NEUROPROTECTIVE EVALUATION OF DALBERGIA SISSOO ROXB. LEAVES AGAINST CEREBRAL ISCHEMIA/REPERFUSION (I/R) INDUCED OXIDATIVE STRESS IN RATS

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ABSTRACT

Human brain is the most evolved complex structure in the body. Neurodegenerative disorders such as Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) are relatively common and represent a substantial medical and societal problem. Presently available pharmacological treatment for neurodegenerative disorders are symptomatic and do not alter the course or progression of the neurodegenerative disorders. Hence, this study was carried out with an interest and contribution for herbal medicines as essential potent drug. Dalbergia sissoo (family: Fabaceae) is an Asian deciduous rosewood tree. It is the state tree of Punjab state (India) called as Shisham used for aphrodisiac, abortifacient, expectorant, anthelmintic and antipyretic, memory enhancer etc. The leaf extract of Dalbergia having more concentration of flavonoids and its neuroprotective effect in animals is unclear. To validate the ethno therapeutic claims of the plant, ethanolic extract of Dalbergia sissoo leaves was evaluated by checking brain weight, antioxidant levels, histopathological and TTC staining studies in cerebral ischemia induced rats. The extracts (ethanolic 300, 600 mg/kg) were compared to negative control (global cerebral ischemic rats). It is observed that prior treatment of Dalbergia sissoo extract (DSE) (300mg/kg and 600mg/kg, p.o. for 10days) markedly reversed the brain weight, antioxidant levels and restored to normal levels as compared to I/R groups. Moreover, brain coronal sections staining and histopathological studies revealed protection against ischemic brain damage in the DSE treated groups. From the results obtained, it is evident that the traditional herbal leaf extract had a significant neuroprotective activity.

Keywords:
Brain, edema, cerebral ischemia, oxidative stress, Dalbergia sissoo, bilateral carotid artery occlusion, histopathology

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INTRODUCTION

Neurodegenerative disorders are heterogeneous group of diseases of nervous system, including the brain, spinal cord, and peripheral nerves that have many different etiologies. Many are hereditary, some are secondary to toxic or metabolic processes, and others result from infections. Due to the prevalence, morbidity, and mortality of the neurodegenerative diseases, they represent significant medical, social, and financial burden on the society [1]. The average life expectancy of many populations throughout the world now extends late into the eight decade and the prevalence of most neurodegenerative disorders (NDD) increases drastically with advancing age. Aging and age-related disorders are known to contribute to neurodegeneration and cognitive decline with progressive deterioration of physiological functions and metabolic processes [2].

Cerebro vascular disease (CVD) is a neurodegenerative brain dysfunctions related to disease of the blood vessels supplying the brain that includes some of the most common devastating disorders such as ischemic stroke, hemorrhagic stroke and cerebro vascular anoma. It is reported that CVD represents major cause of disability causing two lacks deaths each year. World Health Organization (WHO) data reflects 5.7 million deaths from CVDs out of 58 million global deaths in 2005. It is postulated that CVDs will be the second most frequent cause of projected deaths in the year 2020. Ischemic stroke has been shown as substantial public health problem that leads to long-term disability in major industrialized countries. Indeed, it is the third leading cause of death after heart disease and cancer. A number of drugs with potential neuroprotective activity have been used in the treatment of stroke. However, there is no clinically effective therapy for the management of acute stroke except tissue-plasminogen activator (t-PA). Recently, intense interest has been focused on the neuroprotective properties of series of natural products. In particular, some natural products act as neuroprotectant by enhancing the survival of neurons while preventing their death and apoptosis [3].

Oxidative stress is one of the primary factors that exacerbate damage by cerebral ischemia. Several components of reactive oxygen species (ROS) that are generated after ischemia-reperfusion injury play an important role in neuronal loss after cerebral ischemia. Superoxide and hydroxyl radicals are potent in producing destruction of the cell membrane by inducing lipid peroxidation. Inducible nitric oxide synthase (iNOS) is upregulated after ischemia injury. It results in NO production. This excess NO reacts with superoxide to form peroxynitrite radical which causes neuronal death. The brain is vulnerable to oxidative stress injury because of intensr ROS metabolites, polyunsaturated fatty acids, low antioxidant capacity and non-replicating nature of neuronal cell [4].

