Effect of ethanolic extract of root of *Pongamia pinnata* (L) pierre on oxidative stress, behavioral and histopathological alterations induced by cerebral ischemia–reperfusion and long-term hypoperfusion in rats

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Possible effect of an ethanolic root extract of *Pongamia pinnata* (L) Pierre (*P. pinnata*) on oxidant-antioxidant status and histopathological changes in acute ischemia-reperfusion injury in the rat forebrain have been investigated. Further, its effect was also assessed on long-term cerebral hypoperfusion-induced changes in anxiety, cognitive and histopathological parameters. Cerebral post-ischemic reperfusion is known to be associated with generation of free radicals. In the present study, bilateral common carotid artery occlusion (BCCAO) for 30 min followed by 45 min reperfusion produced increases in lipid peroxidation, superoxide dismutase (SOD) activity and a fall in the total tissue sulfhydryl (T-SH) levels. The ethanolic extract of roots of *P. pinnata* (50 mg kg⁻¹, po for 5 days) attenuated the ischemia-reperfusion-induced increase in lipid peroxidation, SOD activity and a fall in T-SH levels. The extract also ameliorated histopathological changes and inflammatory cell infiltration in the frontoparietal region of the rat brain. The extract (50 mg kg⁻¹, po for 15 days) was also found to alleviate the long-term hypoperfusion-induced anxiety and listlessness (open field paradigm). There was an improvement of learning and memory deficits (Morris’ water maze testing). It also attenuated reactive changes in forebrain histology like gliosis, lymphocytic infiltration, astrocytosis and cellular edema. Results suggest protective role of *P. pinnata* in ischemia-reperfusion injury and cerebrovascular insufficiency states.

**Keywords:** Behavior, Cerebral hypoperfusion, Cognition, Oxidative stress, *Pongamia pinnata*, Reperfusion injury

The plant, *Pongamia pinnata* (L) Pierre (Leguminosae) (Synonym *Pongamia glabra* Vent.) (Karanj in Hindi) is widely used in several Ayurvedic medicinal formulations for its diverse medicinal properties¹,². The ethanolic extract of *P. pinnata* roots is reported to have antiinflammatory, gastroprotective and antistress activity³,⁴. It is known to have antioxidant property⁵ and has also been reported to reverse alterations in central cholinergic markers of cognition in experimentally demented rats⁶.

Oxygen free radicals play an important role in cerebral ischemia. Vascular reperfusion, subsequent to transient occlusion of carotid artery results in worsening of the histopathological damage if reperfusion is achieved after some critical period of occlusion (i.e. ischemia)⁷. The oxygen free radicals initiate lipid peroxidation and inflict damage on macromolecular components of the cell⁷,⁸. In contrast to acute ischemia-reperfusion, a chronic partial/total reduction in cerebral blood flow and brain energy metabolism causes behavioral and cognitive defects⁹–¹².

In view of the reported antiinflammatory, antioxidant, cognition-enhancing and antistress properties of *P. pinnata*⁶,³,⁴, it will be of interest to investigate its effect on cerebral ischemia-reperfusion injury and long-term hypoperfusion.

**Materials and Methods**

Drug and reagent—1, 1, 3, 3-Tetraethoxypropane (TEP, Merck, Germany), thiobarbituric acid (TBA), NADH, nitroblue tetrazolium (NBT) and phenazine methosulfate (PMS), (Sigma, USA) were used. All other chemicals and reagents were of the highest analytical grades available locally.

Pharmacognostically identified roots of *P. pinnata* were obtained from Regional Research Institute (Ayurveda), Patna. A voucher specimen has been kept

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in the Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University. A sample of *P. pinnata* root (900 g) was crushed, powdered by grinding, extracted with ethanol and then concentrated under steam bath. The yield was 1.11% (9.99 g of dried extract was obtained at the end). The extract tested positive for steroids, glycoside and flavonoids (done at Indian Herbs Ltd, Saharanpur). Animals received this extract added to a glycoside and flavonoids (done at Indian Herbs Ltd, Saharanpur). Animals received this extract added to a suspension of 1% gum acacia in double distilled water at a dose of 50 mg kg\(^{-1}\) d\(^{-1}\) po. This particular dose was chosen on the basis of earlier reports\(^3\).

