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Effect of chlorophyll and aqueous extracts of *Bacopa monniera* and *Valeriana wallichii* on ischaemia and reperfusion-induced cerebral injury in mice

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Bilateral carotid artery occlusion followed by reperfusion produced significant cerebral infarction and impaired short-term memory, motor co-ordination and lateral push response. Individual pretreatments with chlorophyll and aqueous extracts of *B. monniera* and *V. wallichii* markedly attenuated ischaemia-reperfusion induced cerebral injury in terms of decreased infarct size, increase in short-term memory, motor in coordination and lateral push response. The results suggest that chlorophyll and aqueous extracts of *B. monniera* and *V. wallichii* prevent ischaemia-reperfusion induced cerebral injury with comparable potency.

Keywords: *Bacopa monniera*, Cerebral injury, Chlorophyll, *Valeriana wallichii*

A burst of free radical formation has been demonstrated during cerebral ischaemia[1,2] and at the onset of reperfusion after cerebral ischaemia[3,4]. Brain is more prone to the damage due to oxidative stress because neurons are rich in polyunsaturated fatty acids and that the levels of endogenous antioxidant enzymes in neuronal tissue are low[5,6]. Therefore, oxidative stress may contribute to neuronal cell death due to ischaemia and reperfusion. Synthetic antioxidants have been evaluated and have been shown to be protective in animal models of cerebral ischaemia and reperfusion-induced injury[7-9].

Chlorophyll is the green pigment present in all photosynthetic plants. Recent reports suggest that water soluble sodium-copper salt of chlorophyll has reactive oxygen species scavanging property *in vitro* and *ex vivo*[10,11]. *Bacopa monniera* Wettst (Family: Scrophulariaceae), is a perennial, creeping herb whose habitat includes wetlands and muddy shores. Its common names include Water Hyssop and brahmi (brahmi is also the Ayurvedic name given to *Centella asiatica* and other herbs). Besides, this plant is also known as Thyme-leafed gratiola and Moneywort. Synonyms include *Herpestris monniera*, *Moniera euneifolia*, *Lysimachia monniera* and *Bacopa monniera*. The leaves of the plant are succulent and relatively thick. Leaves are oblanceolate and are arranged oppositely on the stem. The flowers are small and white, with four or five petals. Its ability to grow in water makes it a popular aquarium plant. It can even grow in slightly brackish conditions. Propagation is often achieved through cuttings. It commonly grows in marshy areas throughout India, Nepal, Sri Lanka, China, Taiwan, and is also found in Florida and other southern states of USA, where it can be grown in damp conditions by the pond or bog garden. Famed in Ayurvedic medicine, brahmi has antioxidant properties. It has been reported to reduce oxidation of fats in the blood stream, which is a risk factor for cardiovascular diseases. It has been used for centuries to help benefit epilepsy, memory capacity, increase concentration, and reduce stress-induced anxiety. It is listed as a nootropic, a drug that enhances cognitive ability. In India, this plant has also been used traditionally to consecrate new born babies in the belief that it will open the gateway of intelligence. Recent studies suggest bacopa may improve intellectual activity[12]. Besides, *B. monniera* extract is reported to have an antioxidant effect in the rat frontal cortex, striatum and hippocampus[9] and is found to inhibit calcium influx via voltage and receptor operated calcium channels[13]. *Valeriana wallichii* DC. (Family : Valerianaceae) commonly known as Indian valerian or 'Tagara' is one of the important plant species of commerce. It is native to India (Himalayas). Indian valerian is used in various pharmaceutical preparations for the treatment of migraine and now been under evaluation for its other CNS effects[14]. The active constituents present in the

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root of *V. wallichii* are valerenic acid, valerenol, valerenone, valtrate and isovaltrate. Flavonoids (6-methylapigenin and hesperidin) isolated from *V. wallichii* have been shown to have CNS effects besides their antioxidant properties[15]. Therefore, the present study has been designed to investigate the effect of chlorophyll and aqueous extracts of *B. monniera* and *V. wallichii* on ischaemia and reperfusion induced cerebral injury in mice.