Pharmaceutical approach for novel prevention and treatment strategies of neurodegeneration involves the use of neuroprotective agents in order to delay or stop neuronal cell death or to strengthen cellular defense system. But effective therapies still remain elusive [5]. In view of this, several plants have been selected based on their use in traditional systems of medicine for augmenting neurological health, which appear to offer very promising outcomes for neuroprotection. A number of natural compounds are being used as brain tonic to help restore debilitated conditions. Since plants produce significant amount of antioxidants, they represent a potential source of new compounds with antioxidant activity.

*Dalbergia sissoo* Roxb.(family-Fabaceae) also called ‘shisham’ is used time immemorial for treatment of various ailments like burning sensations, dysentery, dyspepsia, leucoderma, and skin ailment, antiinflammatory, memory enhancer and leaves have significant levels of flavonoids which showed antioxidant activity twice of commonly used antioxidants like vitamin C and selenium [6]. Pharmacologically, it is understood that DS has combination of many phytoconstituents that are beneficial in curing mental inefficiency, illness and useful in the management of various other disorders. Hence, in this context, the therapeutic potential of the botanical, *Dalbergia sissoo* Roxb. (family:Fabaceae) was investigated to modulate neurotoxicant induced oxidative response in rat brain.
MATERIALS AND METHODS

Drugs and Chemicals

Trichloroacetic acid(TCA), 2-thiobarbituricacid(TBA), 5-5- dithiobis (2 nitrobenzoicacid) (DTNB), Phenazine methosulphate, Nicotinamide adenine dinucleotide (NADH), Nitro blue tetrazolium (NBT) were purchased from Sigma-Aldrich Co., Spruce Street, St. Louis, MO, USA. 2,3,5-triphenyltetrazoliumchloride (TTC) was purchased from Hi-Media, Mumbai. All other chemicals were of the highest purity commercially available.

Plant Authentication

The fresh leaves of Dalbergia sissoo Roxb. were collected from GKVK Karnataka, India in the month of July 2012. The plant was identified and authenticated by Mr. KP Sreenath, taxonomist Department Botany, Bangalore University, Bangalore, India and voucher specimen of the plant was kept in the college herbarium.

Plant Extraction

The collected fresh leaves of around 1.5 kg were shade dried or tray dried for 2 weeks and then grinded to a fine powder. In the continuous hot extraction method, the plant leaves powder was extracted in ethanol for 3 regular days at temperature of 78-80 °C. The mixture was subsequently filtered and concentrated under reduced pressure at 40 °C in rotary flush evaporator. The extract yield was 26%w/w [7]. The extract was stored in desiccator.

Preparation of Dalbergia sissoo Leaf Extracts Suspension

Weighed quantity of ELDS was suspended in distilled water using 0.5% v/v Dimethyl sulphoxide and administered orally to rats. The Suspension of extract was prepared freshly every day. The extract was administered at a constant volume of 1ml for each animal [8].

Preliminary Phytochemical Investigation

The extracts were used for preliminary phytochemical screening with a battery of chemical tests viz., Molisch’s, Fehling’s benedicts and Barfoed’s test for carbohydrates; Biuret and Millon’s tests for proteins; ninhydrin’s test for amino acids; Salkowski and Libermann-Burchard’s reactions for steroids; Borntrager’s test for anthraquinone glycosides; foam test for saponin glycosides; Shinoda and alkaline tests for flavonoids glycosides; Dragendorff’s, Mayer’s, Hager’s and Wagner’s tests for alkaloids; and ferric chloride, lead acetate tests for tannins and phenols. The result showed presence of all chemical constituents except alkaloids [9].

Acute Oral Toxicity Studies

Acute toxicity studies were carried out on mice accordingly, alcoholic extracts at dose of 50, 100, 300, 1000, and 3000mg/kg body weight were administered to separate groups of the mice (n=6) after overnight fasting. Subsequent to administration of ELDS, the mice observed closely for the first 3hours for toxic manifestations like increased motor activity, salvation, clonic convulsions, coma and death. The observations are made at regular intervals for 24hr. The animals were observed for 1week. The study revealed that ELDS was not toxic up to 3000 mg/kg body weight [10].

Animals

Adult female Wistar rats (180 – 220 g) bred in animal house of PES College of Pharmacy, Bangalore, were used. The animals were procured from Ragavendra enterprises, Bangalore maintained on a 12 h light: 12 h
dark cycle and free excess of food and water. Animals were acclimatized to laboratory conditions before the test. The experimental protocols were approved by the Institutional Animal Ethics Committee (PESCP/IAEC/02/11, Date:14/12/11) and conducted according to CPCSEA guidelines, Govt. of India.