**Animals**—The present study was conducted on inbred Charles Foster (CF) male albino rats (18 months old) weighing 250-300 g, obtained from the central animal house of the Institute of Medical Sciences, Banaras Hindu University, Varanasi. They were kept in the departmental animal house in colony cages at an ambient temperature of 25±2°C and 45-55% RH with 10:14 hr light: dark cycles. They had free access to standard rodent pellet diet and drinking water and were fasted for 18-24 hr before surgery (water was allowed *ad libitum*). The study protocol was approved by the Institutional Ethical Committee and the Principles of laboratory animal care guidelines (NIH Publication No. 86-23, revised 1985) were followed throughout.

**Experimental procedure**—The surgical technique used in the present experiments for induction of cerebral ischemia by bilateral common carotid artery occlusion (BCCAO) was adapted from the earlier published method of Iwasaki *et al.*\(^{13}\). Rats were anaesthetized by ketamine (100 mg kg\(^{-1}\), ip). Both common carotid arteries were carefully exposed through a midline skin incision in the neck.

Acute ischemia-reperfusion injury was produced by blocking bilateral common carotid arteries (BCCA) for 30 min (lifting arteries with loose ligature with the help of thread) and reperfusion for 45 min was allowed by just untying the ligature and releasing the thread. Body temperature was maintained at about 37°C during the period with the help of a heating lamp. This protocol was adopted on the basis of earlier reports\(^8,14\). At the end of reperfusion injury, the animals were sacrificed by decapitation and the frontoparietal part of cerebral cortex from both the hemispheres were dissected out and transferred to appropriate homogenizing medium for biochemical estimation.

For long-term hypoperfusion studies, BCCAs were doubly ligated with 3-0 silk sutures and cut in between\(^{15}\). The skin was then sutured and animals were returned to their home cage. On the day 15, 60 min after the last dose of *P. pinnata*, all animals were subjected to behavioral testing and histopathological studies.

For acute studies, the animals were divided into 4 groups of 6 animals each. Group I served as sham-operated control (underwent all surgical procedure except BCCAO). In Group II, *P. pinnata* (50 mg kg\(^{-1}\) d\(^{-1}\), po) was administered to sham-operated animals to determine the effect of drug per se. Group III animals underwent 30 min BCCAO and 45 min reperfusion. In Group IV (treatment) animals, *P. pinnata* was administered 60 min before subjecting the animals to ischemia-reperfusion and thereafter continued for 5 days in the dose of 50 mg kg\(^{-1}\) d\(^{-1}\), po.

For the long-term hypoperfusion studies, animals were again divided into 4 groups of 6 animals each. First group served as sham-operated control. In second group, *P. pinnata* (50 mg kg\(^{-1}\) d\(^{-1}\), po) was administered during the experimental period in sham-operated animals (per se). Third group animals underwent permanent BCCAO and received vehicle only (hypoperfusion group). In the fourth group, *P. pinnata* was administered 60 min before BCCAO. *P. pinnata* was then continued upto 15\(^{th}\) post surgical day in sham-operated per se and hypoperfused (treatment group) animals. On day 15 (60 min after the last dose of drug in drug treated groups), animals of all groups were subjected to behavioral assessment paradigm and subsequently they were sacrificed by guillotine and brain samples were collected for histopathological studies.

**Biochemical analysis**—At the end of experiments animals were sacrificed by decapitation and the frontoparietal part of the cerebral cortex from both the hemispheres were separated. After rinsing with ice-cold normal saline, the brain tissue were transferred to the appropriate homogenizing medium and analyzed for the biochemical parameters of the oxidant-antioxidant status i.e. thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD) activity and tissue total sulfhydryl (T-SH) level. All the procedures on the brain samples were performed on ice or ice bath and samples were kept in the freezer compartment (−20°C) of refrigerator. All biochemical parameter were measured in the
frontoparietal part of the cerebral cortex of both the hemispheres.

**Lipid peroxidation**—Estimation of lipid peroxidation was done by measuring the lipid peroxidation product TBARS (thio barbituric acid reactive substances) following the method of Ohkawa et al.¹⁶. TEP was used as an external standard and the level of lipid peroxidation was expressed as nanomoles TBARS mg⁻¹ of protein.

