Neuroprotective profile of mulberry leaf extract in focal cerebral ischemia model in rats

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ABSTRACT

The cerebral ischemia causes severe degree of oxidative stress as a result of reperfusion injury. An attempt has been made to analyze the preventive and curative potential of mulberry leaf extract, in focal cerebral ischemia model in rat, as it is rich source of novel chemicals possessing antioxidant and neuroprotective properties. The aqueous extract (MLE-AR-14) pre-treatment one hour prior to ischemia significantly (65\%) prevented the ischemic cerebral damage. It also tended to revert the behavioral deficit caused due to brain damage. The biochemical markers of oxidative stress MDA and GSH were also significantly altered towards basal levels. The most important and exciting finding is the effectiveness of MLE-AR-14 administration after six hours post cerebral injury. The extract at a dose of 100 mg/kg p.o. caused reduction in cerebral infarct by 54\% indicating its curative potential in cerebral stroke. The oxidative end product MDA was reduced by 42\% and the level of GSH was elevated by 54\% on post-treatment.

Thus, it seems that MLE-AR-14 has a potent neuroprotective profile and also has modulatory effect on cellular and biomolecular processes in preventing redox status to arrest apoptotic and necrotic processes underlying focal cerebral ischemia, as evidenced by histological parameters. The post-ischemic efficacy of the extract suggests that it may be clinically exploited in cerebral stroke.

Key words: Mulberry, Cerebral ischemia, stroke, neuroprotection, antioxidants.

1. INTRODUCTION

Cerebral stroke is a clinical syndrome characterized by abrupt loss of brain functions in humans due to blood clots, plaque formation in the blood vessels or hemorrhage in the brain that impairs blood supply to the brain. The rapid depletion of oxygen and glucose results in widespread cellular damage in the vicinity of the affected brain area. Usually an ischemic episode is followed by spontaneous reperfusion and serious adverse effects may arise due to re-oxygenation, leading to overproduction of reactive oxygen species (ROS) and leading to oxidative stress in the affected brain regions\textsuperscript{1-3}. The oxidative stress is an important factor in brain damage during post-ischemic reperfusion. Therefore, antioxidants may act as a major defense by preventing or attenuating the damage caused by free radicals. Numerous antioxidants are neuroprotective in stroke models and significant evidence is available supporting the role of dietary antioxidants and polyphenolic compounds from botanical sources like green tea extract, \textit{Ginkgo biloba} extract and resveratrol to combat ischemia-reperfusion induced oxidative stress\textsuperscript{4}. Several plant candidates like \textit{Morus alba}, \textit{Withania somnifera}, \textit{Centella asiatica}, \textit{Tinospora cordifolia} and \textit{Convolvulus pluricaulis} are best sources of potential therapeutic agents\textsuperscript{5}.

Mulberry, a plant belonging to a family of Moraceae, is predominantly grown in eastern, southern and southeastern Asia. The extracts of mulberry leaves, stems, root bark and fruits have been used for various ailments in the traditional system of medicine in Asian countries\textsuperscript{6-8}. The plant is reported to contain a number of biologically active phytoconstituents viz. tannins, phytosterols, sitosterols, saponins, triterpenes, flavanoids, benzoferan derivatives, morusimic acid, anthocyanins, anthroquinones, glycosides and oleanolic acid as the main active principles\textsuperscript{9}. The hypoglycemic and hypotensive effect of mulberry extracts has been attributed to the presence of phenolic mulberrofurans found in the plant\textsuperscript{10}. The antioxidant and anti-apoptotic potential of mulberry extract is due to the free radical scavenging property of the flavonoids present there in, which consequently leads to the regulation of nitric oxide generation, Bcl-2 and Bax proteins, mitochondrial membrane depolarization and caspase-3 activation\textsuperscript{11}. The mulberry-leaf extract is effective in suppressing the progression of atherosclerosis by inhibiting the oxidation of LDL-cholesterol, which is a major factor in the development of atherosclerotic plaque\textsuperscript{12}. The study by Naowaboot and colleagues, 2009, showed that white mulberry leaf extract helps to restore the vascular reactivity, besides producing anti-hyperglycemic, antioxidant and antiglycation effects in chronic diabetic rats\textsuperscript{13}. The clinical studies in humans have also shown mulberry leaf powder’s potential to decrease serum triglyceride, LDL, and CRP levels in mild dyslipidemic patients without causing any adverse reactions\textsuperscript{14}.