**Experimental Protocol for Global Cerebral Ischemia/Reperfusion-Induced Oxidative Stress**

The Albino-Wistar rats of either sex (150–200g) were divided into 4 groups of six rats each and fed with drug or vehicle for 10 days prior to the experiment and treated as follows:

Group 1: Receives 1ml of vehicle (0.5% DMSO) alone for 10 days (p.o), no ischemia on 11th day.

Group 2: Receives 1ml of vehicle (0.5% DMSO) alone for 10 days (p.o), on 11th day bilateral carotid artery occlusion for 30 min followed by 1hr reperfusion.

Group 3: Receives 1ml of *D. siscoo* leaves extract 300 mg/kg alone for 10 days (p.o.), on 11th day bilateral carotid artery occlusion for 30 min followed by 1hr reperfusion.

Group 4: Receives 1ml of *D. siscoo* leaves extract 600 mg/kg alone for 10 days (p.o.), on 11th day bilateral carotid artery occlusion for 30 min followed by 1hr reperfusion.

**Induction or Global Cerebral Ischemia and Reperfusion (i/r)**

Rats were anesthetized with 25% urethane (6ml/kg) (i.p). Animals were placed back, a midline ventral incision was made in neck. Trachea of animal was exposed followed by right and left common carotid arteries were located. Both carotid arteries were exposed with special attention paid to separating and preserving the vagus nerve fibres. A cotton thread was passed below carotid artery and a surgical knot was put on both arteries for 30 min induced ischemia. After 30 min thread was removed for reperfusion for 1hr. Body temperature was maintained at 37±0.5 °C throughout surgical procedure. Sham control had no BCA occlusion. After induction the animals were sacrificed brain weight, biochemical parameters, and TTC staining were performed [4, 11].

**Animal Autopsy and Isolation of Brains**

After the treatment period, experimental and control rats were sacrificed by decapitation under mild anesthesia. Brains were immediately isolated, rinsed in ice cold saline to remove blood and stored at -20°C immediately until used for weight in assays described below.

**Brain Weight and Water Content**

After autopsy the brain of the 4 groups of animals were removed and immediately weighed in preweighed glass vials and the wet weight recorded. The percentage water content of each brain was calculated.

**Biochemical ESTIMATIONs**

**Preparation of Tissue Homogenate**

The whole brain dissected out, blotted dry and immediately weighed. The brain regions cerebral cortex (Ct), cerebellum (Cb), hippocampus (Hc) and striatum (St) were subsequently dissected from the intact brain carefully on ice plate (4 ± 2 °C). A 10% brain homogenate was prepared with ice-cold phosphate buffered saline (0.1 M, pH 7.4) using Teflon-glass homogenizer. The homogenate was centrifuged at 10,000 rpm at -4°C for 15 min and the pellet discarded. The supernatant obtained was used for the quantification of antioxidant levels like GSH, CAT, LPO, TT, SOD, total protein levels [12].

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Catalase (CAT)

In brief, the incubation mixture contained in a final volume of 2.0 ml, 0.1 ml of diluted homogenate, 1.0 ml of phosphate buffer and 0.4 ml of distilled water to which 0.5 ml of H$_2$O$_2$ solution was added to initiate the reaction, while the H$_2$O$_2$ solution was left out in control tubes. After incubating for 1 min at 37°C the reaction was stopped by addition of 2 ml of potassium dichromate acetic acid reagent. The samples were kept in boiling water bath for 15 minutes, finally cooled and the absorbance measured at 570 nm against control. The catalase content was calculated by using molar extinction coefficient $= 58.03 \times 10^{-3}$ M$^{-1}$ cm$^{-1}$ and the values are expressed as nMoles/mg protein [13].

Lipid Peroxidation (LPO)

Briefly, the reaction mixture contained 0.1 ml of brain regions homogenate/ mitochondria (1 mg protein), 1.5 ml of 20% acetic acid (pH 3.5), 1.5 ml of 0.8% thiobarbituric acid (0.8% w/v) and 0.2 ml SDS. Following these additions, tubes were mixed and heated at 95 °C for one hour on a water bath and cooled under tap water before mixing 1 ml of distilled water and 5 ml mixture of n-butanol and pyridine (15:1). The mixture was centrifuged at 2200g for 10 min. The amount of MDA/TBARS formed was measured by the absorbance of upper organic layer at a wave length of 532 nm. The results are expressed as nmol MDA/mg protein. The absorbance of the clear pink color supernatant was measured at 532 nm against appropriate blank. The amount of lipid peroxidation was determined by using molar extinction coefficient 1.56 x 10$^5$ M$^{-1}$ cm$^{-1}$ and the results were expressed as nMoles MDA/g of protein [14].

