Clinical and Experimental Pharmacology and Physiology (2003) **30**, 399–404

EVALUATION OF *WITHANIA SOMNIFERA* IN A MIDDLE CEREBRAL ARTERY OCCLUSION MODEL OF STROKE IN RATS

Geeta Chaudhary,* Uma Sharma,† Naranamangalam R Jagannathan† and Yogendra K Gupta*

*Departments of *Pharmacology and †NMR, All India Institute of Medical Sciences, New Delhi, India*

SUMMARY

1. Stroke causes brain injury in millions of people worldwide each year. Despite the enormity of the problem, there is currently no approved therapy that can reduce infarct size or neurological disability. One of the approaches that can be used in limiting the neurological damage after stroke is the use of prophylactic treatment in patients with a high-risk of stroke. The present study was undertaken to investigate the effect of the Indian herbal plant *Withania somnifera* as a prophylactic treatment in the middle cerebral artery (MCA) occlusion model of stroke in rats.

2. Two groups of male Wistar rats were pretreated with a hydroalcoholic extract of *W. somnifera* (1 g/kg, p.o.) for 15 and 30 days. Thereafter, rats were subjected to focal ischaemia by occlusion of the MCA using an intraluminal thread. After 2 h MCA occlusion, reperfusion was allowed by retracting the thread. Animals were assessed for ischaemic changes using diffusion-weighted imaging 30 min after reperfusion. Twenty-four hours later, rats were subjected to motor performance tests and were subsequently killed for the estimation of the marker of oxidative stress malondialdehyde (MDA). The control group received vehicle and a similar protocol was followed.

3. Significant motor impairment, with elevated levels of MDA, was observed in vehicle-treated MCA-occluded rats. In addition, diffusion-weighted imaging showed increased signal intensity in the right hemisphere compared with the contralateral hemisphere. Treatment with *W. somnifera* for 15 days did not improve motor performance or decrease the elevated levels of MDA. However, when the pretreatment time of *W. somnifera* was increased to 30 days, it prevented motor impairment and significantly decreased the raised levels of MDA compared with vehicle-treated rats. In the *W. somnifera* (30 days)-pretreated group, the percentage hemispheric lesion area in diffusion-weighted imaging was significantly attenuated (17 ± 2%) compared with the vehicle-treated MCA-occluded group (30 ± 4%).

4. Because *W. somnifera* has been documented to have anti-oxidant properties, the protection afforded by *W. somnifera* could be due to its anti-oxidant effect. The present study provides first evidence of the effectiveness of an Indian herb in focal ischaemia.

Key words: cerebral ischaemia, Indian herb, rats, *Withania somnifera*.

INTRODUCTION

Ischaemic stroke is a leading cause of death and long-term disability worldwide, yet no satisfactory treatment is available. The only drug that is used clinically is recombinant plasminogen activator and only in a few selected patients.[1] In contrast, neuroprotective agents, despite proving effective in animal models of stroke, have largely failed to fulfil their promise in clinical trials and, as such, currently there is no neuroprotective drug that is being used clinically.[2] This has led to an upsurge in experimental studies to evaluate combination therapy and prophylactic treatment in stroke. Combining neuroprotective drugs with thrombolytics has demonstrated encouraging results in animal models but, as yet, there are no results available from clinical trials.[1] We have demonstrated in experimental model of stroke that the combination of melatonin and meloxicam shows better effects compared with the two drugs used alone.[3]

Moreover, it has been seen that in animal models of stroke, in the case of many drugs, pretreatment yields better outcomes than postonset treatment. Further suggestions from clinical trials have shown that very early treatment after stroke may be necessary for the drugs to be effective.[4] Therefore, in a subgroup of patients who are at substantial risk of ischaemic stroke (e.g. patients with a mild episode of stroke, transient ischaemic attacks), prophylactic neuroprotection may offer a useful treatment approach. The agent to be used prophylactically should be efficacious, safe, orally administered and affordable.[5]

Herbal drugs have been described in the ancient systems of medicine for the treatment of various ailments. These are currently be re-evaluated by extensive research on different plant species and their therapeutic principles. Because herbal drugs have a relatively higher therapeutic window, fewer side-effects and are economical, they have gained lot of acceptance in recent years and are potential candidates for the prophylactic treatment of stroke.