The plant leaf extract has been used in some studies to explore its neuroprotective effect. Further, the accumulation of gamma-aminobutyric acid (GABA) in mulberry leaf as a result of the anaerobic treatment...
enhances the neuroprotective effect against cerebral ischemia. Recently, it was found that anthocyanin, a flavonoid pigment in mulberry fruit, offered protection against cerebral ischemia. In another study, it was demonstrated that the antioxidative flavanoid, Quercetin-3-O-β-D-glucopyranoside from mulberry leaf significantly decreased certain stroke parameters like hemoglobin, strokin, cortexin, frontalin, temporalin, parietalin, occipitalin, brain ventuculin, hemorrhagic clot in rabbits. Further, Cynidin-3-O-beta- D-glucopyranoside isolated from mulberry fruit also offered neuroprotective effect in vivo using a transient focal cerebral ischemia model of brain injury. Hence, presence of novel chemicals in mulberry having antioxidant, dyslipidemic, antihypertensive with potent neuroprotective properties prompted us to analyse the preventive as well as curative anti-stroke activity of mulberry leaf extracts using middle cerebral artery occlusion (MCAO) model of focal cerebral ischemia in rats.

2. MATERIALS AND METHODS

Preparation of aqueous leaf extract of Mulberry:
Fresh leaves from the mulberry variety AR-14 was obtained from mulberry plantation in Babasheb Bhimrao Ambedkar University campus, Lucknow. The leaves were processed and powdered using standard methods. The resulting powder was then passed through an 80-mesh sieve and stored in a sealed aluminum pouch at 4 °C, till further use.

The mulberry leaf powder about 2 grams was soaked in 200 ml of boiling water for 20 minutes on a rotating shaker (200 rpm). The mixture was cooled at room temperature then filtered through Whatman No.1 filter paper and lyophilized. The freeze-dried solid leaf extract, MLE-AR-14 was stored in plastic tubes at −20 °C to protect from light. The solid extract was dissolved in double distilled water prior to use in all the experiments.

Experimental animals: Adult male Sprague Dawley rats, weighing 260±20 g, were used in the present study. The animals were procured from the National Laboratory Animal Centre of CSIR- Central Drug Research Institute, Lucknow. All animal experiments were performed strictly in compliance with the guidelines for the care and use of animals after necessary approval of the Institutional Animal Ethical Committee of the institute. Rats were allowed food and water ad libitum and housed in the experimental room in the animal house under proper laboratory condition.

Induction of Middle Cerebral Artery Occlusion:
Focal cerebral ischemia was induced by occluding the middle cerebral artery (MCA) using the modified method of the intraluminal technique routinely used in the lab. Briefly, the animals were anesthetized with chloral hydrate (300 mg/kg i.p.). Then left common carotid artery (CCA) was exposed through the midline incision in the neck region and was traced rostrally to expose the external carotid artery (ECA) and internal carotid artery (ICA). A 4.5 cm long 3-0 mono filament (Ethicon, Johnsons & Johnsonsons Ltd., Mumbai) with rounded tip was gently introduced into the internal carotid artery through a small nick in the ECA and gently advanced about 2 cm from the ICA origin till a mild resistance was felt. The protocol of 2 hours ischemia and 24 hour reperfusion period (2/24 I/R) was used for developing cerebral stroke. The monofilament was retracted after 2 hours and allowed reperfusion for 24 hours. In sham operated rats, a similar surgical procedure was performed except for the insertion of monofilament. Post-surgery, the experimental animals were returned to cages with free access to food and water.

