Protective effect of biflavones from *Araucaria bidwillii* Hook in rat cerebral ischemia/reperfusion induced oxidative stress

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Abstract

Oxidative stress is implicated in the pathogenesis of ischemic brain injury. Flavonoids from various herbal extracts have been shown to be neuroprotective in experimental models of cerebral ischemia/reperfusion (I/R). The present study was designed to investigate the neuroprotective effect of the biflavone rich fraction from *Araucaria bidwillii* Hook (ABH) (Family: Araucariaceae) in I/R induced oxidative stress. The I/R was induced by occluding bilateral common carotid arteries (BCCAO) for 30 min, followed by 24 h reperfusion. BCCAO caused significant depletion in superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and significant increase in lipid peroxidation (LPO) in various brain regions. The neurological deficit and sensory motor function were also decreased significantly by BCCAO group as compared to sham group animals. All the alteration induced by cerebral ischemia was significantly attenuated by 7 days’ pretreatment with biflavone fraction (BFR) at the dose of 100 and 200 mg/kg, comparable to that given by Vitamin E (200 mg/kg). Consistent with neurobehavioral deficits, pretreatment with biflavones at higher doses significantly reduced ischemia-induced neuronal loss of the brain. In conclusion the biflavone rich fraction from *A. bidwillii* was found to protect rat brain against I/R induced oxidative stress, and attributable to its antioxidant properties.

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1. Introduction

Growing evidence supports the participation of oxidative stress in brain injury mediated by cerebral ischemia and stroke [1,2]. The roles of reactive oxygen species (ROS) and lipid peroxidation (LPO) have been proposed to be important factors in reduction of cerebral blood flow and the reperfusion period [3–5]. The polyphenolics including flavonoids, which are found in many herbal extracts have been shown to be strong ROS scavengers, antioxidants and protectors of neurons from lethal damage in \textit{in vitro} [6–8]. In addition, the neuroprotective effects of flavonoids in herbal extracts and their physiologically relevant conjugates against ischemia/reperfusion (I/R)-induced oxidative damage have also been reported [9–11]. Phenolic antioxidants from medicinal plants have also been evaluated \textit{in vivo} as neuroprotective agents in animal models of I/R induced oxidative stress. The plant *Araucaria bidwillii* Hook (ABH) (Family: Araucariaceae) is a tree popularly known as “monkey puzzle” and is distributed in high altitudes of the Western Ghats of Nilgiris and northern part of Himalayan regions in India. The genus *Araucaria* is cited in Indian and Thai folk medicine for its sedative and hypnotic properties and treatment of central nervous system illness [12]. The alcoholic leaf extract of ABH is recommended for pain, inflammation, anxiety, sleeplessness, and other neurological disorders [13,14]. The analgesic and anti-inflammatory activity of the leaf extract has been reported [15]. Administration of alcoholic extract of leaf has been shown to increase the blood clotting time in rats [16]. The genus ABH is rich in biflavones as major chemical compounds as shown by others [17,18] studies with plants like *Hypericum perforatum*, *Panax ginseng*, and *Ginkgo biloba* and plant derived biflavones have been extensively conducted for age related cognitive and motor decline [19,20], cerebral ischemia/reperfusion injuries [21] and other brain pathologies [22]. The aim of our present study was to evaluate the biflavone rich fraction (BFR) of the
alcoholic leaf extract of ABH on cerebral ischemia/reperfusion induced oxidative stress in rats.

2. Materials and methods

2.1. Animals

Inbred Wistar strain male albino rats weighing 200–225 g were used for this study. Animals were housed in groups of 6–7 in colony cages at an ambient temperature of 25 ± 2°C and 45–55% relative humidity with 12 h light/dark cycle. They had free access to pellet chow (Brook Bond, Lipton India) and water ad libitum. The experimentations on animals were approved by the Institutional Animal Ethical Committee (IAEC) under the regulation of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi.

2.2. Chemicals

Adrenaline bitartrate, thiobarbituric acid, NADH were purchased from Sisco research laboratories, India. Hydrogen peroxide was purchased from E. Merck, Mumbai, India. Pentobarbitone, Vitamin E was used purchased from central drug house, Delhi, India. The other chemicals and solvents used were of analytical grade purchased from commercial suppliers.