Withania somnifera, referred to as ‘Aswagandha’ in the Indian system of medicine, is a central nervous system (CNS)-active herb that has been used for various neurological disorders. It has not,

Correspondence: Professor YK Gupta, Neuropharmacology Laboratory, Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, 110029, India. Email: ykg@hotmail.com

Received 26 August 2002; revision 21 January 2003; accepted 26 January 2003.

however, been evaluated for its potential in stroke. Studies with *W. somnifera* have indicated that it exerts an anti-ageing effect and has significant anxiolytic and antidepressant activity.[6] We have demonstrated the protective effect of *W. somnifera* against cancer and cancer chemotherapy induced neutropenia.[7,8] The other pharmacological actions exerted by *W. somnifera* include anti-inflammatory, antistress, haemopoeitic immunomodulatory and anti-oxidant effects.[9–11] The aim of the present study was to investigate the effect of chronic treatment with *W. somnifera* in an acute ischaemic model of stroke in rats and to ascertain whether it can be of prophylactic value in high-risk stroke patients.

METHODS

Animals

Adult male Wistar rats, 300–350 g, were used in the present study. The animals were procured from the Central Animal House facility at the All India Institute of Medical Sciences, New Delhi. Rats were group housed in polypropylene cages (38 × 23 × 10 cm) with not more than five animals per cage and were maintained under standard laboratory conditions with natural dark and light cycles. Rats were allowed free access to standard dry rat diet (Golden Feeds, Delhi, India) and tap water. All procedures described were reviewed and approved by the Institutional Animal Ethics Committee.

Drug schedule

Rats were divided into four groups: (i) sham; (ii) vehicle-treated middle cerebral artery (MCA)-occluded rats; (iii) *W. somnifera* 15 days pre-treatment group; and (iv) *W. somnifera* 30 days pretreatment group. Each group consisted of 12 animals.

A standardized hydroalcoholic extract of *W. somnifera* was obtained from Dabur Research Foundation, Ghaziabad, India. The extract was administered at a dose of 1 g/kg, p.o., between 10.00 and 11.00 h every day for 15 or 30 days. The weight of the rats was recorded every day before drug administration. On 16th and 31st days in the two groups given *W. somnifera*, focal cerebral ischaemia was induced by occlusion of the MCA. The vehicle-treated group received distilled water for 30 days and was subjected to MCA occlusion. In the sham-operated group, rats were operated on and the filament occluding the MCA was pulled within 60 s.

Middle cerebral artery occlusion procedure to induce focal cerebral ischaemia

Rats were anaesthetized with chloral hydrate (dissolved in distilled water) at a dose of 400 mg/kg, i.p. Briefly, the right common carotid artery and right external carotid artery were exposed through a ventral midline neck incision and were ligated proximally and permanently under an operative magnifying glass. A 4.0 monofilament nylon thread (Ethicon; Johnson & Johnson, Aurangabad, India), with its tip rounded by heating quickly near a flame, was advanced from the external carotid artery into the lumen of the internal carotid artery until the resistance was felt approximately 17 mm, which ensures that the suture entered the anterior cerebral artery, thus occluding the origins of the anterior cerebral artery, the MCA and the posterior communicating artery. The nylon filament was allowed to remain in place for 2 h. After 2 h, the filament was retracted so as to allow reperfusion of the ischaemic region.[12]

Measurement of physiological parameters

Rectal (core) temperature was recorded using a rectal thermometer and maintained at 37 ± 1°C throughout the surgical procedure and upto 2 h after reperfusion a using thermostatically regulated heating lamp. For the monitoring of arterial blood pressure, the left femoral artery was exposed and polyethylene tubing was inserted for continuous monitoring of blood pressure using a Coulbourn polygraph (Coulbourn Instruments, Allentown, PA, USA).

Motor performance tests

Motor activity of rats was assessed 24 h after MCA occlusion using the grip test, rota rod, foot fault test and closed field activity test.

Grip test

The grip test was performed according to the methods of Moran *et al.*[13] An apparatus with a 50 cm string pulled taut between two vertical supports and elevated 40 cm from a flat surface was used. The rat was placed on the string at a point midway between the supports and evaluated according to the following scale: 0, falls off; 1, hangs onto the string with two forepaws; 2, hangs onto the string with two forepaws but also attempts to climb onto the string; 3, hangs onto string by two forepaws plus one or both hind paws; 4, hangs onto string by all paws plus the tail wrapped around the string; 5, escapes.