Experimental groups and treatment schedule: A different set of eight experimental groups were made consisting of 6-10 rats in each group. It includes sham, placebo (gum accacia), MLE-AR-14 (50 and 100 mg/kg p.o.) and resveratrol (50 and 100 mg/kg p.o.) as standard agent. The focal cerebral ischemia/reperfusion injury of (2/24hrs) was used throughout the study. The schedule of treatment for preventive effect includes treatment with test substance or standard compound 1 hour prior to ischemia and for curative effect, 6 hour post reperfusion injury.

Assessment of Ischemia/Reperfusion injury: The ischemic brain damage was characterized by neurobehavioural as well as by biochemical and histological parameters.

Neurobehavioral studies: Rats were examined for neurologic deficits (ND) post 2/24 h I/R injury. The neurobehavioral deficits were scored on a 10 point scale by evaluating four parameters. The deficit is symbolized in the form of neurobehavioral incoordination and characterized by flexion (1 point), contra-lateral circling (2 points), hemiparesis (3 points) and non-spontaneity (4 points). Thus, an impact of ischemic injury is indicated by enhancement in the behavioral deficit scores. The neurobehavioral scores obtained after careful assessment of each animal were averaged as neurological deficit scores for each experimental group. The neurological deficit was also used as an inclusion/exclusion criterion and I/R rats showing no sign of neurological deficits were excluded from the study.

Biochemical Studies:
Malondialdehyde estimation in blood: The malondialdehyde (MDA) an important biomarker of lipid peroxidation was measured in blood serum by the method of Colado, 1997. It is based on its reaction
with thio-barbituric acid (TBA). Two molecules of TBA react stoichiometrically with one molecule of MDA to form a pink pigment that has absorption at 532 nm. Thiobarbituric acid reactive substances (TBARS) were and used as a measure of MDA in the sample.

Rats were anaesthetized with anaesthetic ether and about 3 ml blood was withdrawn from the retro-orbital plexus using the fine glass capillary tube. Trichloroacetic acid, 30% followed by 5N HCl, 2% 2-thiobarbituric acid and triple distilled water were added to the serum obtained after the samples were spun down at 13,000 rpm for 10 min at 25°C addition of. Vigorous vortexing was done after every step. Post incubation at 90°C for 15 min in the water-bath, samples were then spun down at 3000 rpm for 10 minutes to settle down the particulate material. The absorbance of the clear, pink supernatant was determined spectrophotometrically at 532 nm. The readings were interpolated in the standard graph for determining the MDA contents for each samples tested.

**Glutathione estimation in blood:** Glutathione (GSH) is a major intracellular antioxidant found in high concentration in the brain. Hydrogen peroxide is a potent oxidizing agent that is reduced by glutathione in a reaction catalyzed by glutathione peroxidase. GSH was estimated in blood by the 5, 5'dithiobis-(2-nitrobenzoic acid) (DTNB)-glutathione reductase coupled assay essentially as described by Anderson, 198532.

Blood was withdrawn from the retro-orbital plexus of the anesthesized rats using a glass capillary, an anticoagulant, sodium citrate was added. Blood aliquots taken in different tubes were added with 6% acetic acid and 10% sulphasalysilic acid. Vigorous vortexing was done after every step. The plasma obtained after spinning the sample at 13,000 rpm for 10 min at 25°C, phosphate buffer, triple distilled water and DTNB was added followed by vortexing. After incubation at 37°C for 15 min., the intensity of the yellow coloured chromogen formed by reaction of GSH with DTNB was quantified at 412 nm in a spectrophotometer and the values thus obtained were interpolated in the standard graph for determining GSH.

**Histological studies:**

**TTC Staining:** Brain damage following cerebral ischemia/reperfusion injury was visualized with 0.5% 2, 3, 5 triphenyl tetrazolium chloride (TTC) staining33. TTC stains the live mitochondria of the cell, which appears bright red in colour. The dead or infarct tissue remains unstained by this dye owing to lack of oxygen and appears white.