Materials and Methods

Male inbred BALB/c mice weighing 25±2g maintained on standard laboratory diet (Kisan Feeds Ltd., Bombay, India) having free access to tap water were employed in the present study. They were housed in the departmental animal house and were exposed to 12 hr cycle of light and dark. All the animals used in study were naïve to the elevated plus-maze test. The experiments were conducted in a semi-sound proof laboratory. The animal experiments were carried out as per the guidelines of institutional ethical committee.

Plant material, drugs and chemicals— Powdered 'whole' plant material of *B. monniera* and *V. wallichii* was obtained in the form of capsules from Himalaya Drug Co., India. Leaves of *Spinacia oleracea* were obtained from the cultivated farms in North India. The plant specimens were authenticated by Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, India. Chloral hydrate (Riedel-deHaen, Germany) was dissolved in distilled water. All other chemicals used in present study were of analar quality. All drug solutions were freshly prepared before use.

Preparation of aqueous extracts of B. monniera and V. wallichii— Plant material (2g) of *B. monniera* and *V. wallichii* each was grounded to a fine powder using a mortar and pestle and was then stirred vigorously in a volume of 30 ml warm distilled water for 20 min. The aqueous extract thus obtained was transferred in a china dish and evaporated to dryness by heating. The extract so obtained was scrapped off, weighed and reconstituted in normal saline. The yields of *B. monniera* and *V. wallichii* were 0.40 g and 0.30, g respectively from 2 g of the plant material.

Isolation of chlorophyll pigments and preparation of their salt— Fresh leaves of spinach (2g) were grounded with 95% acetone in a pestle and mortar repeatedly by changing the acetone extract with fresh acetone each time until the residue became white. The crude acetone extract was then centrifuged and the supernatant was separated. Fifteen ml of this supernatant was taken in a separating funnel to which was added equal amount of petroleum ether. It was gently mixed by simple rotating for a period of 15 min. Then 15 ml water was added and the mixing was continued. The separating funnel was then placed in a stand until two distinct layers were formed. The lower layer was discarded and the upper layer was given several steps of washings with distilled water until water layer became clear. Remaining petroleum ether solution was taken in a different separating funnel to which was added 10 ml of 10% solution of KOH and 20 ml water, which was mixed with vigorous shaking. The *p*H was brought to neutral. The water layer was separated and evaporated to dryness. The end product was weighed and reconstituted in distilled water. The yield of potassium salt of chlorophyll was found to be 90 mg. The end product was qualitatively tested for purity by spectroscopic techniques[16].

Global cerebral ischaemia and reperfusion— Mice were anaesthetized with chloral hydrate (400 mg/kg, ip). A midline ventral incision was made in the throat. Right and left common carotid arteries were located and freed from surrounding tissue and vagus nerve. A cotton thread was passed below each of the carotid artery. Global cerebral ischaemia was induced by pulling the ends of thread with constant weight. After 5 min of cerebral ischaemia, weight on the thread was removed to allow the reflow of blood through carotid arteries. The incision was sutured back in layers[17]. After completion of surgical procedure, the animals were shifted individually to their home cage and were allowed to recover until the 24 hr of reperfusion period was over. Body temperature of mice was maintained at 37^{o}C by heated surgical platform from the onset of anesthesia until the recovery from surgery. All the surgical instruments used in the surgical procedure were sterilized. The behavioral tests were performed in a semi-sound proof laboratory 12 and 24 hr after ischaemia.

Assessment of cerebral infarct size— At the end of 24 hr reperfusion after the global cerebral ischaemia, animals were sacrificed by spinal dislocation and the brain was removed. Brain was sliced into uniform sections of about 2-3 mm thicknesses. The slices were incubated in 1% triphenyltetrazolium chloride (TTC) at 37^{o}C in

0.2 M tris buffer (pH 7.4) for 20 min[18]. TTC stained the viable cells deep red leaving the infarcted cells unstained dull yellow. A transparent plastic grid with 100 squares in 1 cm^2 was placed over it. Average area of each brain slice was calculated by counting the number of square on either side. Similarly, number of squares falling over non-stained dull yellow area was also counted. Infarcted area was expressed as a percentage of total brain volume. Investigator estimating the infarct size was not aware about the type of treatment received by the animal.