**Superoxide dismutase** (SOD)—SOD was estimated by adopting the procedure of Kakkar et al.¹⁷. The inhibition by SOD of reduction of nitroblue tetrazolium to blue colored chromogen in presence of phenazine methosulphate and NADH was measured at 560 nm. One unit of enzyme activity was defined as enzyme concentration required to inhibit the absorbance at 560 nm of chromogen production by 50% in one min under assay condition and expressed as specific activity in milliunits mg⁻¹ of protein.

**Total tissue sulfhydryl groups** (T-SH)—Total T-SH groups in brain were measured following the method of Sedlack and Lindsay¹⁸. The level of T-SH groups was expressed as moles of SH 100⁻¹ g of wet tissue weight.

**Estimation of brain protein**—The protein content of brain tissue was estimated using the method of Lowry et al.¹⁹.

**Behavioral testing**

**Open field test**—Locomotor activity was evaluated in an open field paradigm²⁰. The open field was made of plywood and consisted of a floor (96 × 96 cm) with high walls (61 × 61 cm). Entire apparatus was painted black except for 6 mm thick white lines that divided the floor into 16 squares. The entire room except the open field was kept dark during the experimentation. The open field was lit by a 60 watt bulb focusing on to the field from a height of 100 cm from the floor. Each animal was placed individually in one corner of the apparatus and for the next 5 min, it was observed for ambulations (number of squares crossed), total period of immobility (in sec), number of rearings, groomings and fecal pellets.

**Morris’ water maze test**—The maze consisted of a black circular pool²¹ (diameter 2.14 m, height 80 cm) filled to a depth of 44 cm with water (25°C±1°C). Water was made opaque by adding Indian ink. On day 15 after surgery, spatial learning and memory was tested in water maze. On 14th day the rats received habituation (exposure in water maze for 1 min) in which there was no platform present. Then, on day 15, a circular platform (9 cm in diameter) was kept hidden 2 cm below water level in the center of one of the quadrants. The platform remained in the same position during training days (reference memory procedure) (escape trial). At the beginning of each session, a random sequence of four starting poles along the perimeter of the pool was generated. All animals followed this sequence for that session. Each rat was placed in the water facing the wall at the start location and was allowed 90 sec to find the hidden platform. The animal was allowed a 20 sec rest on the platform. The latency to reach the platform was recorded. If the rat was unable to locate the hidden platform, it was lifted out and placed on the platform for 20 sec. The procedure was repeated for all the four start locations.

Two sessions of 4 trials each were conducted on first day of testing separated by 4 hr and one session of 4 trials was conducted on the next day. After that, the platform was removed and a probe trial (without platform) was conducted 4 hr later. Each rat was placed in the pool at the same randomly selected starting pole and swimming path was observed and time spent in the quadrant of pool which initially contained platform was recorded.

On completion of the probe trial, a black platform that extended 1 cm above the surface of water was placed in a quadrant other than that chosen for the submerged platform. Each rat was then given 4 trials of 90 sec to locate it. The latency to reach the platform was recorded (working memory procedure).

**Histopathological examinations**—At the end of experimental procedures and behavioral testing, rats were sacrificed and the brains were taken out. The brains were transferred to 10% formalin. Sections (<5 µm thick) of the frontal forebrain were prepared and stained by hematoxylin and eosin for microscopical examination.

**Statistical analysis**—Statistical analysis was performed by one-way Analysis of Variance (ANOVA) followed by post hoc Tukey Test for biochemical parameters and behavioral observations. A P-value of <0.05 was considered statistically significant.

**Results**

**Biochemical Observations**—Acute BCCAO for 30 min followed by 45 min reperfusion induced a 2-fold increase in lipid peroxidation (TBARS), 2.1-fold SOD
activity and a 35% fall in T-SH levels. Pretreatment of rats subjected to cerebral ischemia-reperfusion injury with *P. pinnata* root extract attenuated the increases in lipid peroxidation (*P* <0.01) and SOD levels (*P* <0.01) and prevented the fall in T-SH (*P* <0.05) (Table 1).

**Behavioral observations—Open field test:** Animals with permanent BCCAO (hypoperfusion group) showed marked alterations in locomotor activities in open field paradigm. Permanent BCCAO was associated with reduced number of ambulations (*P* <0.01), rearings (*P* <0.05) and groomings (*P* <0.01) along with an increase in the period of immobility (*P* <0.01) (Table 2). *P. pinnata* pretreatment prevented these alterations.

*P. pinnata* per se did not have any effect on any of the parameters of the test (Table 2).