The brains were rapidly dissected out after trans-cardiac perfusion with saline. The brain tissue was sectioned into 2 mm thick serial coronal sections. All the brain slices were incubated for 20 min in a 0.5% solution of TTC at 37°C. The stained sections were digitally photographed and the infarct tissue of each brain section was traced and quantified by computerized image analysis software (Biovis Image Plus). The infarct area of each brain section was summed up and averaged and this was divided by the pooled average of ipsilateral brain area to obtain the percentage of brain infarction.

**Hematoxylin and Eosin (HE) Staining:** HE staining is a powerful tool to differentiate brain damage in the form of apoptotic and necrotic cell death on the basis of alteration in cellular morphology following I/R injury (24). The rats after 2/24 hours of I/R injury were subjected to neurobehavioral assessment prior to euthanizing them by anesthetic ether. This was followed by trans-cardiac perfusion with normal saline followed by 4% paraformaldehyde, thereafter the brain was immediately taken out in chilled medium. The cerebral hemispheres were sectioned in the coronal plane and processed for paraffin embedding. Sections of 4-6 μm thickness were cut using a microtome (Leica, Germany) and were transferred on poly-L-Lysine coated glass slides. The sections were deparaffinized by xylene treatment and rehydrated sequentially by immersing the slides through graded concentrations of ethanol (95%, 85%, 70% and 50%) for 3 minutes each and then washed with saline and phosphate buffered saline (PBS, pH 7.4) for 5 minutes each. The slides were dried and processed for HE staining for morphometric analysis under light microscope (Leica, Wetzlar, Germany) at 40X magnification. A minimum of 10 microscopic fields were examined of each slide to obtain a true picture of cellular architecture of affected brain tissue.

**Cresyl Violet staining:** To further confirm the regional distribution of cerebral damage, cresyl violet (CV) staining was also done. CV acetate solution is used to stain Nissl substance in the cell body and dendritic processes of the viable neurons. The Nissl substance (rough endoplasmic reticulum) appears dark blue due to the staining of ribosomal RNA, giving the cytoplasm a mottled appearance.

Basically the preparation of brain section slide is same as described previously under HE staining section. The sections were then dipped in CV staining solution for few seconds and air dried. Similarly, the sections were given few short exposures to n-butanol and then dipped in acetone followed by xylene. The stained sections were mounted with DPX and examined under light microscope (Leica, Germany) to assess the brain parts affected by I/R injury (25).

**Statistical Analysis:** The comparisons of results among different groups were made using the One-way Analysis of Variance (ANOVA) followed by Newman-Keuls
3. RESULTS

Assessment of Neuroprotective profile: The neuroprotective effect was assessed by improvement in neurobehavioral deficit and cerebral infarct size. The biochemical indices GSH level was up regulated and MDA level was deregulated in treated rats with mulberry extract.

Neurological Deficit: A major prerequisite for the present study was the successful occlusion of middle cerebral artery by the nylon monofilament. This was verified by observing ND, which is a direct consequence of ischemic (I/R) injury. The MCAO group of rats after 24 hour of reperfusion showed a score of 7 on a 10 point scale of ND, indicating severe impairment in behavioral and motor functioning due to brain damage by I/R.

One hour pre-treatment with MLE-AR-14 at 50 mg improved ND by 37% whereas, the 100 mg dose resulted in significant improvement by about 50% in neurological deficit scores in ischemic rats post 24 hrs of reperfusion. The standard marker, resveratrol also showed significant reduction in ND by 63% and 77% respectively with 50 mg and 100 mg doses (Fig. 1a).

The post-treatment of MLE-AR-14 at a dose of 50 mg and 100 mg resulted in 40% and 57% improvement in neurological deficit scores in ischemic rats as compared to 65% and 71% observed with post-treatment with resveratrol with same dose. Thus the MLE-AR-14 was quite neuroprotective in preventing the brain damage caused by I/R injury. The relative marked effect of antioxidant resveratrol may be due to high dose of standard used whereas, 50 mg /dose was adequate as it is purified compound (Fig. 1b).