Reduced Glutathione (GSH)

The assay is based on the principle of Ellman’s reaction. The sulfhydryl group of glutathione reacts with DTNB (5,5′-dithiobis-2-nitrobenzoic acid) and produces a yellow colored 5-thio-2-nitrobenzoic acid (TNB). Measurement of the absorbance of TNB at 412 nm provides an accurate estimation of glutathione in a sample. Briefly, 0.5 ml of homogenate is mixed with 0.1 ml of 25% TCA to precipitate proteins and centrifuged at 4000 rpm for 5 min. Then 0.3 ml of the supernatant was mixed with 0.5 ml of 0.1 M phosphate buffer (pH 7.4) and 0.2 ml of 10 mM DTNB. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blank. The glutathione content was calculated by using extension coefficient 13.6 x 10$^3$ M$^{-1}$ cm$^{-1}$. The values are expressed as nMoles/mg protein [15].

Super Oxide Dismutase (SOD)

The assay mixture contained 0.1 ml of sample, 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml of phenazine methosulphate (186 μM), 0.3 ml of nitro blue tetrazolium (300 μM), 0.2 ml of NADH (750 μM). Reaction was started by addition of NADH. After incubation at 300°C for 90 sec, the reaction was stopped by the addition of 1 ml of glacial acetic acid. The mixture was allowed to stand for 10 min. The color intensity of the chromogen was measured at 560 nm against blank and concentration of SOD was expressed as units/min/mg of protein [16, 17].

Total Thiols (TT)

The assay is based on the formation of a relatively stable yellow product when sulphydryl groups react with DTNB. Briefly, 0.2 ml of brain tissue supernatant was mixed with 0.36 ml of 0.1 M phosphate buffer (pH = 7.4), 40 μl of 10 mM DTNB and 1.4 ml of methanol. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blanks. The total thiol content was calculated by using molar extinction coefficient $= 13.6 \times 103$ M$^{-1}$ cm$^{-1}$ and the values are expressed as nMoles/mg protein [18].
Total Protein
The total protein of brain tissue was determined by Biuret method in ERBA diagnostic kit [19, 20].
Total protein (g/dl) = Absorbance of test/Absorbance of standard*concentration of standard (g/dl)

TTC Staining
Animals were deeply anesthetized and transcardially perfused with ice cold normal saline. The brains were removed, sliced coronally into 2 mm thick section and incubated in phosphate buffered saline (pH 7.4) containing 2% of 2, 3, 5-triphenyltetrazolium chloride (TTC) for 30 min at 37° C in dark and followed by fixing in 10% neutral buffered formalin(27) for overnight. The images of the stained sections were acquired by scanning with a high resolution scanner, and the lesion area was compared visually [21].

Histopathological Studies
A section of the brain was fixed with 10% formalin and embedded in paraffin wax and cut into sections of 5 μm thickness. The sections were stained with haemotoxylin and eosin dye for histopathological observations. Depending on the model, either hippocampal or striatal neurons were observed for morphological changes [22].

Statistical Evaluation
The data were expressed as Mean ± S.E.M. Statistical comparisons were performed by one way ANOVA followed by Dunnett’s post-test using Graph Pad Prism version 5.0.

RESULTS AND DISCUSSION

Table: 1 Effect of DSE on brain weight in ischemia induced Neurotoxicity in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wet weight of brain(gm)</th>
<th>Dry weight of brain(gm)</th>
<th>% of water content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control group</td>
<td>2.56±0.02b</td>
<td>2.17±0.06c</td>
<td>15.23</td>
</tr>
<tr>
<td>Inducing group (Ischemia)</td>
<td>2.31±0.01</td>
<td>1.64±0.02</td>
<td>28.94</td>
</tr>
<tr>
<td>Low dose (D.S 300mg/kg)+Ischemia</td>
<td>2.44±0.02c</td>
<td>1.93±0.06</td>
<td>21.06</td>
</tr>
<tr>
<td>High dose (D.S 600mg/kg)+Ischemia</td>
<td>2.43±0.01c</td>
<td>1.83±0.07</td>
<td>24.64</td>
</tr>
</tbody>
</table>