Short term memory evaluation using elevated plus maze—Plus maze consisted of two open (16×5 cm) and two enclosed (16×5×12 cm) arms, connected by a central platform (5×5 cm). The apparatus was elevated to a height of 25 cm above the floor. A fine line was drawn in the middle of the floor of each enclosed arm. All the animals were given a single trial on plus maze. Each mouse was individually placed at the end of open arm facing away from central platform of the maze. The time taken by the mouse to enter from open arm with all the four legs into the enclosed arm was taken as transfer latency time (TLT). In case the animal did not enter the enclosed arm within 90 sec, it was gently pushed into the enclosed arm and TLT of 90 sec was assigned to it. The animal was allowed to explore the maze for an additional 10 sec after the measurement of TLT[19]. The animal was put to elevated plus maze test for three consecutive days. TLT recorded on the third day served as an index of short-term memory. Animal was then subjected to cerebral ischaemia for 5 min followed by reperfusion for 24 hr and was again put to elevated plus maze test.

Inclined beam-walking test— Inclined beam-walking test was employed to evaluate fore and hind limb motor coordination[20]. Each animal was individually placed on a metallic bar 55 cm long and 1.5 cm wide, inclined at an angle of 60^o from ground. The motor performance of mouse was on a scale ranging from 0 to 4. A grade of 0 was assigned to animal that could readily traverse the beam, grade 1 was given to animal demonstrating mild impairment, grade 2 was assigned to animal demonstrating moderate impairment, grade 3 was given to animal demonstrating severe impairment and grade 4 was assigned to animal completely unable to walk on the beam. Inclined beam-walking test was performed before global cerebral ischaemia and 12 and 24 hr after cerebral ischaemia.

Lateral push test— Lateral push test was employed to assess the postural deficit[21]. Animal was placed on a rough surface for firm grip and evaluated for resistance to lateral push from either side of shoulder. The test was performed before global cerebral ischaemia and 12 and 24 hr after global cerebral ischaemia and reperfusion. Mice with increased or decreased resistance to lateral push after global ischaemia were assigned + or – score, respectively.

Experimental protocol— A total of six groups of eight animals each were employed in the present study. Sham Group (Group I)**:** mice were subjected to surgical procedure and a thread was passed below both carotid arteries but the arteries were not occluded. After 5 min, thread was removed and the animal was sutured back and allowed to recover for 24 hr. Control ischaemia/reperfusion (I/R) Group (Group II): mice were subjected to 5 min global cerebral ischaemia followed by reperfusion for 24 hr. Chlorophyll salt treated I/R Group: Group III comprised of mice injected chlorophyll salt 400 mg/kg, ip 60 min before subjecting the mice to global cerebral ischaemia. *B. monniera* (Bm) extract treated I/R Groups: Group IV and group V comprised of mice injected *B. monniera* (Bm) extract 200 mg/kg, ip and 400 mg/kg, ip respectively, 60 min before subjecting the mice to global cerebral ischaemia. *V. wallichii* extract treated I/R Group: group VI consisted of mice injected *V. wallichii* extract 400 mg/kg, ip, 60 min before subjecting the mice to global cerebral ischaemia. Pilot experiments using infarct size as the end point were performed to choose the dose(s) of chlorophyll salt and extracts of *B. monniera* and *V. wallichii* employed for the study. Elevated plus maze test was performed 24 hr after induction of cerebral ischaemia. Inclined beam walk test and lateral push test was performed 12 and 24 hr after induction of cerebral ischaemia.

Statistical analysis— Statistical analysis for infarct size and TLT was done using one-way ANOVA followed by Dunett's test and Tukey's multiple range test as post-hoc analysis. Statistical significance for lateral push and beam walking were calculated using Chi square and Wilcoxon Rank sum test, respectively. A value of $P<0.05$ were considered to be statistically significant.

Results

Effect of chlorophyll salt and aqueous extracts of B. monniera and *V. wallichii* on *ischaemia and reperfusion-induced cerebral infarct size*— Global cerebral ischaemia followed by reperfusion produced significant increase in cerebral infarction measured by volume method (34.2%; $P<0.05$) when compared to the sham group (5.9%). Administration of *B. monniera* extract (200 mg/kg, ip and 400 mg/kg, ip) before ischaemia, significantly ($P<0.05$ for both) reduced ischaemia and reperfusion-induced increase in cerebral infarct size by volume method to 9.4 and 7.4%, respectively. Administration of *V. wallichii* extract and chlorophyll salt at the dose of 400 mg/kg, ip prior to ischaemia also significantly attenuated ($P<0.05$ for both) ischaemia and reperfusion-induced cerebral infarct size by volume method to 14.9% and 15.1%, respectively (Fig. 1).