Cerebral infarction: TTC staining is commonly used to assess the brain damage as a result of ischemic insult. The rats showing significant ND were sacrificed and brain sections stained with TTC were used for assessing the cerebral infarct area.

The 2/24 hr I/R injury resulted in almost 65% brain infarct, as white mass in TTC stained sections of rat brains (Fig. 2a). The pre-treatment with 50 mg dose of MLE-AR-14 reduced the cerebral infarct by 34%. The increased dose of 100 mg of extract further enhanced the neuroprotection by 65% whereas, pre-treatment with 50 mg and 100 mg of resveratrol resulted in reduction of brain infarction by about 55% and 76% respectively.

Interestingly, the extract was significantly active even post I/R injury. The treatment with 50 mg of extract after 6 hour of I/R prevented brain damage by 28% whereas, 100 mg dose offered about 54% neuroprotection. The standard compound, resveratrol offered a reduction of 53% and 68% in cerebral infarction at a dose of 50 mg and 100 mg respectively. Thus, it appears that extract is significantly effective even after post ischemic injury (Fig. 2b).

Malondialdehyde levels in blood: The level of lipid peroxidation was measured by estimating its marker, MDA content in the serum of experimental animals. The blood MDA level was found elevated significantly by about 68% following 2/24 I/R injury. Interestingly, the MDA levels were significantly altered by both pre-treatment as well as post-treatment with MLE-AR-14 extract. The downward trend in MDA reduction was 30% with 100 mg dose of MLE-AR-14. The resveratrol pre-treatment with same dose had reducing effect by 50% on blood MDA level.

Interestingly, the MLE-AR-14 was significantly active in combating oxidative stress post reperfusion as compared to pre-treatment. The extract on post-treatment owing to its potent antioxidant activity was able to reduce the MDA levels in ischemic rats by about 42%. The standard drug, resveratrol when post-treated proved slightly more effective in reducing MDA content in the blood of ischemic rats (Fig. 3a).

Glutathione levels in blood: Glutathione is a central component in the antioxidant defense mechanism, acting directly to scavenge reactive oxygen species and also as a substrate for various peroxidases. The I/R induced stress depleted the glutathione level in the blood of stroke animals and showed overall decrease of about 60% as compared to the sham operated rats.

MLE-AR-14 pre-treatment was very effective in averting the depletion of GSH stores following I/R injury. The 100 mg/kg p.o.dose of MLE-AR-14 tended to improve GSH level by 54 % as compared to an increase of 77% by resveratrol pre-treatment with same dose. The post-treatment with the MLE-AR-14 also significantly prevented depletion of glutathione level by 34% (Fig. 3b).

Hematoxylin and Eosin staining: The histological changes observed under light microscopy after inducing 2 hour of ischemia followed by 24 hour of reperfusion showed enormous damaged cells exhibiting features of both necrosis and apoptosis. Necrotic injury stained with HE was characterized by nuclear pyknosis, karyolysis which contained dispersed chromatin clumps, associated with shrunken neurons, increased cytoplasmic eosinophilia (red neurons) and nuclei lacking cellular structures known as ghost neurons (Fig. 4).

Apoptotic cells were identified using characteristics enunciated by Kerr, (1972)26, and these
were characterized by protruberances on the cell surface separated with plasmalemma sealing, which produced membrane bound apoptotic bodies of roughly spherical or ovoid shape. The severity of apoptotic injury was visible in sections as rounded or oval apoptotic bodies, typically dark purple-blue masses. Cells containing more than two apoptotic bodies were taken positive for apoptotic changes. There was also evidence of pannecrosis in the striatal and cortical regions of MCAO induced rats as shown by cavitations.