Rota rod

The rota rod was used to evaluate the muscle coordination of rats in both vehicle- and *W. somnifera*-treated MCA occluded rats. On the day of and before MCA occlusion, rats were conditioned to the accelerating rota rod (Ugo Basile, Comerio, Italy). Each animal received a training session on the rota rod set at a constant speed of 8 r.p.m. and was tested until the criterion of remaining on the rotating spindle for 60 s was achieved. Each rat then received a single baseline trial on the accelerating rota rod in which the spindle increased in speed from 4 to 40 r.p.m. over a period of 5 min. At 24 h postocclusion, each rat again received a test trial.[14]

Foot fault test

Rats were placed on an elevated grid floor with a mesh size of 30 mm for 1 min to acclimatize. After acclimatization, rats were placed on the grid for 1 min and the total number of paired steps, as well as the total number of foot fault errors (i.e limb misplaced and fell through the grid), was counted.[14] A similar procedure was followed after 24 h in sham-operated rats, vehicle-treated MCA occluded rats and in drug-treated MCA occluded rats. The percentage error was determined as follows:

$$\% \text{ Foot fault error} = (\text{no. foot fault steps/no. paried steps}) \times 100$$

Magnetic resonance imaging

Magnetic resonance imaging (MRI) studies were performed using an animal MRI scanner (Bruker BIOSPEC; Bruker, Fallanden, Switzerland) 30 min after reperfusion in both vehicle- and drug-treated MCA-occluded rats. Experiments were performed at 4.7 T, using a 69 mm circularly polarized birdcage volume resonator. The ischaemic region was identified by acquiring multislice T_2-weighted pilot images using rapid acquisition with a rapid enhancement sequence (TR = 2000 msec, TE = 25 msec, slice thickness = 2 mm and number of slices = 7). The 2 mm slice thickness was used because decreasing the slice thickness would have decreased the signal to noise intensity ratio and, in order to increase it, the number of averages would have to be increased, thereby increasing the acquisition time. After identification of the area of interest, diffusion-weighted images were acquired using a stimulated echo diffusion-weighted pulse sequence. Diffusion-weighted images were acquired with the following acquisition parameters: TR = 2000 msec, TE = 40 msec, TM = 30 msec, gradient duration = 10 msec and gradient strength = 25, 50 and 75 mT/m. Contrast of images of diffusion-weighted images were adjusted using the grey scale. Images that were acquired at 25 mT/M showed best contrast and were used for image analysis. The signal intensity of the ischaemic and contralateral non-ischaemic hemispheres was determined using the standard image analysis program provided by the manufacturer (Bruker BIOSPEC).

The area of ischaemic tissue damage was calculated by dividing the area of the hyperintense region with the area of the ipsilateral hemisphere and was expressed as percentage hemispheric lesion area (% HLA).[15,16]

Estimation of oxidative stress markers

For assessing the level of oxidative stress, malondialdehyde (MDA) was estimated after 24 h of MCA occlusion. Rats were decapitated under ether anaesthesia and the brains quickly removed, cleaned by rinsing with chilled saline and stored for 2 days at −70°C until biochemical analysis.

Measurement of lipid peroxidation

Malondialdehyde (an indicator of lipid peroxidation) was estimated as described by Okhawa *et al.*[17] Briefly, brain tissues were homogenized with 10 times (w/v) 0.1 sodium phosphate buffer (pH 7.4). Acetic acid (1.5 mL of 20%, pH 3.5) thiobarbituric acid (1.5 mL of 0.8%) and sodium dodecyl sulphate (0.2 mL of 8.1%) were added to 0.1 mL processed tissue sample. The mixture was then heated at 100°C for 60 min. The mixture was cooled with tap water and 5 mL *n*-butanol : pyridine (15 : 1% v/v) and 1 mL distilled water was added. The mixture was shaken vigorously. After centrifugation at 2000 *g* for 10 min, the organic layer was withdrawn and absorbance was measured at 532 nm using a spectrophotometer.

Statistical analysis

Data are presented as the mean±SEM. ANOVA with Bonferroni test was used for statistical analysis.