Effect of chlorophyll salt and aqueous extracts of B. monniera and V. wallichii on ischaemia and reperfusion-induced Impairment of short-term memory and motor performance— Global cerebral ischaemia followed by reperfusion produced significant increase ($P<0.05$) in percentage TLT (103.1%) as compared to sham group (6.0 %). Administration of *B. monniera* extract (200 mg/kg, ip and 400 mg/kg, ip) significantly ($P<0.05$ for both) reduced ischaemia and reperfusion-induced increase in percentage TLT to 15.7 and -38.8%, respectively. Administration of *V. wallichii* extract and chlorophyll salt prior to ischaemia also significantly attenuated ($P<0.05$ for both) ischaemia and reperfusion-induced increase in percentage TLT to 75.9% and 35.0%, respectively (Fig. 2).

Global cerebral ischaemia followed by reperfusion produced significant increase ($P<0.05$ for both time points) in motor incoordination in mice noted after 12 hr (1.8 mean score) and 24 hr (2.2 mean score) of reperfusion as compared to the mean of sham group (0.2 mean score each), a value which was deleted from every other group's mean value. Administration of *B. monniera* extract at doses of 200 mg/kg, ip (0.4 and 0.6 mean score) and 400 mg/kg, ip (0.2 and 0.1 mean score) and chlorophyll salt at dose of 400 mg/kg, ip (0.7 and 0.7 mean score) markedly prevented ($P<0.05$ for each comparison) ischaemia-reperfusion induced increase in motor incoordination after 12 and 24 hr of reperfusion. However, administration of *V. wallichii* extract before ischaemia slightly attenuated ischaemia and reperfusion-induced motor incoordination (mean score) but the results were not statistically significant (Fig. 3).

All animals in sham group demonstrated resistance to lateral push. Seven out of eight mice subjected to cerebral ischaemia followed by reperfusion in control

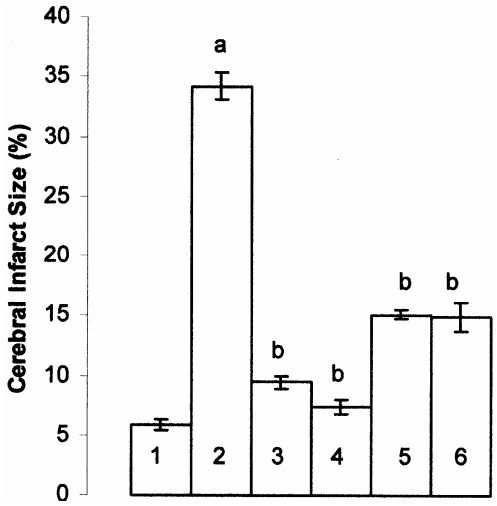

Fig. 1— Effect of chlorophyll and extracts of *B. monniera* and *V. wallichii* on ischaemia and 24 hrs of reperfusion induced cerebral infarct size in mice. [1=sham, 2=control, 3=*B. monniera* (200 mg/kg), 4= *B. monniera* (400 mg/kg), 5= chlorophyll (400 mg/kg), 6= *V. wallichii* (400 mg/kg). Values are percent infarct expressed as mean ± SE. Statistical analysis for infarct size was done using one-way ANOVA, followed by Dunett's test and Tukey's multiple range test as post-hoc analysis. a=p<0.05 vs. sham; b=*P* <0.05 vs. control.]