However, this cellular damage was significantly reduced in the brain sections of rats treated with MLE-AR-14 showing only a few apoptotic cells in both pre-treated and post-treated groups. And, relatively, similar reduced number of cavitations were observed in the resveratrol pre-treated group. The post treated rat brains either with MLE-AR-14 or resveratrol had very few cells showing apoptotic and necrotic features in both striatal and cortical brain regions. There was no evidence of such histological abnormalities in the brain sections of sham operated rats.

Cresyl-violet staining: Cresyl-violet staining showed a significantly reduced cellular damage in both ischemic cortical and striatal sections of ischemic rats, treated with MLE-AR-14. The treatment with resveratrol was also equally effective in controlling the brain damage. The neuronal cells in the non-ischemic contralateral hemisphere were morphologically intact. Thus, these results very well corroborate with that of HE results (Fig. 5).

DISCUSSION

Cerebral stroke is characterized by sudden reduction of blood flow to affected part of the brain due to blockade of blood vessels or owing to hemorrhage. This results in deprivation of oxygen and glucose leading to breakdown of metabolic machinery in the affected part of the brain. The cerebral ischemia activates various overlapping cascades responsible for brain damage. The important ones are excitotoxicity, oxidative stress, intracellular signaling, inflammation, unfolded protein response and altered gene expression, and these mechanisms modulate cell survival and damage.

The aqueous mulberry leaf extract produced significant preventive as well as curative anti stroke effects in focal cerebral ischemia model of rat. The most significant finding is that it offered neuroprotection even six hours post reperfusion injury. The results have also demonstrated that, it has also significantly improved the neurobehavioral deficits and significantly reduced the cerebral infarct area. Thus, the antioxidant defense mechanism of MLE-AR-14 seems to be quite effective in reducing the overall deleterious effects of cerebral ischemia. Moreover, HE and cresyl violet staining of affected brain tissue also showed that MLE-AR-14 significantly protected cellular damage leading to neuroprotection.

Mulberry has been widely used in Chinese medicine since ancient times. The pharmacological properties of the plant may be attributed to the presence of high amounts of polyphenolic compounds and secondary metabolites. Mulberry leaf extract contains many known polyphenolic compounds including rutin, quercetin, glucopyranoid, aastragalin, kaempferol etc., which exerts potent antioxidant activity. Therefore, it is reasonable to believe that presence of such polyphenolic compounds in mulberry leaf extract would exert potent antioxidant activity to prevent the ischemic brain damage.

One of the most important factors responsible for brain damage induced by cerebral ischemia is oxidative stress caused by the reactive oxygen species (ROS) generated during hypoxia and reperfusion. During oxidative stress, rapid overproduction of free radicals overwhelms the detoxification and scavenging capacity of cellular antioxidant enzymes resulting in a severe and immediate damage to cellular proteins, DNA and lipids leading to brain dysfunction and cell death. In order to protect tissues against the devastating consequences of ROS, all cells exhibit defense mechanisms that involve SOD, catalase, glutathione reductase and glutathione peroxidase. These antioxidant enzymes vitamin E, glutathione, superoxide dismutase and catalase are free radical scavengers.

With respect to stroke patient recovery, the most common neurological sequelae in patients with brain injuries and in animal models of cerebral ischemia are functional behavioral deficits. The degree of sensorimotor dysfunction is an important indicator of severity of brain injury. A considerably severe neurobehavioral deficit was demonstrated in rats following MCAO. The results indicate that MLE-AR-14 treatment significantly improved neurobehavioral deficits when administered orally one hour prior to ischemic insult or six hour post reperfusion. The improvement in ND is indicative of the neuroprotective activity of the extract.

The level of MDA, an oxidative stress marker and a byproduct of lipid peroxidation was found to be elevated after focal cerebral I/R injury. This was indicative of oxidative stress exerted during focal cerebral ischemic injury in rats. Resveratrol treatment significantly reversed the increased MDA levels. Similar effect was exhibited by the MLE-AR-14 extract. Therefore, attenuating effect of MLE-AR-14 on MDA may be attributed to free radical scavenging activity of the extract as well as to its protective effect.
against lipid peroxidation in cellular membranes and DNA damage due to free radicals.