Student's *t*-test was used for comparison of signal intensity in the diffusion-weighted images. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of *W. somnifera* treatment on bodyweight

The mean weight range in the vehicle- and *W. somnifera*-treated groups before the start of the experiments was not significantly different. The bodyweight of rats in both groups was noted each day. The mean weight gain after 15 and 30 days pretreatment in the *Withania somnifera*-treated groups was not significantly different compared with the vehicle-treated MCA-occluded rats.

After MCA occlusion, there was a significant fall in bodyweight in the sham-operated, vehicle-treated MCA-occluded and *W. somnifera*-pretreated rats compared with the insignificant change seen in normal rats within 24 h.

Effect of *W. somnifera* on grip test

The effect of *W. somnifera* was assessed on the basis of neurological scores in the grip test. The neurological score significantly decreased 24 h after MCA occlusion in vehicle-treated rats compared with sham-operated rats (1.2 ± 0.2 and 3.6 ± 0.2, respectively). In the *W. somnifera* (15 days)-pretreated group, the neurological deficit was still observed (1.8 ± 0.4) and although values were greater than in the vehicle-treated rats, statistical significance was not reached. However, when *W. somnifera* was administered for 30 days, the neurological deficit was prevented and scores were significantly different than those for the vehicle-treated MCA-occluded rats (2.8 ± 0.24; $P < 0.05$; Table 1).

Effect of *W. somnifera* on rota rod

The mean value of time spent on the spindle of a rota rod by sham-operated rats 24 h after surgery was 184 ± 10 s. In the vehicle-treated MCA-occluded rats, motor performance was significantly less (80 ± 8 s; $P < 0.05$) compared with sham-operated rats. In the *W. somnifera* (15 days)-pretreated MCA-occluded rats, there was no significant improvement in the time spent on the rotating spindle (128 ± 12 s). On increasing the period of pretreatment of *W. somnifera* to 30 days, a significant improvement was observed in the time spent on the rotating spindle (153 ± 10 s; $P < 0.05$; Table 1).

Effect of *W. somnifera* on the percentage of foot fault errors

The foot fault error test was used to evaluate the muscular integrity of rats. In sham-operated rats, the mean foot fault error was $12 \pm 1\%$, which was significantly increased in vehicle-treated MCA-occluded rats ($69 \pm 8\%$). In the *W. somnifera* (30 days)-pretreated group, there was a significant decrease in foot fault errors ($30 \pm 9\%$; $P < 0.05$) compared with vehicle-treated MCA-occluded rats, whereas the effect after 15 days pretreatment was not statistically significant ($46 \pm 10\%$; Table 1).

Effect of *W. somnifera* on brain MDA levels

Middle cerebral artery occlusion significantly increased the levels of MDA in the vehicle-treated group compared with sham-operated rats (690 ± 28 *vs* 220 ± 21 nmol/g wet tissue; $P < 0.05$). In the *W. somnifera* (15 days)-pretreated rats, there was no significant difference in the levels of MDA compared with sham-operated rats (545 ± 30 nmol/g wet tissue). However, in the *W. somnifera* (30 days)-pretreated group, there was a significant decrease in MDA levels compared with vehicle-treated MCA-occluded rats (360 ± 37 nmol/g wet tissue; $P < 0.05$; Table 1).

Effect of *W. somnifera* pretreatment on diffusion-weighted imaging

The ischaemic region was manifested as an increased signal intensity on diffusion-weighted imaging scans with a high b-value.

Table 1 Motor performance tests and levels of markers of oxidative stress in *Withania somnifera*-pretreated middle cerebral artery occluded rats

	Sham-operated	Vehicle-treated MCA-occluded rats	*Withania somnifera* (1 g/kg) 15 days	30 days
Grip test	2.4 ± 0.2	1.2 ± 0.2*	1.8 ± 0.4	$2.8 \pm 0.2^{\dagger}$
Rota rod performance test (s)	184 ± 10	80 ± 8*	128 ± 12	$153 \pm 10^{\dagger}$
Foot fault test (%)	12 ± 1	69 ± 8*	46 ± 10	$30 \pm 9^{\dagger}$
MDA (nmol/g wet tissue)	220 ± 21	690 ± 28*	545 ± 30	$360 \pm 37^{\dagger}$

*$P < 0.05$ compared with sham-operated rats; $^{\dagger}P < 0.05$ compared with vehicle-treated middle cerebral artery (MCA)-occluded rats.
MDA, malondialdehyde.