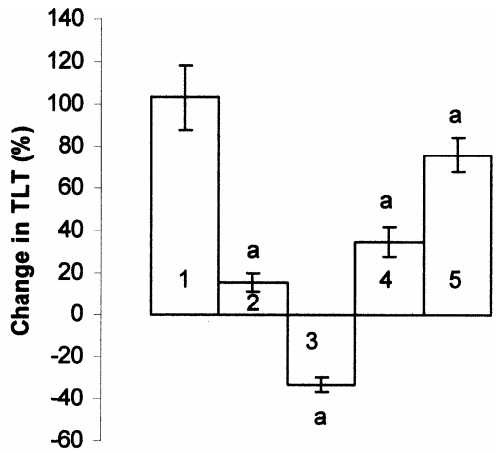

Fig. 2— Effect of chlorophyll and extracts of *B. monniera* and *V. wallichii* on ischaemia and 24 hrs of reperfusion induced impairment of short term memory in mice. [1=control, 2=*B. monniera* (200 mg/kg), 3= *B. monniera* (400 mg/kg), 4= chlorophyll (400 mg/kg), 5= *V. wallichii* (400 mg/kg). Values are percentage of transfer latency time expressed as mean ± SE. Statistical analysis for TLT was done using one-way ANOVA followed by Dunett's test and Tukey's multiple range test as post-hoc analysis. a=*P*<0.05 vs. control.]

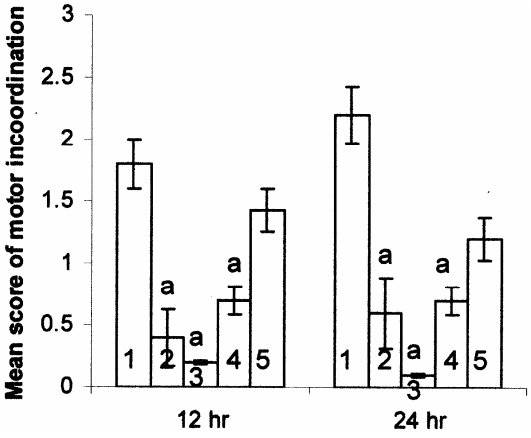

Fig. 3— Effect of chlorophyll and extracts of *B. monniera* and *V. wallichii* on ischaemia and reperfusion induced impairment of motor coordination noted after 12 and 24 hr of reperfusion in mice. [1=control, 2=*B. monniera* (200 mg/kg), 3= *B. monniera* (400 mg/kg), 4= chlorophyll (400 mg/kg), 5= *V. wallichii* (400 mg/kg). Values are graded scores of motor incoordination expressed as mean ± SE. Wilcoxon Rank sum test was used to test the statistical significance of difference between various groups. a=$P<0.05$ vs. control.]

group demonstrated loss of the resistance to lateral push response. However, prior administration of *B. monniera* extract to mice subjected to cerebral ischaemia followed by reperfusion, in groups IV and V, at dose levels of 200 mg/kg, ip and 400 mg/kg, ip attenuated their ischaemia/ reperfusion induced loss of the ability to resist to the lateral push stimuli as observed 24 hr after cerebral ischaemia (i.e. 5 out of 8 and 6 out of 8 mice showed resistance to lateral push for the two dose groups, respectively). Moreover, prior administration of chlorophyll salt and *V. wallichii* extract to mice subjected to cerebral ischaemia followed by reperfusion, in group III and group VI, attenuated their ischaemia/ reperfusion induced loss of the ability to resist to the lateral push stimuli as observed 24 hr after cerebral ischaemia (i.e. 6 out of 8 and 5 out of 8 mice showed resistance to lateral push for the treatment groups, respectively).

