reperfusion (I/R) induction

All the rats were anesthetized by an i.p. of chloral hydrate (360 mg/kg) and subjected them to ischemia. In which by a incision in the neck, left and right carotid arteries were exposed and ischemic condition was developed by applying clamp to carotid arteries for the duration of 1

hr. thereafter circulation was restored by declamping the arteries and reperfusion was permitted for the period of 1 hr. moreover during the period of study temperature was maintained at 37°C to avoid hypothermia [12].

Preparation of tissue homogenate

At the end of protocol all the rats were sacrificed by cervical dislocation. The brain of each rat was isolated and homogenized in both ice cold solution and phosphate buffer solution for biochemical estimation.

Estimation of oxidative stress parameters

In brain homogenate lipid peroxide level was estimated in terms of MDA level. In which absorbance was estimated at 535 and 520 nm by using UV spectrophotometer. The concentration of MDA was estimated by calculation the differences in the absorbance and plotted it on standard curve [13].

Estimation of nitric oxide (NO) was done as per the method described by Miranda et al. in which the reduction of nitrate to nitrite was estimated at 540 nm [14].

Superoxide dismutase (SOD) was estimated in the brain tissue of STZ treated AD rats by using riboflavin sensitized method. The alteration in absorbance was observed for 4 min at 460 nm [15].

Activity of glutathione peroxidase (GPX) enzyme was estimated as per the method described by Paglia and Valentine. In this assay activity of enzyme is directly proportional to decrease in the optical density at 340 nm [16].

Estimation of cytokines in tissue homogenate

Cytokines such as TNF- α and IL-10 were estimated in the tissue homogenate by using ELISA kits (RayBiotech, Inc., USA). The absorbance for the reactant solution was estimated at 450 nm [17, 18].

Estimation of nuclear factor- κ B p65 (NF- κ B p65) in tissue homogenate

NF- κ B p65 was estimated in the brain tissue homogenate by using ELISA kit. In this assay cell membranes were busted out by using freeze thaw cycle and then by centrifuging it at

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Table 1. Effect of *S. suberectus* on parameters of oxidative stress in the brain tissue of ischemic rats

Sr. No.	Group	MDA ($\mu\text{M}/100$ mg of wet tissue)	NO ($\mu\text{M}/100$ mg of wet tissue)	SOD (U/100 mg of wet tissue)	GPX (U/100 mg of wet tissue)
1.	Control	2.49 \pm 0.26	9.82 \pm 0.85	2.71 \pm 0.27	179.4 \pm 12.52
2.	I/R group	7.31 \pm 0.61 [@]	18.6 \pm 1.15 [@]	0.86 \pm 0.09 [@]	79.1 \pm 8.31 [@]
3.	I/R + SS (100 mg/kg)	5.27 \pm 0.38*	15.9 \pm 0.91*	1.59 \pm 0.11**	98.8 \pm 7.75**
4.	I/R + SS (200 mg/kg)	3.75 \pm 0.31**	13.5 \pm 0.73**	2.15 \pm 0.18**	126.9 \pm 9.41**

Result of the study expressed as means \pm SD (n=10), [@]P<0.01 than control, *P<0.05, **P<0.01 than I/R group.

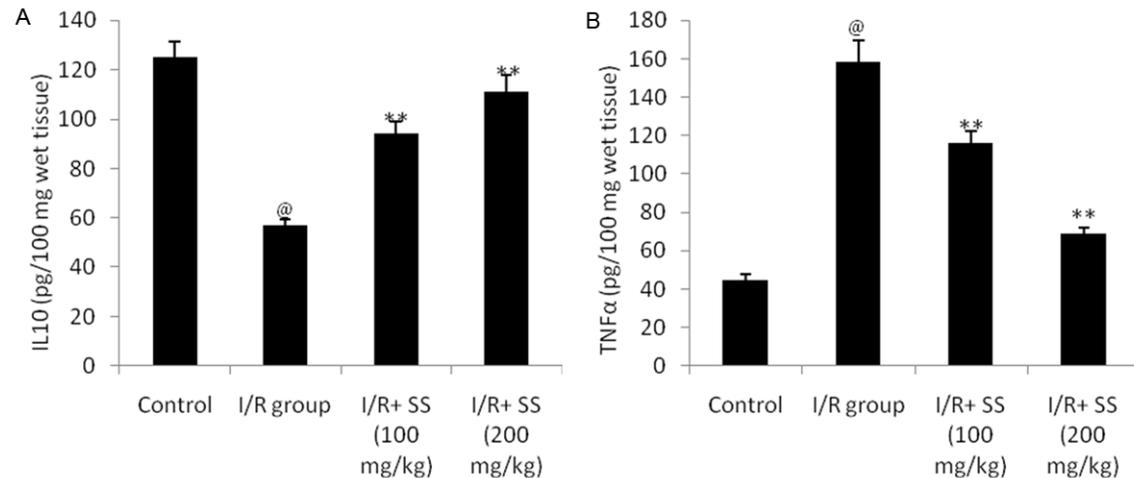


Figure 1. Effect of *S. suberectus* on the level of cytokines in the brain tissue of ischemic rats. Result of the study expressed as means \pm SD (n=10), [@]P<0.01 than control; **P<0.01 than I/R group.