2.3. Extraction of the plant material and separation of biflavones rich fraction

The fresh leaves of ABH were collected from the Botanical Garden, Udhagamandalam, Nilgiris District, Tamil Nadu. The plant material was authenticated at the Survey of Medicinal Plants and Collection Unit, Nilgiris District, Tamil Nadu, India. A voucher specimen (JUNPSL 2002-01) has been retained in the School of Natural Product Studies, Jadavpur University. The shed dried, coarse powdered leaves (500 g) of A. bidwillii were extracted separately in the soxhlet extraction apparatus using ethanol (95%). The resultant alcoholic extract was concentrated by rotary vacuum evaporator. The extracts were then freeze-dried and stored in a vacuum desiccator (yield 13.16%, w/w). The dried alcoholic extract was suspended in water. The alcoholic extract was mixed with n-hexane and the n-hexane portion was discarded after separation. To the aqueous portion, dichloromethane was added and the dichloromethane portion was discarded after separation and the aqueous portion was collected and extracted ethyl acetate. The ethyl acetate portion was collected and solvent was completely removed by rotary vacuum evaporator. The yield of the ethyl acetate fraction was 1.125% (w/w). The ethyl acetate fraction was subjected to qualitative chemical test and thin layer chromatography studies and showed positive test for flavonoids.

2.4. Thin layer chromatographic studies (TLC) of ethyl acetate fraction

Pre coated silica gel GF254 Plate 15 cm × 20 cm (E. Merck, Mumbai, India) was used as the stationary phase. The ethyl acetate fraction was dissolved in ethanol. This fraction was applied by means of a Linomat IV sample applicator to the plates about 1 cm above the edge. The chromatogram was developed up to 10 cm with toluene:ethyl acetate:formic acid:water (3.5:5:1:0.5) as the solvent system in a CAMAG twin trough chamber [23,24]. The developed TLC plate was sprayed with natural products-poly ethylene glycol reagent (NP/PEG) and observed under UV-light. From the thin layer chromatographic studies, the presence of various flavonoids was observed with Rf values between 0.06 and 0.58. The Rf value of flavonoids present in ethyl acetate fraction was compared with literature values of biflavones and gave a good agreement [25–27]. The ethyl acetate fraction was labeled as biflavone rich fraction (BFR).

2.5. Acute toxicity study and gross behavior in rats

Acute toxicity study – up and down procedure – was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD). The maximum upper limit dose 2000 mg/kg of BFR was administered orally to rats. Animals were observed individually after dosing. Observation included mortality and clinical signs, such as changes in skin fur, eyes and mucous membranes. The gross behaviors, e.g. body positions, locomotion, rear ing, tremors, gait was observed. The effect of BFR on passivity, grip strength, pain response, stereotypy, vocalization, righting reflex, body weight and water intake was assessed [28]. Pilot study was carried out with various doses (10, 100, 200 and 400 mg/kg, per oral route to rats) of BFR in animal models of pain and inflammation [29]. At doses of 100 and 200 mg/kg, it was active and at 10 mg/kg it was inactive. The dose 400 mg/kg was equipotent with 200/mg/kg dose. Based on this observations two different doses (100 and 200 mg/kg) of BFR was selected in ischemia/reperfusion induced oxidative stress model.

2.6. Induction of cerebral ischemia/reperfusion (I/R) injury in rats

Induction of cerebral ischemia/reperfusion was carried out by method described previously, with slight modification [30]. The animals were treated with atropine sulphate (0.5 mg/kg, i.p.) as preanaesthetic medication and anaesthetized with chloral hydrate (350 mg/kg, i.p.). Both common carotid arteries were exposed over a midline incision, and a dissection was made between the sternocleidomastoid and the sternohyoid muscles parallel to the trachea. Each carotid artery was freed from its adventitial sheath and vagus nerve, which was carefully separated and maintained. The induction of ischemia was performed by occluding bilateral common carotid arteries (BCCAO) with clamps for 30 min followed by 24 h reperfusion and the skin was closed with stitches using waxed silk suture. During the BCCAO, animals were observed to match the following criteria: maintenance of dilated pupils, absence of a cornea reflex when exposed to strong light stimulation, and maintenance of rectal temperature at (37 ± 0.5) °C. Animals not matching these criteria or with seizures were all excluded. Sham control animals received the same surgical procedures, except BCCAO were not occluded. After the completion of reperfusion period the animals were assessed for neurological outcome and then sacrificed.

2.7. Administration of the extracts and drugs

2.7.1. Effect of BFR on endogenous brain antioxidants in rats without IS/R

In this experiment animals were divided into three groups each having six animals. The first group served as control, second and third groups were treated with BFR 100 and 200/mg/kg, respectively.