Discussion

Global cerebral ischaemia and reperfusion model employed in the present study is reported to simulate the clinical situation of cerebral ischaemia[22]. Prolonged global cerebral ischaemia results in neuronal death irrespective of post ischaemic reperfusion[23]. Cerebral ischaemia has been reported to impair short-term memory because hippocampal neurons are susceptible to the deleterious effects of ischaemia and reperfusion[24] and hippocampus is involved in regulation of short-term memory. Cerebral ischaemia is further documented to impair sensorimotor ability as well[25]. Therefore, in the present investigation, elevated plus-maze test was employed to assess short-term memory and inclined beam walking test and resistance to lateral push test for evaluation of sensorimotor ability. In the present study, global cerebral ischaemia reperfusion produced a significant rise in infarct size and induced impairment of short term memory as well as of motor coordination. These findings are in line with earlier report[26]. Further, ischaemia-reperfusion induced cerebral injury and impairment of the behavioural manifestations are prevented by administration of chlorophyll salt. Chlorophyll salt has been documented to possess reactive oxygen species scavenging property[11]. Besides, chlorophyllin, a water-soluble derivative of chlorophyll, has also shown to be an effective antioxidant against membrane damage *in vitro* and *ex vivo*[10]. A burst of free radical formation during cerebral ischaemia-reperfusion injury causes the ischaemic neuronal injury[3,4]. Thus, it may be put forth that the antioxidant activity of chlorophyll may be mediating its observed ameliorative effect on the ischaemic brain. Administration of the aqueous extract of *Bacopa monniera* prior to global cerebral ischaemia attenuated ischaemia and reperfusion induced cerebral infarct size and related behavioural deficit in terms of the impairment of short term memory, motor in coordination and decrease in resistance to lateral push. Some recent studies have indicated an antioxidant effect of bacosides (triterpenoid saponin isolated from *Bacopa monniera*) against chronic toxin induced oxidative damage in rat brain[27]. Besides, *B. monniera* extract has also been demonstrated to have antioxidant effect in the rat frontal cortex, striatum and hippocampus[9, 28]. Thus, the neuroprotective effect of *B. monniera* extract salt may be ascribed to having its reactive oxygen species scavenging property. Moreover, treatment with aqueous extract of *Valeriana wallichii* was also seen to inhibit neuronal injury elicited by ischaemic insult to the brain but only in terms of infarct size and percentage change in TLT. These observations suggest a mild neuroprotective effect of the extract of *V. wallichii.* Further investigations are however, required to confirm and elucidate this observed effect. However, flavonoids viz. 6-methylapigenin and hesperidin have been shown to be a constituent of *V. wallichii,* which are reported to exert antioxidant effects[15], thus facilitating its possible neuroprotective effect.

In conclusion, chlorophyll salt and the aqueous extracts of *Bacopa monniera* and *Valeriana wallichii*

exert a neuroprotective effect, which may be due to their antioxidant activity. Thus, the plant extracts merit further investigation for identification of their active constituents involved in the observed neuroprotective effect. Although the study indicates the neuroprotective effect of chlorophyll and aqueous extracts of *B. monniera* and *V. wallichii*, the extrapolation of the results to clinical conditions still awaits elaborate experimental validation of the qualitative and quantitative aspects of the potency of the test extracts. Besides, general screening of pharmacological activity of admixtures of plant extracts and isolates may benefit from taking into consideration the fact that the extraction procedures may inadvertently elute chlorophyll, which may be responsible for at least a fraction of their observed potency of the extract.

References

1 Mori T, Asano T, Matsui T, Moramatsu H, Ueda M & Kamiya T, Intraluminal increase of superoxide anion follwing transient focal cerebral ischemia in rats, *Brain Res*, 816 (1999) 350.

2 Ste-Marie L, Vachon P, Vachon L, Bemear C, Guertin M C & Montgomery J, Hydroxyl radical production in the cortex and striatum in a rat model of focal cerebral ischaemia, *Can J Neurol Sci*, 27 (2000) 152.

3 Dirnagl U, Lindauer I, Tehm A, Schreiber S, Pfister H W & Kedel U G, Global cerebral ischemia in the rats, online monitoring of oxygen free radical production using chemiluminescence *in vivo*, *J Cereb Blood Flow Met*, 15 (1995) 929.

4 Kumura E, Yoshimine T, Iwatsuki K I, Yamanaka K, Tanaka S & Hayakawa, T, Generation of nitric oxide superoxide during reperfusion after focal cerebral ischemia in rats, *Am J Physiol*, 270 (1996) 748.

5 Marciniak G & Petty M A, Design and biological evaluation of new antioxidants for use in cerebrovasular disorders, *Drugs Future*, 21 (1996) 1037.

6 Juurlink B H J & Sweeney M I, Mechanisms that result in damage during and flowing cerebral ischaemia, *Neurosci Biobehav Rev*, 21 (1997) 21.

7 Baker K, Marcus C B, Huffman K, Krunk H, Malfory B & Doctrow S R, Synthetic combined superoxide dismutase/catalase mimetics are protective as a delayed treatment in a rat stroke model : a key role for reactive oxygen species in ischemic brain injury, *J Pharmacol Exp Ther*, 284 (1998) 215.

8 Kuroda S, Tsuchidate R, Smith M L, Maples B K & Siesjo B K, Neuroprotective effects of noval nitrone, NXY-059, after transient focal cerebral ischemia in the rat, *J Cereb Blood Flow Met*, 19 (1999) 778.