5000 RPM for the period of 5 min at 2°C. Supernatant of the homogenate was separated and used it immediately for the assay. Optical density of the resultant solution was estimated with in 5 min at 450 nm [19].

Estimation of caspase 3 in tissue homogenate

Caspase 3 level was determined by using a ELISA kit. The absorbance of final solution was estimated at 450 nm by microplate reader [20].

Estimation of ATP in tissue homogenate

ATP level in the brain tissue was estimated by ELISA kit and the intensity of resultant solution was determined at 450 nm [21].

Estimation of DNA damage

Damage of DNA was estimated by Comet assay method. In this assay brain sample was crushed and suspended it with PBS and stirred for the duration of 5 min. the filtered sample of 100 μl of cell suspension was mixed with 600 μl of

agarose and thereafter spreading this mixture on a slide which was pre coated with agarose. These slides were kept at 4°C to get solidify and then place it in a lysing solution for the period of 60 min. Slides were placed in electrophoresis chamber, which was filled with alkaline buffer and to allow for 20 min to unwind the DNA. Electrophoresis was allowed for the duration of 20 min at 180 mA and 25 V. Slides were washed with 0.4 M Tris HCl buffer. Thereafter staining was done by adding ethidium bromide solution on each slide and pattern of DNA fragment for each sample was observed through fluorescence microscope. The Comet 5 image software (Kinetic Imaging Ltd., UK) was used for the estimation of cellular DNA damage [22].

Statistical analysis

All the values of these experiments were articulated as mean \pm SEM and the data was statistically analyzed by one-way ANOVA and thereafter applied to Dunnett post hoc test. P<0.05 was considered statistically significant.

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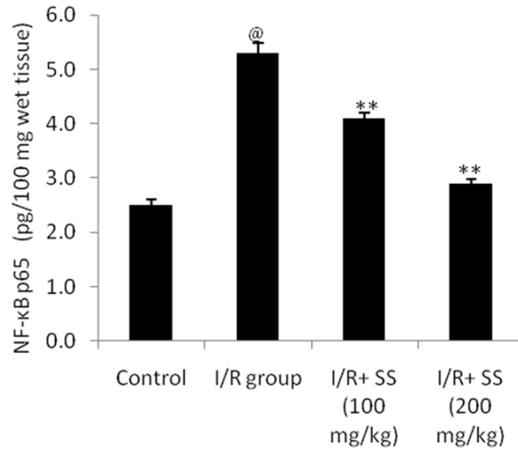


Figure 2. Effect of *S. suberectus* on the level of NF-κB p65 in the brain tissue of ischemic rats. Result of the study expressed as means \pm SD (n=10), [@]P<0.01 than control; ^{**}P<0.01 than I/R group.

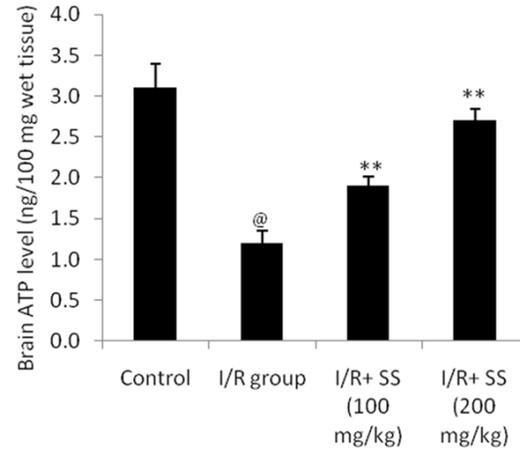


Figure 4. Effect of *S. suberectus* on the level of ATP in the brain tissue of ischemic rats. Result of the study expressed as means \pm SD (n=10), [@]P<0.01 than control; ^{**}P<0.01 than I/R group.

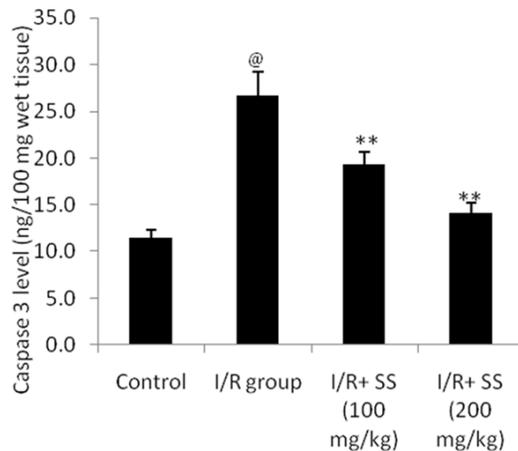


Figure 3. Effect of *S. suberectus* on the level of caspase 3 in the brain tissue of ischemic rats. Result of the study expressed as means \pm SD (n=10), [@]P<0.01 than control; ^{**}P<0.01 than I/R group.