Each value are expressed as mean ± SEM (n = 6), a P < 0.001, b P < 0.01, c P < 0.05 when compared to ischemic rats. One-way ANOVA followed by Dunnett’s post test
Table No: 2 Effect of DSE on Biochemical properties in ischemia treated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Catalase (µm H$_2$O$_2$/min/mg of protein)</th>
<th>Lipid peroxidation (nMoles of MDA/g protein)</th>
<th>Reduced glutathione (nMoles/min/mg of protein)</th>
<th>Super oxide dismutase (units/min/mg of protein)</th>
<th>Total thiol (nMoles/mg of protein)</th>
<th>Protein estimation (g/dl of total protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control group</td>
<td>65.40±3.24a</td>
<td>236.2±5.04a</td>
<td>2.80 ±0.17a</td>
<td>19.55±0.28b</td>
<td>31.77±1.04b</td>
<td>9.17±0.64b</td>
</tr>
<tr>
<td>Inducing group (Ischemia)</td>
<td>29.57±1.41</td>
<td>569.4±20.01</td>
<td>0.54±0.10</td>
<td>11.32±0.77</td>
<td>15.43±0.50</td>
<td>3.37±0.45</td>
</tr>
<tr>
<td>Low dose (D.S 300mg/kg)+Ischemia</td>
<td>44.28±1.30c</td>
<td>333.1±12.90b</td>
<td>1.33±0.04c</td>
<td>13.56±0.53</td>
<td>21.58±1.40c</td>
<td>5.65±0.09</td>
</tr>
<tr>
<td>High dose (D.S 600mg/kg)+Ischemia</td>
<td>59.58±0.70b</td>
<td>304.6±5.65a</td>
<td>1.97±0.04b</td>
<td>17.01±0.91c</td>
<td>27.43±1.06b</td>
<td>7.55±0.12b</td>
</tr>
</tbody>
</table>

Each value are expressed as mean ± SEM (n = 6), a P < 0.001, b P < 0.01, c P < 0.05 when compared to ischemic rats. One-way ANOVA followed by Dunnett’s post test

**TTC Staining**

The extent of depletion of Succinate dehydrogenase (SDH, a mitochondrial enzyme) was determined by TTC staining, a SDH inhibitor which produce deep red color on reaction with the enzyme. Ischemia induced group exhibited a large lesion in the striatal region indicating the massive depletion of SDH. Pretreatment with DSE at both the doses markedly attenuated the depletion of this enzyme in ischemic animals. The brain sections of DSE treated animals showed decreased disappearance of red color in the striatal region, indicating the antagonistic effect striatal degeneration.
**Fig 1**: TTC staining images showing effect of DSE on ischemia induced lesion area. Red arrow mark indicating the affected area of the brain striatal region in corneal slices of ischemic rat brain. In DSE pretreated rats showing less or no lesion area in rat brain section.
Histopathology

Control group: The CA3 region shows intact pyramidal cells in tight clusters [Fig. 1, Arrow]. The interconnected neuropil fibers in CA3 region appear intact. The CA1 region shows intact pyramidal cells along with intact neuropil fibers [Fig.2, Arrow].

Ischemia inducing group: The CA1 region [Fig. 1, Arrow] shows degeneration of most of the pyramidal cells with intact neuropil fibers. The CA3 region [Fig. 2, Arrow] shows degeneration of the pyramidal cells along with focal loss of neuropil fibers.

Low dose DSE (300mg/kg) + Ischemia: The CA3 region shows intact pyramidal cells [Fig. 1, Arrow]. The interconnected neuropil fibers in CA3 region appear intact. The CA1 region shows intact pyramidal cells along with intact neuropil fibres [Fig.2, Arrow].

High dose DSE (600mg/kg) + Ischemia: The CA3 region shows intact pyramidal cells with interconnected neuropil fibers [Fig. 1, Arrow]. The CA1 region shows intact pyramidal cells along with intact neuropil fibres [Fig.2, Arrow].
DISCUSSION

The present study revealed neuroprotective potential of DSE at 300 mg/kg and 600 mg/kg. We estimated LPO, SOD, CAT, GSH and total thiol levels in the brain tissue as an index to assess the severity of oxidative damage. In the present study, the selected dose levels 300 and 600 mg/kg were devoid of any sign of toxicity in animals. Oxygen is essential for aerobic life, but it is also precursor to the formation of harmful reactive oxygen species (ROS). Free radicals in the living organism are generated both enzymatically and non-enzymatically, leading to the formation of ROS. It has been reported that hydroxyl and peroxynitrite are the most potent ROS that can damage proteins, lipids and nucleic acids, resulting in the inactivation of some enzyme activities, disruption of ion homeostasis and modification of genetic apparatus and apoptotic death [23].