**Morris’ water maze test:** Table 3 shows that there was no difference between sham-operated control and *P. pinnata* per se groups with respect to any of the parameters. All rats were able to locate the hidden platform during the sessions of escape trial. The hypoperfused animals took more time than sham-operated control in finding submerged platform. The increases in the time taken in finding the platform were significant in both the second and third trials

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### Table 1—Effect of *P. pinnata* (50 mg kg⁻¹ po for 5 days) on biochemical parameters of oxidative stress in rat forebrain following cerebral ischemia-reperfusion injury (30 min BCCAO followed by 45 min reperfusion injury)

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nM mg⁻¹ protein)</th>
<th>SOD milliunits mg⁻¹ protein</th>
<th>T-SH (×10⁻⁵ M mg⁻¹ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Sham-operated</td>
<td>1.93 ± 0.13</td>
<td>340.92 ± 42.19</td>
<td>3.73 ± 0.20</td>
</tr>
<tr>
<td>II Per se</td>
<td>1.95 ± 0.10</td>
<td>369.18 ± 35.29</td>
<td>3.80 ± 0.43</td>
</tr>
<tr>
<td>III Ischemia-reperfusion</td>
<td>3.94 ± 0.26**</td>
<td>730.30 ± 65.29**</td>
<td>3.31 ± 0.12**</td>
</tr>
<tr>
<td>IV <em>P. pinnata</em> extract treated</td>
<td>2.20 ± 0.15**</td>
<td>490.70 ± 61.49**</td>
<td>3.31 ± 0.12*</td>
</tr>
<tr>
<td>F (3, 20)</td>
<td>32.99</td>
<td>11.41</td>
<td>6.25</td>
</tr>
</tbody>
</table>

Gr. I and Gr. IV are compared with Gr. III. Gr. II is compared with Gr. I. 
*P* values: * <0.05; ** <0.01 (ANOVA followed by Tukey test).

### Table 2—Effect of *P. pinnata* (50 mg kg⁻¹ po for 5 days) on open field parameter in long-term hypoperfused rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ambulations (number)</th>
<th>Immobility (s)</th>
<th>Rearing (number)</th>
<th>Grooming (number)</th>
<th>Fecal pellets (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Sham-operated control</td>
<td>62.83 ± 4.76</td>
<td>26.00 ± 2.00</td>
<td>25.16 ± 1.70</td>
<td>7.50 ± 0.42</td>
<td>3.40 ± 1.21</td>
</tr>
<tr>
<td>II Per se</td>
<td>64.50 ± 5.11</td>
<td>26.50 ± 1.75</td>
<td>25.83 ± 1.92</td>
<td>7.83 ± 2.50</td>
<td>2.50 ± 0.75</td>
</tr>
<tr>
<td>III Hypoperfusion</td>
<td>30.00 ± 5.55**</td>
<td>58.33 ± 1.52**</td>
<td>18.83 ± 1.20**</td>
<td>4.00 ± 0.58**</td>
<td>2.16 ± 0.40</td>
</tr>
<tr>
<td>IV <em>P. pinnata</em> extract treated</td>
<td>68.33 ± 5.18**</td>
<td>29.00 ± 2.60**</td>
<td>29.16 ± 1.81**</td>
<td>5.83 ± 0.75*</td>
<td>2.83 ± 0.65</td>
</tr>
<tr>
<td>F (3, 20)</td>
<td>9.03</td>
<td>75.12</td>
<td>6.57</td>
<td>9.16</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

Gr. I and Gr. IV are compared with Gr. III. Gr. II is compared with Gr. I. 
*P* values: * <0.05; ** <0.01 (ANOVA followed by Tukey test).

### Table 3—Effect of *P. pinnata* (50 mg kg⁻¹ po for 5 days) on learning and memory in long-term hypoperfused rats in Morris’s water maze

<table>
<thead>
<tr>
<th>Groups</th>
<th>Escape latency (Sessions)</th>
<th>Probe trial</th>
<th>New platform trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Sham-operated control</td>
<td>65.33 ± 5.66</td>
<td>26.50 ± 1.76</td>
<td>34.16 ± 4.00</td>
</tr>
<tr>
<td>II Per se</td>
<td>62.83 ± 5.13</td>
<td>28.16 ± 1.47</td>
<td>35.00 ± 3.80</td>
</tr>
<tr>
<td>III Hypoperfusion</td>
<td>69.66 ± 4.72</td>
<td>41.33 ± 2.33</td>
<td>36.50 ± 4.75</td>
</tr>
<tr>
<td>IV <em>P. pinnata</em> extract treated</td>
<td>64.83 ± 5.42**</td>
<td>26.83 ± 2.00**</td>
<td>20.16 ± 0.94**</td>
</tr>
<tr>
<td>F (3, 20)</td>
<td>Not significant</td>
<td>26.08</td>
<td>26.35</td>
</tr>
</tbody>
</table>