Result

Effect of *S. suberectus* on parameters of oxidative stress

Effect of aqueous extract of stem of *S. suberectus* on oxidative stress parameters was shown in **Table 1**. It was observed that level of MDA and NO significantly increases up to 7.31 and 9.82 μ M/ 100 mg of wet tissue respectively in the brain tissues of I/R group rats compared to control group rats. Whereas, the level of SOD and GPX found to be decreased upto 0.86 and 79.1 U/ 100 mg of wet tissue respectively compared to control group of rats. Treatment with

S. suberectus significantly decreases (P<0.01) the MDA and NO level in the brain tissues of ischemic rats compared to I/R group. Moreover activity of SOD and GPX increases significantly (P<0.01) compared to I/R group.

Effect of *S. suberectus* on cytokines

As shown in **Figure 1**. Cerebral ischemia significantly decreases the level of IL-10 and increases the TNF- α in the brain tissues of I/R group compared to control group. There were significant increase in the level of IL 10 and decrease in TNF- α in the brain tissue of SS treated group compared to I/R group of rats.

Effect of *S. suberectus* on the level of NF-κB p65

Effect of *S. suberectus* extract on the level of NF-κB p65 in the brain tissue of cerebral ischemic rats was shown in **Figure 2**. The result suggested that treatment with *S. suberectus* extract significantly decreases the NF-κB p65 level in the brain tissue of cerebral ischemia rats compared to I/R group of rats. This decrease in the NF-κB p65 level with with *S. suberectus* extract treatment was found to be in a dose dependent manner.

Effect of *S. suberectus* on the level of Caspase-3

Estimation of caspase 3 level in the brain tissue of ischemic rat was illustrated in **Figure 3**. There were significant (P<0.01) decreases in

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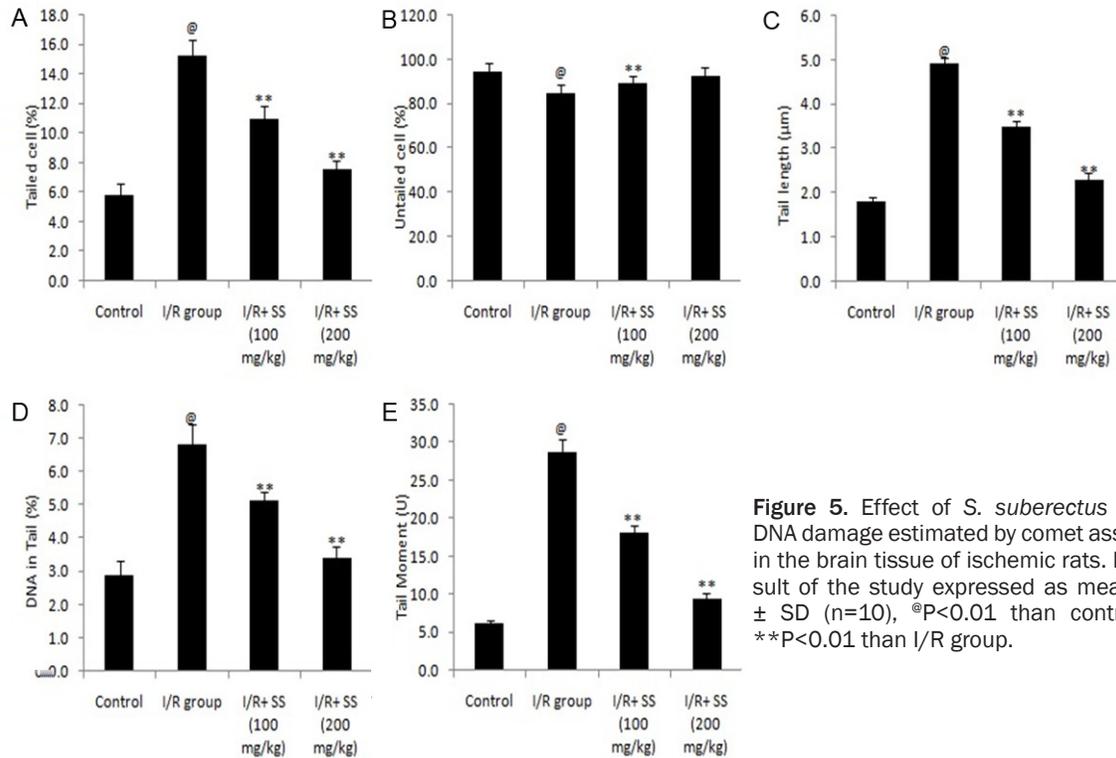


Figure 5. Effect of *S. suberectus* on DNA damage estimated by comet assay in the brain tissue of ischemic rats. Result of the study expressed as means \pm SD (n=10), [@]P<0.01 than control; ^{**}P<0.01 than I/R group.

the caspase 3 in *S. suberectus* extract treated cerebral ischemic rats compared to I/R group.