Focal ischaemia was seen in all vehicle-treated MCA-occluded rats. The MRI signal intensity of the ischaemic region (right hemisphere) was compared with the corresponding identical region of the brain in the contralateral hemisphere (left hemisphere). In vehicle-treated MCA-occluded rats, the signal intensity in the right hemisphere was high 195 ± 13 arbitrary units (AU) compared with the contralateral hemisphere 149 ± 21 AU for the same brain slice (Fig. 1b). In the *W. somnifera* (30 days)-pretreated group, the signal intensity was high; however, it was not significantly different compared with the contralateral side. The mean value of signal intensity in the ischaemic hemisphere was 184 ± 5 AU compared with 164 ± 7 AU for the contralateral side (Fig. 1c).

The %HLA in vehicle-treated MCA-occluded rats was 30 ± 4%. In *W. somnifera* (30 days)-treated rats, the %HLA was 17 ± 2%, which was significantly less compared with vehicle-treated MCA-occluded rats ($P < 0.05$; Table 2; Fig. 1).

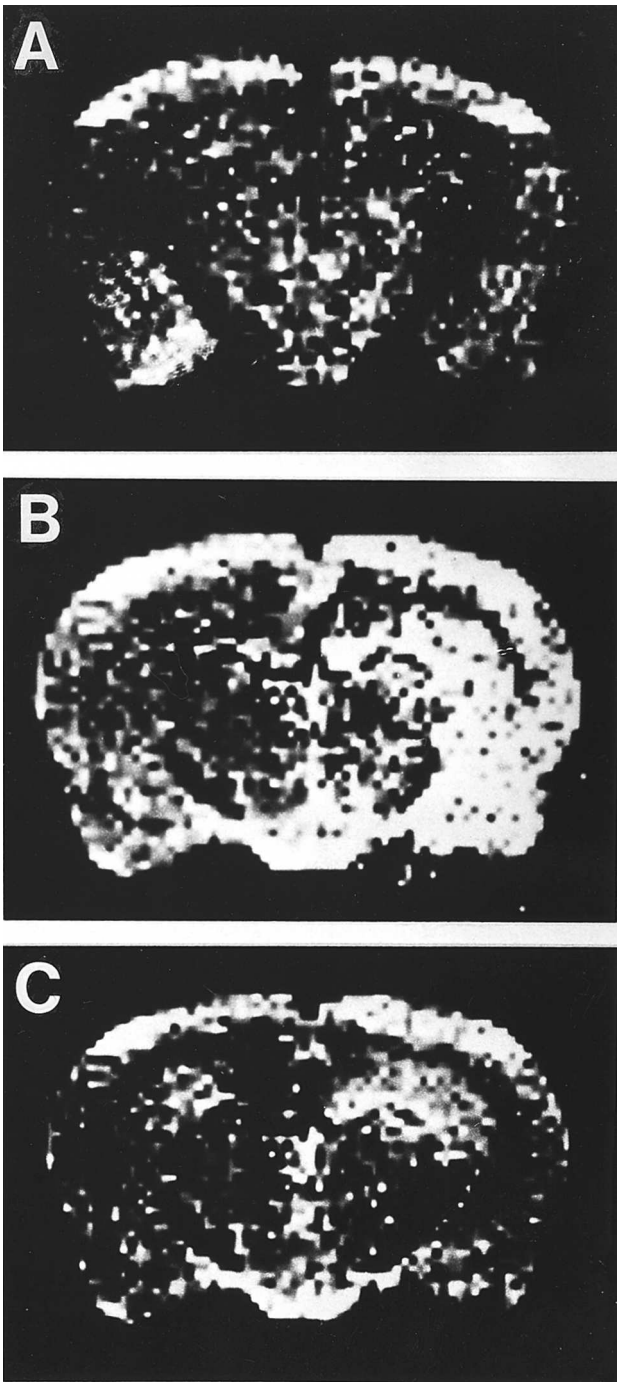

Fig. 1 Demonstrates the diffusion-weighted imaging scans. (a) Normal rat, (b) vehicle-treated middle cerebral artery (MCA)-occluded rat (30 min after reperfusion) and (c) *Withania Somnifera* pretreated MCA-occluded rat (30 min after reperfusion).