9 Bhattacharya S K, Bhattacharya A, Kumar A & Ghosal S, Antioxidant activity of *Bacopa monniera* in rat frontal cortex, striatum and hippocampus, *Phytotherapy Res*, 14 (2000) 174.

10 Kamat J P, Boloor K K & Devasagayam T P, Chlorophyll as an effective antioxidant against membrane damage *in vitro* and ex vivo, *Biochim Biophys Acta*, 1487 (2000) 113.

11 Kumar S S, Devasagayam T P, Bhushan B, Verma N C, Scavenging of reactive oxygen species by chlorophyll: an ESR study. *Free Rad Res*, 35 (2001) 563.

12 Chopra R N, Nayar S L & Chopra I C, *Glossary of Indian medicinal plants* (Council of Scientific and Industrial Research), New Delhi, 1956, 32.

13 Dar A & Channa S, Calcium antagonistic activity of *Bacopa monniera* on vascular and intestinal smooth muscles of rabbit and guinea-pig, *J Ethnopharmacol*, 66 (1999) 167.

14 Houghton P J, The biological activity of valerian and related plants, *J Ethnopharmacol*, 22 (1988) 122.

15 Marder M, Viola H, Wasowski C, Fernandez S, Medina J H & Paladini A C, 6-methylapigenin and hesperidin: new valeriana flavonoids with activity on the CNS, *Pharmacol Biochem Behav*, 75(3) (2003) 537.

16 Witham F H, Blade D F, Devlin R M, *Experiments in plant physiology* (Van Nostrand Reinhold Company, New York) 1971, 55.

17 Himori N, Wantanabe H, Akaike N, Kurasawa M, ltoh J & Tanaka Y, Cerebral ischemia model with conscious mice. Involvement of NMDA receptor activation and derangement of learning and memory ability, *J Pharmacol Methods*, 23 (1990) 311.

18 Bochelen D, Rudin M & Sauter A, Calcineurin inhibitors FK506 and SDZASM 981 alleviate the outcome of focal cerebral ischemic/reperfusion injury, *J Pharmacol Exp Therap*, 288 (1999) 653.

19 Itoh J, Nabeshima T & Kameyama T, Utility of an elevated plus maze for the evaluation of memory in mice. Effects of nootropics, scopolamine and electroconvulsive shock, *Psychopharmacol*, 101 (1990) 27.

20 Feeney D M, Boyeson M G, Linn R T, Murray H M & Dail W G, Responses to cortical injury: I. Methodology and local effects of contusions in the rat, *Brain Res*, 211 (1981) 67.

21 Bederson J B, Pitts L H, Tsuji M, Nishimur M C, Davis R L & Bartowski H, Rat middle cerebral artery occlusion : evaluation of the model and development of a neurologic examination, *Stroke*, 17 (1986) 472.

22 Alonsode de Lecinana M, Diez-Tejedor E, Cearcellar F & Roda J M, Cerebral ischemia : from animal studies to clinical practice. Should the methods be reviewed? *Cerebrovasc Dis*, 11 (1) (2001) 20.

23 Neumar R W, Molecular mechanism of ischaemic neuronal injury, *Annals Emergency Med*, 36 (2000) 483.

24 Jenkins L W, Povlishock J T, Lewelt W, Miller J D & Becker D P, The role of postischemic recirculation in the development of ischemic neuronal injury following complete cerebral ischemia, *Acta Neuropathol*, 5 (1981) 205.

25 Dobkin B H, The rehabilitation of elderly stroke patients, *Clin Geriat Med*, 7 (1991) 507.

26 Gupta R, Singh M & Sharma A, Neuroprotective effect of antioxidants on ischaemia/reperfusion-induced cerebral injury, *Pharmacol Res*, 48(2) (2003) 209.

27 Anbarasi K, Vani G, Balakrishna K & Devi C S, Effect of bacoside A on brain antioxidant status in cigarette smoke exposed rats, *Life Sci*, 78 (2006) 1378.

28 Russo A, Izzo A A, Borrelli F, Renis M & Vanella A, Free radical scavenging capacity and protective effect of *Bacopa monniera* L. on DNA damage, *Phytother Res*, 17 (2003) 870.