2.7.2. Effect of BFR on endogenous brain antioxidants in rats with IS/R

In this experiment animals were divided into five groups each having six animals. The first group served as control. The second group was I/R induced animals, whilst the third, fourth I/R groups were pretreated with BFR at 100 and 200 mg/kg, respectively. The fifth group was group pretreated with Vitamin E (200 mg/kg). The BFR and Vitamin E were pre-administered orally for 7 days, 1 h before ischemia and on the second day of the reperfusion period as suspension in 0.1% carboxy methyl cellulose (CMC).

2.9. Assessment of neurological deficit

Neurological deficits in I/R and drug treated group were measured after 24 h of reperfusion period. Neurological outcome were scored on a five-point scale. No neurological deficit (right = 2, falling to right = 3, did not walk spontaneously and semi conscious = 4 [31].

2.10. Assessment of sensory motor function

2.10.1. Hanging wire test

This task was used as a measure of grasping ability and forelimb strength of the rats after I/R induced brain injury [32]. In this test I/R rats (control and drug
treated) were suspended by the forelimbs on a wire stretched between 2 posts 60 cm above a foam pillow. The time (in s) until the animal fell was recorded.

10.2. Rotord test
In rotord test experiment, control, I/R and drug treated I/R rats were kept on a rotord apparatus and the speed was maintained at 10 rpm. The motor coordination was evaluated as the ability of rat to hold the rotating rotor. The time for which the rat held the rotor was calculated and the result was expressed in s. The cut off time was 3 min.[33].

11. Preparation of brain tissue for estimation of oxidative stress marker

The brain of each animals was removed after completion of neurobehavioral experiment, weighed and the various brain regions of cortex, hippocampus, striatum and cerebellum were dissected out and homogenized with 10 times (w/v) ice cold phosphate buffer saline (50 mM pH 7.8) in teflon glass homogenizer. The homogenate was centrifuged at 1000 rpm 4°C for 3 min and the supernatant divided into two portions, one of which was used for measurement of lipid peroxidation (LPO). The remaining supernatant was again centrifuged at 12,000 rpm at 4°C for 15 min and used for the measurement of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH). Protein was measured by the method of Lowry et al. [34].

11.1. Measurement of lipid peroxidation (LPO)
The LPO end product malondialdehyde (MDA) was measured by the method of Ohkawa et al. [35]. 0.1 ml of brain homogenate was treated with 20% of 1.5 ml of acetic acid (pH 3.5), 1.5 ml thio barbituric acid and 0.2 ml sodium dodecy sulphate (8.1%). The mixture was then heated at 100°C for 60 min. The mixture was cooled and 5 ml of n-butanol–pyridine mixture (15:1) was added followed by 1 ml of distilled water. The mixture was shaken vigorously. After centrifugation of the mixture at 4000 rpm for 10 min, the organic layer was taken and its absorbance was measured at 532 nm. The concentration of MDA formed is expressed as n mole/mg of protein.

11.2. Estimation of superoxide dismutase (SOD)
SOD activity in I/R and drug treated various brain regions was measured by Saggu et al. [36]. Superoxide dismutase measurement was carried out by the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome. A mixture of 2.80 ml of sodium carbonate (0.05 mM) buffer (pH 10.2), 100 μl of EDTA (1.0 mM) and 20 μl of brain homogenate or sucrose (blank) were incubated at 30°C for 45 min. Thereafter, reaction was initiated by adding 100 μl of adrenaline solution (9.0 mM). The change in the absorbance was recorded at 480 nm for 8–12 min. Similarly, SOD calibration curve was prepared by taking 10 units/ml a standard solution. One unit of SOD produced approximately 50% inhibition of auto-oxidation of adrenaline. The results are expressed as SOD μmol/min/mg of protein.

11.3. Estimation of catalase (CAT)
Catalase measurement was carried out by the ability of CAT to oxidize hydrogen peroxide (H2O2). 2.25 ml of potassium phosphate buffer (65 mM, pH 7.8) and 100 μl of the brain homogenate were incubated at 25°C for 30 min. A 650 μl H2O2 (7.5 mM) was added to the brain homogenate to initiate the reaction. The change in absorption was measured at 240 nm for 2–3 min and the results were expressed as CAT μmol/min/mg of protein [37].