Glutathione, an important endogenous antioxidant, prevents damage to important cellular components caused by reactive oxygen species. A decreased GSH level is considered to be indicative of oxidative stress. In this study, GSH level in the MCAO rats was significantly reduced compared to sham group. The GSH level of the resveratrol treated groups was significantly higher than the MCAO groups. Likewise, MLE-AR-14 also proved to be quite effective in ameliorating the level of GSH in ischemic rats. These results indicated that MLE-AR-14 remarkably attenuated neuronal oxidative stress. This antioxidant effect may be attributed to the presence of high amounts of quercetin, oxyresveratrol, 5,7-dihydroxycoumarin 7-methyl ether, cyanidin 3-glucoside, cyaniding 3-rutinoside, Mulberroside A etc. in the mulberry extract.

Neurons die under ischemic conditions when delivery of oxygen and glucose are not sufficient to meet their metabolic demands for a long period. The histologically defined infarct area in the brain indicates the end point of ischemic damage. However, treatment with MLE-AR-14 as well as resveratrol proved to be effective in decreasing the brain infarct size as displayed by TTC staining.

Histopathological studies also reveal that the ischemic core region undergo irreversible process i.e. necrosis giving rise to morphological injury like development of cosinophilic cytoplasm and shrunken nuclei in more than 80% of neurons after vascular occlusion. However, the ischemic penumbra, the brain areas situated at a larger distance to the occluded vessel exhibit apoptosis with only mild ischemic damage with a possibility of reversal of the ischemic cellular damages. The MCAO induced rats in our study, showed a high extent of cellular ischemic damage in the apoptotic region. However, the damage was reverted by the administration of MLE-AR-14 as revealed by HE and Cresyl violet staining.

Figure 1. The effect of (a) 1 hr pre-treatment and (b) 6 hr post-treatment with MLE-AR-14 and resveratrol at a dose of 50 mg/kg and 100 mg/kg p.o., on neurological deficit scores in rats. #p < 0.001 compared with the sham and ***p < 0.001 compared with MCAO. (n=8-10 animals each)

Figure 2a. TTC stained brain sections showing area of infarction in MCAO, Pre- and post-treatment with MLE-AR-14 and resveratrol rats. Both extract and resveratrol significantly reduced brain infarction at 24 hours post reperfusion.
Fig. 2b. The effect of (a) 1 hr pre-treatment and (b) 6 hr post-treatment with MLE-AR-14 and resveratrol at a dose of 50 mg/kg and 100 mg/kg p.o., on brain infarction in rats. #p<0.001 compared with the sham and ***p<0.001 compared with MCAO. (n=8-10 animals each)

Fig. 3. The effect of 1 hr pre-treatment and 6 hr post-treatment with MLE-AR-14 and resveratrol at a dose of 100 mg/kg p.o., on (a) MDA level and (b) GSH level in rats. #p<0.001 compared with the sham and ***p<0.001 compared with MCAO. (n=8-10 animals each)

Fig. 4. Photomicrographs of HE stained 6 µm brain sections showing ischemic brain damaged area of MCAO treated, pre-treated (1 hour) and post-treated (6 hour) with MLE-AR-14 and resveratrol in rats at a dose of 100 mg/kg p.o. as compared to the sham operated group.
4. CONCLUSIONS

Mulberry plant is rich source of chemicals with potent anti-oxidant activity and has been traditionally used for various ailments. Therefore, it was selected to investigate its neuroprotective effect in focal cerebral ischemia. The aqueous mulberry leaf extract offered both preventive as well as curative anti stroke potential. The neuroprotective effect was showcased by amelioration in oxidative damage, betterment in behavioral dysfunction, reduction in cerebral infarct. The neuroprotective effect of MLE-AR-14 may be attributed owing to its potent antioxidant activity.

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