Gr. I and Gr. IV are compared with Gr. III. Gr. II is compared with Gr. I. 
*P* values: * <0.05; ** <0.01 (ANOVA followed by Tukey test).
(P < 0.01). Treatment with *P. pinnata* prevented this delay in escape latencies in second and third sessions (P < 0.01) but not during the first session. The data of probe trial revealed that the hypoperfused rats spent less time in quadrant of former platform than did the sham-operated rats (P < 0.05). This change was significantly reversed by *P. pinnata* treatment (P < 0.05). The hypoperfused animals took a longer time to locate the new platform than the sham-operated rats (P < 0.01). *P. pinnata* treated animals took less time in finding the new visible platform than the hypoperfused animals (P < 0.01).

**Histopathological observations**

*Acute cerebral ischemia-reperfusion injury*—Fig. 1 shows normal forebrain architecture of sham-operated control animals (the forebrain architecture obtained in the sections of those rat brain samples in which no changes are expected as these animals did not undergo any ischemia-reperfusion injuries and hence treated as normal control histological pictures against which the alterations in other groups are compared). When the animals were subjected to 30 min BCCAO followed by 45 min reperfusion there was alteration in forebrain histology in the form of inflammatory cell infiltration (Fig. 3). *P. pinnata* per se did not produce any change in the histological architecture of forebrain (Fig. 2). *P. pinnata* (50 mg kg⁻¹, po × 5 days) partially reduced this acute injury induced change in architecture (Fig. 4).

*Long-term hypoperfusion injury*—Fig. 5 shows the normal histological picture of the brain in sham-operated controls. Long-term hypoperfusion produced marked alterations in the form of increased size and number of glial cells with nuclear enlargement and inflammatory changes resulting from lymphocytic

Figs 1-4—Acute cerebral ischemia-reperfusion injury. Histological appearance of the forebrain. 1 – Sham-operated control animals. Note the normal histology of the brain. 2 – *P. Pinnata* per se group. Note the normal histology of the brain. 3 – Subjected to 30 min BCCAO followed by 45 min reperfusion. Note the reactive changes in the form of mild inflammatory cell infiltration. 4 – Reperfused forebrains treated with *P. Pinnata*. Note the partial reduction in the injury induced reactive changes (H & E, 100×).
infiltration and cellular edema (Fig. 7). P. pinnata per se did not produce any change in the histological structure of the forebrain (Fig. 6). P. pinnata treatment partially reduced these hypoperfusion-induced changes as reflected by less lymphocytic infiltration and reduced glial cell proliferations (Fig. 8).

**Discussion**

The BCCAO for 30 min followed by 45 min reperfusion causes ischemia-reperfusion biochemical injury. Generation of free radicals initiates lipid peroxidation that is reflected as increased level of TBARS. Increased SOD activity, a marker of oxidative stress, also suggests the same. The biochemical, behavioral and histopathological observations of this study are in agreement with those reported earlier. Similarly, a fall in T-SH levels reflects consumption of tissue thiols. As such, a fall in GSH (a non-protein sulphydryl) levels during cerebral reperfusion injury is well reported. Sulphydryl compounds are among the most important endogenous antioxidants. They have a role in the maintenance of cellular proteins and lipids in their functional states. When these are consumed, the toxic effects of oxidative insult are exacerbated resulting in increased membrane and cell damage.