Brain edema has also been studied in ischemia to assess the impact of brain damage. Cerebral edema occur as the result of ionic imbalance across the cellular membrane due to energy failure results in neuronal depolarization, which alters ionic gradients of Na⁺, K⁺, Cl⁻, Ca²⁺. As glutamate increases in extracellular space peri-infract depolarization occur so water shift occur resulting in cerebral edema. The DSE reversed the effect of brain water content in ischemia reperfusion animals compared to treated animals. These resulting in potential therapeutic value in cerebrovascular diseases including stroke.

An interesting finding of the present study was amelioration of LPO by DSE, an effect that could be attributed to its capacity to scavenge free radicals, as shown by the observed restoration of the antioxidant enzyme activities. We may recall that ROS produces malondialdehyde (MDA), an end product of LPO, a process that leads to dysfunction of membrane bound receptors and enzymes [24]. Moreover, recent studies showed that global cerebral ischemia and reperfusion significantly augmented MDA levels in animals. In consistent with this, we found that the ischemia and reperfusion resulted into augmentation of LPO process and thereby elevated the MDA levels in the brains of animals. There is strong indirect evidence that free radical production appears to be an important mechanism of brain injury after exposure to I/R [25].

To further characterize the neuroprotective actions of DSE, we evaluated effect of DSE on endogenous enzymatic and non-enzymatic antioxidant levels in the brain homogenates. It is well established that antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) play central role in the defense mechanisms against free radical- induced oxidative stress. Similarly, non-enzymatic antioxidants such as ascorbic acid (vitamin C), a-tocopherol (vitamin E), glutathione (GSH), and carotenoids have to inhibitory role in the oxidative damage induced by free radicals [26]. In the present study, prior treatment of DSE significantly increased GSH levels. It can be recalled that GSH acts directly by detoxifying ROS and indirectly as a substrate for various peroxidases thus, protecting the cells against oxidative damage. Dysfunction of the GSH system has been implicated in a number of neurodegenerative diseases such as Alzheimer and Parkinson’s diseases, Huntington’s disease etc potential contributor to oxidative damage following temporary ischemia in rodents. In the current study, GSH levels were moderately reduced due to I/R insult which is in consistence with other studies [27]. Furthermore, administration of DSE showed significant increase in SOD levels in brain. It has been shown that the SOD, a major antioxidant enzyme located in cytoplasm and mitochondria of cells, forms the principal defense system against excess O₂ production by direct scavenging of O₂ during reperfusion [28].

We also evaluated effect of DSE on the CAT levels in the rat brain. As compared to the control animals DSE-treated group showed significant increase in the CAT levels. Some studies reported that I/R in rats lowered CAT activity [29]. However, in our experiments, the mean CAT activity was significantly decreased in I/R group and is similar in case of total thiol. Furthermore, the histopathological studies revealed significant decrease in neutrophil infiltration, pyramidal cells degeneration in CA1 and CA3 region and brain damage by the DSE as compared to control group. Additionally, the cerebral infraction area was very less DSE-treated
groups as compared to control groups indicating protection from I/R damage in the brain tissue. These results indicated neuroprotection by DSE against BCCA occlusion-induced I/R brain damage.

CONCLUSION

As evident by the results of the present study, it seems that DSE has enhanced the defense mechanism, thereby reducing the damaged produced by global ischemia. It prevented the neuronal loss from ischemia induced brain tissue and showed antioxidant activity in reperfusion induced oxidative stress. We suggest that the mechanism with which DSE has normalized the damage is probably by various chemical constituents like flavonoids, glycosides, tannins and saponins etc. Biochanin A, quercitin, kamferol are one of the major constituent of DSE having more antioxidant properties than vitamin E biologically. So research studies are to be done to isolate constituents and to explore their targets responsible for preventing neuronal damage.

AUTHORS’ STATEMENTS

Competing Interests

The authors declare no conflict of interest.
REFERENCES