DISCUSSION

Although the ancient Indian literature, Ayruveda, describes the effectiveness of numerous plants in the treatment of neurological disorders, there has been no experimental work performed to date to evaluate the effectiveness of medicinal plants against cerebral ischaemia. However, Chinese herbal preparations, such as radix multiorhizzae, tetramethylpyrazine, tetrandrine and ginseng, have been studied extensively in cerebral ischaemia.[18–22] In human ischaemic stroke, recirculation occurs frequently after focal ischaemia, particularly in the case of cerebral embolism and transient ischaemic attacks, which is a warning signal of stroke. Moreover, recurrence is a very prevalent phenomenon seen in patients who had suffered from one episode of stroke. Therefore, the use of a prophylactic approach becomes important in such patients. Aspirin has been commonly used as for the secondary prevention of stroke due to its antiplatelet properties. Although aspirin is cheap and effective, its side-effect profile (e.g. increased risk of gastric bleeding and bleeding tendency) requires the need for assessment of an alternative strategy.

We have recently demonstrated the effectiveness of prophylactic treatment of *trans*-resveratrol, a potent anti-oxidant derived from red wine, in preventing neurological damage caused by occlusion of the MCA in rats.[23]

Herbs with potential neuroprotective activity can be appropriate candidates for prophylactic treatment because they are considered to be relatively safe, are able to be administered orally and are relatively inexpensive. *Withania somnifera* is a CNS-active Indian herbal plant evaluated for various ailments, including neurological disorders, such as Alzheimer's disease, anxiety and depression.[9] The present study was conducted to evaluate the effectiveness of *W. somnifera* against cerebral ischaemia induced by occlusion of the MCA in rats. Because recirculation occurs frequently after cerebral ischaemia, to resemble this clinical setting, ischaemia–reperfusion was induced in rats by mechanical clipping of the MCA or photothrombotic occlusion of the vessels. However, the limitation of these models is that they involve craniotomy.[24] Therefore MCA occlusion by intraluminal suture, which is a relatively non-invasive method of inducing cerebral ischaemia, was used in the present study.

Table 2 Signal intensity measured by diffusion-weighted imaging in vehicle- and *Withania somnifera*-pretreated middle cerebral artery occluded rats

	Signal intensity	
	Contralateral	Ipsilateral
Middle cerebral artery occluded rats	149 ± 21	195 ± 13*
Withania somnifera (1 g/kg, p.o., for 30 days)	164 ± 7	184 ± 5

*$P < 0.05$ compared with the contralateral hemisphere.

It was observed that 2 h MCA occlusion followed by reperfusion decreased the motor performance, as evidenced by a decrease in the rota rod performance test and spontaneous locomotor activity and an increase in the neurological score in the grip test and the percentage of foot fault errors. There was also an increase in the levels of MDA, indicating oxidative stress. The ischaemia was confirmed using diffusion-weighted imaging, which showed increased hyperintensity in the hemisphere in which the MCA was occluded. The hyperintensity seen in the MCA-occluded rats may be because there is a rapid failure of energy metabolism and associated ion pumps, leading to the accumulation of sodium and water within the cells (cytotoxic oedema), in the ischaemic lesion area. The accumulation of water in cells results in reduced water molecule motion and the region appears hyperintense.[25] In the treatment groups in the present study, rats were pretreated orally with a hydroalcoholic extract of *W. somnifera*. A hydroalcoholic extract was chosen for the present study because it has been demonstrated to contain both the contents of the aqueous and the alcoholic extracts.[26] Pretreatment of rats with *W. somnifera* (1 g/kg) for 15 days did not improve the neurological deficit of rats, nor did it decrease the elevated levels of MDA compared with vehicle-treated MCA-occluded rats.

When the pretreatment period of *W. somnifera* was increased to 30 days, a significant improvement in the motor performance test was observed. This implies that the region controlling motor coordination (caudate putamen as well as the cortex) was less affected in *W. somnifera*-treated rats compared with vehicle-treated MCA-occluded rats. This effect was also demonstrated by diffusion-weighted imaging. Diffusion-weighted imaging is a reliable and sensitive technique to detect early ischaemic changes as regions of increased signal intensity (i.e. decreased water diffusitivity) and is a sensitive tool to evaluate the therapeutic efficacy of neuroprotective agents *in vivo*.[15,27] In *W. somnifera*-pretreated rats, the signal intensity of the right cerebral hemisphere was not significantly high compared with the contralateral hemisphere. The infarct volume was also significantly decreased compared with the vehicle-treated MCA-occluded rats, indicating a beneficial effect of pretreatment of *W. somnifera.*