11.4. Estimation of glutathione (GSH)
Glutathione was measured according to the method of Ellman [38]. The equal quantity of brain homogenate was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.1 ml of this supernatant, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5,5-dithiobis(2-nitrobenzoic acid) and 0.4 ml of double distilled water were added. The mixture was vortexed and absorbance read at 412 nm within 15 min. The concentration of reduced glutathione was expressed as nmol/mg of protein.

12.12. Histopathological examination
At the end of behavioral testing rats were sacrificed by decapitation and the brains were removed. The intact whole brain was transferred to formalin (10%, v/v). The tissue was cut into 3 mm thickness and its blocks were embedded in paraffin. The brain section (5–10 μm) thickness were prepared and stained with hematoxylin and eosin.

12.13. Statistical analysis

Results are reported as mean ± S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall P-value was found statistically significant (P < 0.05), further comparisons among groups were made according to post hoc Tukey’s test. All statistical analyses were performed using SPSS statistical Version 8 software package (SPSS Inc., USA). The diagrammatic representation of the data was performed by using Graph pad PRISM, Version 3 software.

3. Results

3.1. Effect of BFR in acute toxicity and gross behaviors in rats

The rats treated with BFR at the dose of 2000 mg/kg were well tolerated and exhibited normal behavior. Rats were alert with normal grooming, touch response, pain response and there was no sign of passivity, stereotypy, and vocalization. There was no abnormal change in motor activity, secretory signs as well as their body weight and water intake.

3.2. Effect of BFR in sensory motor function and brain antioxidants of rats

There were no significant differences in basal reading of neurobehavioral activities, sensory motor function, oxidative marker MDA and antioxidants in the control rats treated with BFR at the dose of 100 and 200 mg/kg as compared to control.

3.3. Effect of BFR and Vitamin E on neurological score and sensorimotor function

The neurological scoring and sensory motor functions of the I/R and drug treated rats after 24 h of reperfusion are shown in Table 1. Induction of I/R exhibited significant neurological deficit and reduction in sensorimotor coordination as compared to control group. Treatment with BFR

<table>
<thead>
<tr>
<th></th>
<th>Neurological score</th>
<th>Hanging wire test (s)</th>
<th>Rotorod (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham control</td>
<td>0</td>
<td>38.6 ± 5.4</td>
<td>80.63 ± 3.98</td>
</tr>
<tr>
<td>I/R + control solution</td>
<td>3.00 ± 1.0</td>
<td>32.2 ± 4.8</td>
<td>24.00 ± 6.21*</td>
</tr>
<tr>
<td>I/R + BFR 100 mg/kg</td>
<td>3.00 ± 1.1</td>
<td>32.4 ± 5.4</td>
<td>26.66 ± 8.14</td>
</tr>
<tr>
<td>I/R + BFR 200 mg/kg</td>
<td>2.40 ± 1.3</td>
<td>34.4 ± 5.2</td>
<td>36.33 ± 4.14#</td>
</tr>
<tr>
<td>I/R + Vitamin E</td>
<td>2.00 ± 1.2</td>
<td>36.6 ± 4.4</td>
<td>41.66 ± 5.68#</td>
</tr>
</tbody>
</table>

Values are represented as mean ± S.E.M. *P < 0.05 vs. control, #P < 0.05 vs. I/R.
(200 mg/kg) was significantly ($P < 0.05$) increase the time spent in rotorod test as assessed by the sensory motor function of I/R rats as compared to I/R group. Vitamin E treated I/R rats showed significant ($P < 0.05$) increase in fall off time from the rotorod.

3.4. Effect of BFR and Vitamin E in MDA levels

The effect of BFR and Vitamin E in LPO levels was represented in Fig. 1. BCCAO for 30 min followed by 24 h reperfusion resulted in elevation of LPO marker malondialdehyde (MDA) levels throughout cortical and subcortical brain regions as measured 24 h after BCCAO. The amount of MDA formed in cortex, hippocampus, striatum and cerebellum ($3.89 \pm 0.51, 3.78 \pm 0.28, 3.17 \pm 0.50$ and $3.96 \pm 0.39$ nmol/mg of protein) was high as compared to control group. Treatment with BFR (200 mg/kg) and Vitamin E was significantly ($P < 0.05$) lowered the MDA levels in cortex, hippocampus and striatum of the I/R rats brain as compared to I/R group. BFR (100 mg/kg) significantly lowered the MDA levels in cortex and hippocampus of I/R rat brain. The significant effect of the BFR (200 mg) in decreasing the MDA levels in cortex, hippocampus and striatum were comparably equal to Vitamin E.