Effect of *S. suberectus* on the level of ATP

Effect of aqueous extract of *S. suberectus* on the level of ATP in cerebral ischemic rat was shown in **Figure 4**. It was observed that the level of ATP in the brain tissue of *S. suberectus* treated cerebral ischemic rats significantly increases (P<0.01) than I/R group of rats. This decrease in the ATP level was found to be in a dose dependent manner.

Effect of *S. suberectus* on the extent of DNA damage

Figure 5 shows that the effect of *S. suberectus* on DNA damage estimated by comet assay in the brain tissues of ischemic rats. Extent of DNA damage was estimated by determining % of tailed cell, % of untailed cell, length of tail, DNA tail and tail moment of extracted cellular DNA. It was observed that treatment with *S. suberectus* significantly decreases the % of tailed cell and increases the % untailed cell in cerebral ischemic rats compared to I/R group. Moreover, parameters like tail length, DNA tailing and tail moment were found to be signifi-

cantly (P<0.01) decreases in *S. suberectus* treated group compared to I/R treated group of rats.

Discussion

Cerebral ischemia causes the degeneration of neuronal cells as it generates oxidative stress and increases the production of cytokines [23]. Naturally body produces few antioxidant molecules that detoxified the free radicals in normal condition and this harmony get disturb in pathological condition like cerebral ischemia. Here present investigation evaluates the neuroprotective effect of *Spatholobus suberectus* in cerebral ischemia by its strong antioxidant and anti-inflammatory property.

Literature suggested that ischemia increases free radical and reactive oxygen species which exceeded to the production of antioxidant and thus overproduced oxidative species damages nucleic acid and protein [24]. All this result in the DNA fragmentation, damages the cell membrane and its functions. The stem of *Spatholobus suberectus* contain various natural compounds such as dihydroquercetin, butin, neoisoliquiritigenin, plathymenin that posses strong antioxidant property [8]. Result of the

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study suggested that treatment with aqueous extract of stem of *Spatholobus suberectus* significantly decreases ($P < 0.05$, $P < 0.01$) the MDA and NO level and increases in the activity of SOD and GPX in the brain tissues of cerebral ischemic rats compared I/R group.

Moreover, level of NF- κ B and cytokines (IL-10 and TNF- α) was estimated in the brain tissues of cerebral ischemic rats. Reported studies suggested that oxidative stress plays role in the activation of NF- κ B and thereby enhances the expressions of pro inflammatory cytokines such as IL-10 and TNF- α [25]. These cytokine contribute in the injury in cerebral ischemia by activating the cellular adhesion molecule. These activated molecules increases the infiltration of leukocyte and cause increase in inflammation and oxidative stress, which enhances the cerebral damage. The drugs that blocks the activity of TNF- α significantly decreases the infarct volume and brain injury in cerebral ischemia [26]. Results of our study suggested that there were significant ($P < 0.01$) decrease in the expressions of TNF- α and increase in IL-10 in *Spatholobus suberectus* treated group of cerebral ischemic rats compared to I/R group.

Cerebral ischemia results in neuronal damage by activating the apoptosis mechanism and caspase 3 among the caspases plays vital role in the apoptosis [27]. Caspase 3 activation results in cleavage of actin and thereby losses control over the DNase activity. It also breaks the proteins that are important for cell stability and DNA repair and thus causes apoptosis [28]. It was observed that *Spatholobus suberectus* effectively inhibits the expressions of caspase 3 in cerebral ischemic rats in a dose dependent manner. Moreover, effect of *Spatholobus suberectus* on brain ATP was also observed in this study. Mitochondria, power house of cell produces ATP for the energy and any disturbance in the cellular blood flow impairs the oxidative phosphorylation and thus causes decrease in mitochondrial respiration and ATP production [10]. This decreased production of ATP enhances the apoptosis and neuronal damage in cerebral ischemia. Our study result suggested that treatment with *Spatholobus suberectus* significantly increases the brain ATP level and thus prevent the DNA damage in brain tissue of cerebral ischemic rats.

Conclusion

Present study concludes the neuroprotective effect of *Spatholobus suberectus* in cerebral ischemic rats by its antioxidant, anti apoptotic and anti-inflammatory activity.

Disclosure of conflict of interest

None.

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