It has been demonstrated that, during ischaemia–reperfusion, high amounts of free radicals are formed by several pathways, which ultimately leads to cell death. Increased free radical formation, together with a reduced anti-oxidant defence, causes oxidative stress, which plays a pivotal role in the pathogenesis of stroke-associated neuronal injury.[28] The active principles of *W. somnifera* (withanolides) have been demonstrated to increase the expression of the anti-oxidant enzymes if administered chronically.[29] One study has shown that the active principles of *W. somnifera* administered for 21 days at doses of 10 and 20 mg/kg, i.p., induced a dose-related increase in the level of anti-oxidant enzymes superoxide dismutase, catalase and glutathione peroxidase the frontal cortex and striatum of rats.[30] In another study, *W. somnifera* (100 mg/kg, p.o.) prevented the lipid peroxidation induced by pyrogenic substances such as lipopolysaccharides and peptidoglycan.[31] In the present study, the beneficial effect of *W. somnifera* may be because of the attenuation of free radicals by an increased anti-oxidant defence. This was further supported biochemically because the levels of MDA, a marker of oxidative stress, were not elevated in the *Withania somnifera*-treated rats. Because ischaemia-induced neuronal damage is a complex process involving various neurotransmitters and enzymes, such as glutamate, calcium, nitric oxide and cyclo-oxygenase, inhibition of these neurotransmitters and enzymes by *W. somnifera* cannot be ruled out as mechanisms mediating its protective effects.

The present study provides, to the best of our knowledge, the first experimental evidence suggesting a potential benefit of *W. somnifera* treatment in the management of acute ischaemic stroke. The present study indicates that there could be herbs that need to be explored as potential treatments for stroke.

REFERENCES

1. Kaste M. Thrombolysis in ischemic stroke: Present and future. Role of combined therapy. *Cerebrovasc. Dis.* 2001; **11** (Suppl. 1): 55–9.
2. Martinez-Vila E, Sieira PI. Current status and perspectives of neuroprotection in ischemic stroke treatment. *Cerebrovasc. Dis.* 2001; **11** (Suppl. 1): 60–70.
3. Gupta YK, Chaudhary G, Sinha K. Enhanced protection by melatonin and meloxicam combination in middle cerebral artery occlusion model of acute ischemic stroke in rat. *Can. J. Physiol. Pharmacol.* 2002; **80**: 210–17.
4. Albers GW. Rationale for early intervention in acute stroke. *Am. J. Cardiol.* 1997; **80**: D4–10.
5. Fisher M, Jonas S, Sacco RL, Jones S. Prophylactic neuroprotection for cerebral ischemia. *Stroke* 1994; **25**: 1075–80.
6. Bhattacharya SK, Bhattacharya A, Sairam K, Ghosal S. Anxiolytic-antidepressant activity of *Withania somnifera* glycowithanolides: An experimental study. *Phytomedicine* 2000; **7**: 463–9.
7. Prakash J, Gupta SK, Kochupillai V, Singh N, Gupta YK, Joshi S. Chemopreventive activity of *Withania somnifera* in experimentally induced fibrosarcoma tumours in Swiss albino mice. *Phytother. Res.* 2001; **15**: 240–4.
8. Gupta YK, Sharma SS, Rai K, Katiyar CK. Reversal of paclitaxel induced neutropenia by *Withania somnifera* in mice. *Ind. J. Physiol. Pharmacol.* 2001; **45**: 253–7.
9. Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): A review. *Altern. Med. Rev.* 2000; **5**: 334–46.
10. Ziauddin M, Phansalker N, Patki P, Diwanay S, Patwardhan B. Studies on immunomodulatory effects of Ashwagandha. *J.Ethnopharmacol.* 1996; **50**: 69–76.
11. Bhattacharya A, Ghosal S, Bhattacharya SK. Anti-oxidant effect of *Withania somnifera* glycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. *J. Ethnopharmacol.* 2001; **74**: 1–6.
12. Koizumi J, Yoshida Y, Nazakawa T, Ooneda G. Experimental studies of ischemic brain edema: A new experimental model of cerebral embolism in rats in which recirculation can be introduced in the ischemic area. *Jpn. J. Stroke* 1986; **8**: 1–8.
13. Moran PM, Higgins LS, Cordel B, Moser PC. Age related learning deficits in transgenic mice expressing the 721-amino acid isoform of human beta-amyloid precursor protein. *Proc. Natl Acad. Sci. USA* 1995; **92**: 5341–5.
14. Rogers DC, Campbell CA, Stretton JL, Mackay KB. Correlation between motor impairment and infarct volume after permanent and transient middle artery occlusion in the rat. *Stroke* 1997; **28**: 2060–6.
15. Sinha K, Deogaonkar M, Jagannathan NR, Gupta YK. Effect of melatonin on ischemia–reperfusion injury induced by middle cerebral artery occlusion in rats. *Eur. J. Pharmacol.* 2001; **428**: 185–92.
16. Gupta YK, Sinha K, Chaudhary G, Jagannathan NR. Protective effect of adenosine against neuronal injury induced by middle cerebral artery occlusion in rats as evidenced by diffusion-weighted imaging. *Pharmacol. Biochem. Behav.* 2002; **72**: 569–74.