3.5. Effect of BFR and Vitamin E in SOD level

The effects of BFR and Vitamin E on SOD levels in various brain regions are shown in Fig. 2. In the I/R group, depletion of SOD levels was observed in various brain regions ($19.00 \pm 0.17, 17.50 \pm 0.26, 16.30 \pm 0.51$ and $17.40 \pm 0.28$ µmol/min mg of protein) compared to that of control treated animal group. Administration of BFR (100 and 200 mg/kg) significantly ($P < 0.05$) elevated the SOD levels in cortical and sub cortical regions of the I/R rat groups compared to the I/R group. The effect of BFR in restoring the SOD level in the hippocampus was comparable to that given by Vitamin E ($P < 0.05$).

3.6. Effect of BFR and Vitamin E in catalase level

The CAT activity in cortical and sub cortical regions of I/R rat brain was shown in Fig. 3. Decrease in catalase activity was noted in four brain regions ($15.26 \pm 0.29, 13.82 \pm 0.26, 15.80 \pm 0.35$ and $13.60 \pm 0.28$ µmol/min mg of protein) after 24 h of reperfusion. Administration of BFR (100 and 200) and Vitamin E significantly reverse the decreased catalase activity in cortex, hippocampus and striatum regions compared to I/R group alone. BFR (200 mg/kg) also significantly increased the catalase activity in cerebellum region. The reversal was found to be significant ($P < 0.05$). BFR at dose of 100 mg/kg was ineffective to increase the CAT in the cerebellum; this may be explained by inadequate concentration and anti-oxidative capacity of BFR to defend against oxygen free radicals in the cerebellum region against free radicals generated during ischemia.
3.7. Effect of BFR and Vitamin E in reduced glutathione (GSH) level

Fig. 4 represents the effect of BFR and Vitamin E on I/R—induced changes in GSH level in various brain regions. Depletion of GSH level was noted in various brain regions of I/R rats induced by BCCAO for 30 min followed by 24 h reperfusion as compared to sham treated group. There were no significant changes in GSH in the BFR low dose treated group. BFR at 200 mg/kg significantly elevated the GSH level in cortex, hippocampus, striatum and cerebellum of the I/R rats \( P < 0.05 \). Interestingly BFR (200 mg/kg) increased the levels of GSH in hippocampus more than Vitamin E in I/R rats.

3.8. Histology

The histopathology of the brain of I/R group and drug treated groups are shown in Fig. 5. From the histopathological study it was observed that shrinkage of neurons and atrophy of the neurons occurred in brain regions of I/R rats induced by BCCAO for 30 min followed by 24 h reperfusion, compared to control group. This effect was attenuated by administration of BFR (200 mg/kg) and Vitamin E (200 mg/kg).

4. Discussion

The present investigation showed the neuroprotective potential of biflavones rich fraction separated from alcoholic extract of ABH against ischemia/reperfusion (I/R) induced oxidative stress. It is observed that BFR attenuated the impaired neurological deficit and sensory motor functions of the ischemic rats. The activity of BFR appears to work by restoring the altered antioxidants enzymes as well as decrease the production of LPO in various brain regions induced by BCCAO. There is a considerable evidence supports that the role of ROS in the pathogenesis of I/R induced oxidative stress in brain [39,40]. Brain reperfusion after ischemia frequently results in neuronal...
death, which occurs preferentially in some brain regions. This neuronal degeneration has been associated with ROS, which react with cellular macromolecules such as lipids, proteins and nucleic acids leading to oxidative damage of the neurons [41,42]. Thus the endogenous antioxidant enzyme activity of the brain impaired by I/R is particularly important and measurement of those antioxidant enzymes after reperfusion can assess the vulnerability of the particular areas of the brain [43]. Novel therapeutic neuroprotective strategies support the applications of ROS scavengers and induction of endogenous antioxidants drugs, such as natural antioxidants, e.g. plant derived polyphenolic compounds, in mono therapy, or as part of an antioxidant cocktail formulation, for the treatment of neurodegenerative diseases [44–48]. Therefore, the potent radical scavenging properties or LPO inhibiting ability of polyphenolic natural compounds protecting the neurons from oxidative stress may provide useful therapeutic agent for the treatment of neurodegenerative diseases such as I/R induced oxidative stress.