The data obtained in the present study showed that the P. pinnata root extract antagonized ischemia-
reperfusion injury induced raised TBARS levels. Similarly, the extract also reversed the ischemia-reperfusion induced changes in SOD and T-SH activities. These findings support the earlier observation of Annie et al.\textsuperscript{5} where flowers of \textit{P. pinnata} were shown to exert a protective role against cisplatin and gentamycin-induced renal injury and this protective role was explained on the basis of the antioxidant action of the plant. The different extracts of \textit{P. pinnata} are known to contain flavonoids; and flavonoids have been reported to possess antioxidant property\textsuperscript{5,24}. The observed beneficial effect of \textit{P. pinnata} on acute cerebral ischemia-reperfusion injury-induced changes in biochemical parameters may thus be attributed to its flavonoids contents. Production of inflammatory cytokines and recruitment of circulatory polymorphs during reperfusion\textsuperscript{7,25} are well documented. These polymorphonuclear leukocytes are a source of superoxide anions\textsuperscript{26}. Earlier reports showed that ethanolic extract of \textit{P. pinnata} roots has potent anti-inflammatory activity (Comparable to that of phenylbutazone) in carrageenin and PGE\textsubscript{1}-induced edema models\textsuperscript{3,4}. The protective role of \textit{P. pinnata} in reperfusion injury may be attributed to its anti-inflammatory activity.

Long-term hypoperfusion studies have been subjected to critical appraisal, through behavioral analysis\textsuperscript{27,20,21}. The long-term hypoperfused rats in the open field paradigm showed significant reductions in ambulations, rearings and groomings as compared to sham-operated animals. This suggests a propensity towards anxiety and listlessness. \textit{P. pinnata} significantly prevented the long-term hypoperfusion-induced anxiety. Hypoperfused animals also showed deficits of spatial learning and memory as indicated by Morris’ Water Maze data. This is in accordance with earlier reports of ischemia-induced disturbances of spatial learning and memory\textsuperscript{27}. Hypoperfused animals consistently showed longer escape latencies suggesting a defective registration of task (learning). Moreover, probe trial and new platform trial show deficits of reference and working memory in hypoperfused rats. \textit{P. pinnata} alleviated the changes in long-term hypoperfusion-induced anxiety and listlessness, along with an improvement of learning and memory deficits. It is not out of place to mention that \textit{P. pinnata} is known to have nootropic activity\textsuperscript{28} and it modifies the perturbed central cholinergic markers of cognition in experimentally demented rats\textsuperscript{6}.

Histopathological observations made in the present study revealed that acute and long-term BCCAO did not lead to extensive ischemic neuronal changes (such as neuronal cell death). This is consistent with earlier reports\textsuperscript{29,14}. The absence of neuronal loss/death needs consideration. Glial activation, when it occurs, has consistently been shown to precede overt neuronal death\textsuperscript{30,31}. Therefore, it is likely that, damage may be revealed if BCCAO is extended beyond this time point. Alternatively, the subtle histological damage would have been apparent if more sophisticated techniques of evaluation like immunohistochemistry or electron microscopy were used\textsuperscript{32}. Further work is needed to arrive at a final conclusion in this regard. The histopathological findings as observed in the present study are not consistent with the outcome of behavioral analysis. Indeed, it is known that functional deficits may result from transmission failure rather than from ultimate energy failure and/neuronal loss\textsuperscript{33,34,35,29}. Behavioral decline in the absence of overt histological damage and disturbance of cerebral energy parameters has already been reported\textsuperscript{29,34,36,14}. However, the observed reactive changes, that is gliosis, recruitment of macrophages/lymphocytes and perivascular inflammation support the reported ischemia and/or hypoperfusion-induced “reactive microgliosis”\textsuperscript{37,38,14}. The present data showed that \textit{P. pinnata} had partially prevented these reactive changes.

Permanent BCCAO in rats has been used as one of the animal models for neurodegenerative conditions and dementia\textsuperscript{32−34,39}. Reduced blood flow to the extent of 30-45\% in the cerebral cortex and 20\% in hippocampus has been observed 1 week after permanent BCCAO in rats. In addition, reduced glucose utilization by 20-30\% and 15\% in cortex and hippocampus, respectively\textsuperscript{11,12} was also observed. Chronic reduction in cerebral blood flow and brain energy metabolism leads to progressive cognitive deficits\textsuperscript{9}. Reduced brain energy metabolism causes reactive astrocytosis and microglial activation\textsuperscript{38}. Bioflavonoids are known vasodilators\textsuperscript{40}. Therefore, \textit{P. pinnata} by virtue of its bioflavonoid contents is likely to improve cerebral perfusion and microcirculation after long-term BCCAO. This in turn may attenuate behavioral disturbances and reactive changes.
The present study provides additional evidence concerning the anti-oxidative stress and cognition-enhancing property of P. pinnata.

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References