17. Okhawa H, Ohishim N, Yagi K. Assay of lipid peroxides in animals tissue by thiobarbituraic acid reaction. *Anal. Biochem.* 1979; **95**: 351–8.
18. Gong X, Sucher NJ. Stroke therapy in traditional Chinese medicine (TCM): Prospects for drug discovery and development. *Trends Pharmacol. Sci.* 1999; **20**: 191–6.
19. Wu W, Kuang P, Li Z. ET-1 gene expression of rat brain during ischemia and reperfusion and the protective effect of Radix Salviae Miltiorrhizae. *J. Tradit. Chin. Med.* 1997; **17**: 59–64.
20. Kuang P, Li Z, Zhang F, Tao Y, Liu J, Wu W. Protective effect of Radix Salvaie Miltiorrhizae Composita on cerebral ischemia. *J. Tradit. Chin. Med.* 1995; **15**: 135–40.
21. Ho KK, Wen HL, Lee CM. Tetrmethylpyrazine for treatment of experimentally induced stroke in Mongolian gerbils. *Stroke* 1989; **20**: 96–9.
22. Lim JH, Wen TC, Matsuda S *et al*. Protection of ischemic hippocampal neurons by ginsenoside rb1, a main ingredient of ginseng root. *Neurosci. Res.* 1997; **28**: 191–200.
23. Sinha K, Chaudhary G, Gupta YK. Protective effect of resveratrol against oxidative stress in middle cerebral artery occlusion model of stroke in rats. *Life Sci.* 2002; **28**: 655–65.
24. Belayev L, Alonso OF, Busto R, Zhaow W, Ginsberg MD. Middle cerebral artery occlusion in the rats by intraluminal suture: Neurological and pathological evaluation of an improved model. *Stroke* 1996; **27**: 1616–23.
25. Turgut T, Richard AD, Carano MS *et al*. A novel endothelin antagonist, A-127722, attenuates ischemic lesion size in rats with temporary middle cerebral artery occlusion. *Stroke* 1998; **29**: 850–8.
26. Yesilada E, Tsuchiya K, Takaishi Y, Kawazoe K. Isolation and characterization of free radical scavenging flavanoids glycosides for the flowers of *Spartium junceum* by activity guided fractionation. *J. Ethnopharmacol.* 2000; **73**: 471–8.
27. Legos JJ, Erhardt JA, White RF *et al*. SB 239063, a novel p38 inhibitor, attenuates early neuronal injury following ischemia. *Brain Res.* 2001; **892**: 70–7.
28. Siesjo BK. Pathophysiology and treatment of focal cerebral ischemia, part 11: Mechanisms of damage and treatment. *J. Neurosurg.* 1992; **77**: 337–54.
29. Bhattacharya SK, Bhattacharya D, Sairam K, Ghosal S. Effect of *Withania somnifera* glycowithanolides on a rat model of tardive dyskinesia. *Phytomedicine* 2002; **9**: 167–70.
30. Bhattacharya SK, Stayan KS, Ghosal S. Antioxidant activity of glycowithanolides from *Withania somnifera*. *Ind. J. Exp Biol.* 1997; **35**: 236–9.
31. Dhuley J. Effect of ashwagandha on lipid peroxidation in stress induced animals. *J. Ethnopharmacol.* 1998; **60**: 173–8.