Knowing that lipids are most susceptible macromolecules to oxidative stress, from results of our work reveals that the production of MDA was significantly elevated in cortical and sub cortical regions of the rats after 24 h of reperfusion period. Further ischemic animals were exhibited a score of more than 3 in neurological outcome and impaired sensory motor function induced by BCCAO for 30 min followed by 24 h reperfusion. Our present study demonstrates that pretreatment with BFR had markedly reduced the MDA level and inhibits the neuronal injuries from propagating chain reaction of LPO. These results receive further support from recent studies examining the effect of biflavones isolated from plants like G. biloba, Garcinia kola and Hypericum species shown to decrease thiobarbiturate reactive substance formation tested in ex vivo and in vitro methods [49–52].

During cerebral ischemia, a number of events predispose the brain to the formation of ROS such as rapid increase in adenosine triphosphate levels, calcium release from intracellular stores, loss of Ca\(^{2+}\) homeostasis, excitotoxicity, arachidonic acid release and metabolism, mitochondrial dysfunction, acidosis and edema [53–55]. This spectrum can modulate the antioxidants enzymes and its gene expression in I/R insult [43]. Therefore we examined the antioxidants like SOD and CAT, which served as oxidative indices in various brain regions of the BCCAO occluded rats. Decrease in the levels of SOD and CAT were noted in various brain regions of the ischemic rats that indicates participation of superoxide radical which known to produce highly toxic hydroxyl radical through its reaction with H\(_2\)O\(_2\) (Haber–Weiss reaction) [40]. These in turn decreases the SOD through a modification in histidine residue located in the active site of the enzyme [56]. On the other hand this over production of H\(_2\)O\(_2\) can be inactivated by catalase enzyme and there by reduction in CAT. BFR (100 and 200 mg/kg) was found to elevate the activity of two major oxygen radical species metabolizing enzymes in various regions of ischemic brain. At dose of 100 mg/kg, BFR was ineffective to increase the CAT levels in cerebellum region this may be explained by insufficient concentration and antioxidative capacity to defend adequately against oxygen free radicals generated in the cerebellum region during ischemia.

This is supported by previous findings that numerous flavonoids, e.g. those found in green tea extract and other antioxidant preparations containing biflavones and polyphenolics protect the neurons against ischemia-reperfusion induced alteration in activities of intra cellular antioxidant complexes [57–60]. The antioxidant effect of BFR observed in various brain regions of I/R rats explains the potential permeation of BFR across the blood–brain barrier (BBB) and utilization of the BFR on brain. Despite evidences of neuroprotective properties of many biflavonoids, their in vivo biological metabolites have been demonstrated for its uptake by brain endothelial cells as well as permeability to BBB [61]. Such peroxidation processes and overproduction of free radicals may lead to consumption of detoxifying endogenous antioxidants such as GSH. Reduced glutathione (GSH) is one of the primary endogenous antioxidant defense systems in the brain, which removes hydrogen peroxide and lipid peroxides [62]. Decline in GSH levels could either increase or reflect oxidative status [63]. In our experiment, depletion in GSH was observed in various brain regions, i.e. cortex, hippocampus, striatum and cerebellum of ischemic rats. This could be explained by the consumption of GSH due to scavenging of the rapidly generated hydrogen peroxide and lipid peroxides. It has been shown that depletion of GSH in IS/RP injury can be attributed to several factors such as cleavage of GSH to cysteine, decrease in synthesis of GSH and the formation of mixed disulfides with GSSG, causing their cellular stores depleted [64–66]. Interestingly, those rats fed with BFR at the higher dose increased the GSH levels in various brain regions, but the mechanism involved is not known. This study suggest that BFR administration to the normal rat did not showed any effect on the levels of endogenous antioxidant enzymes and oxidative stress marker in various brain regions of normal rat (data not shown). Interestingly BFR exerts its antioxidant effect only in the presence of ischemia and reperfusion induced oxidative stress. This may be due to direct scavenging effect of biflavone on free radical released during ischemia reperfusion (I/R) and also modulate the endogenous antioxidants enzymes mediated neuroprotection. The severe neuronal loss, observed as shrinkage of neurons and atrophy, was observed in the histopathological brain sections of the I/R group. Interestingly this effect was attenuated by the administration of BFR (200 mg/kg) and Vitamin E (200 mg/kg).

5. Conclusion

The present investigation demonstrates the antioxidant effects of BFR tested in cerebral ischemia and reperfusion induced oxidative stress. The results suggest that the BFR from A. bidwillii is protective against ischemia-induced oxidative stress by mechanisms involving inhibition of free radical generation, reactive oxygen species scavenging, modulation of intracellular antioxidants against I/R induced decreases and that this fraction may have potential as a therapy for the oxidative stress—related disorders.
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