STUDIES ON POTENTIAL NEUROPROTECTIVE ACTIVITY OF SELECTED MEDICINAL HERBS

SUMMARY

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Summary

Cerebral ischemia, clinically called stroke is defined as a syndrome characterized by sudden loss of function of a particular part of the body, because of impaired cerebral blood flow (CBF) caused by occlusion or hemorrhage of one of the main artery supplying blood to that part of the brain. Stroke with other cerebro vascular disorders is a major health problem throughout the world and ranks third after cancer and heart diseases. It largely contributes to morbidity, mortality and disability in developed as well as in developing countries. The considerable prevalence of cerebral stroke can be linked to changes in lifestyle; sedentary work habits, lack of exercise, smoking and consumption of alcohol which have led to an increase in incidents of obesity, hypertension, chronic stress, and diabetes mellitus. All these disorders are predisposing factors to cerebral stroke. While cerebral stroke entails high mortality rate, those patients that do survive the episode are left with permanent disabilities that can be devastating placing a burden on family and community. Since brain is the affected organ, a proper and better understanding of the molecular and biochemical mechanisms underlying the cause of neuronal death and survival in cerebral stroke may help to target the long-term function of the brain.

The human brain is the centre of the nervous system and is a highly complex organ. Under normal physiological condition, glucose is the sole source of ATP in the brain, as the brain does not have the capacity to store glucose and its deprivation to the brain tissue, even for a brief duration, can lead to severe pathological changes. In cerebral ischemia, blood supply to the brain is interrupted, leading to oxygen and glucose deprivation in the affected brain region. Due to depletion of oxygen and glucose in ischemic brain tissue, the production of ATP fails leading to breakdown of energy dependent processes necessary for tissue cell survival and results in ionic imbalance that initiates a self-promoting cascade of pathophysiological events like increase of cytosolic Ca^{2+}, excitotoxicity, free radical

formation, mitochondrial dysfunction, protease activation, altered gene expression, inflammation, and apoptosis. Thus it is logical to suggest that if one or more of the processes involved in propagation of these cascades are timely interrupted, some of the affected brain tissue may be rescued.

Recombinant tissue plasminogen activator (r-tPA) is the only drug approved by FDA for treatment of stroke. However, it has a narrow therapeutic window of only three hours hence this drug may not be very effective. Many neuroprotective agents that target cell death pathways have seen failures in clinical settings, therefore, the future challenge of stroke therapy is to translate basic pathophysiological evidence of ischemic neuronal injury into novel neuroprotective therapies either independent or in combination. Therefore, neuroprotective agents with differing modes of action with wide therapeutic window and fewer side effects are immediately required to combat this deadly disease. There are many therapeutic strategies such as neuroprotectants, anti-inflammatory agents, free radical scavengers and neurotrophic agents, which can be used for restoration of brain functions. Interestingly, this can be offered by suitable and effective herbal plants.

In general, diseases have taken a horrendous toll on human life. Modern biomedical research has paved way to new therapies to combat these dreadful diseases but current treatments for most of these diseases are less than adequate. Synthetic medicines to treat these diseases had been a success since early 19^{th} century although, the traditional form of medicine in treating diseases uses plants which are as old as our civilization. The uses of medicinal plants are well documented throughout the history of mankind and they are the best source for medicine. India has a traditionally well-practiced knowledge of herbal medicines. The Indian heritage of traditional medicine and Ayurvedic science takes the advantage of natural resources and is focused on venturing pristine reservoir of herbals.

There are countless herbs grown all across the globe, delivering a host of health benefits. Some of them hold a plethora of essential oils, antioxidants, phytosterols, vitamins, and other nutrient substances that equip our body to fight against many deadliest chronic conditions. Naturally occurring antioxidants are thought to reduce cell damage within the body by countering the potentially dangerous effects of free radicals by scavenging free radicals (per oxide, hydro peroxide or lipid peroxyl, super oxide anion radicals, hydroxyl radicals, hydrogen peroxide and the singlet oxygen) produced during normal and pathological cell metabolism. Antioxidants are therefore, a promising treatment for many diseases especially cerebral stroke cancer and heart diseases because they either inhibit the free radical production, or act as free radical scavengers, or account for free radical degradation, or even upregulate the endogenous antioxidant system thereby reducing the cellular damage.

Currently there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine and in the food industry. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity. The growing interest in assessing the antioxidant capacity of herbal medicine has prompted us to study, potential neuroprotective effect due to the presence of antioxidants in mulberry and curcuma extracts in the MCAO induced focal cerebral ischemia in rats. Many Indian plants have been investigated for their beneficial use as antioxidants or source of antioxidants using presently available experimental techniques.

Oxidative stress and inflammation are central to ischemic pathology. Since mulberry and curcuma extracts having shown anti-inflammatory and anti oxidative properties due to the presence of abundant phytophenols in different models under varied conditions, this study has been undertaken to

investigate the potential of these extracts for offering neuroprotection against cerebral stroke in focal cerebral ischemia model in rats.

While the medicinal benefits of both the plants have been extensively studied, their impressive health promoting phyto-nutrient compounds like polyphenol pigment antioxidants, minerals and vitamins certainly offers a range of health benefits. Poised to be the newest sensation in health-conscious crowds, both the mulberry and turmeric tackle an impressive range of illness and diseases as reviewed in the following section.

Therefore, at the outset prior to assessing the precise neuroprotective activity of mulberry in middle cerebral artery occlusion model of focal cerebral ischemia, an attempt was made first to find out the anti oxidant potential of the various mulberry leaf extracts in rotenone induced oxidative stress model. Further this study will also highlight the neuroprotective effect of curcuma extract and provide information about neuronal survival in various brain regions following I/R stress. The understanding of anti oxidant activity of the extracts during energy deficiency in case of cerebral ischemia may generate knowledge about the extent of ischemic brain damage as a consequence of oxidative stress. The study may also be helpful in developing neuroprotective strategies for clinical exploitation in cases of cerebral stroke in humans.

Male SD rats were subjected to oxidative stress by administering rotenone and the preventive effect of 9 mulberry leaf extracts were studied administering it prior to oxidative stress. Rotenone induced the ROS generation led to brain oxidative stress by lipid peroxidation as revealed by significant 138% increase in MDA content. The results indicate that the maximum attenuating effect was observed with extract MLE-S-146 being 50.49%. Further a decrease of 36.14% in MDA was also observed with extract MLE-AR-14. A reduction in superoxide level offers a defense against cellular damage. As the SOD levels which got elevated due to the increased oxidative stress induced by rotenone were

significantly reduced by pre treatment with various MLEs. There was significant reduction (54.01 %) in the brain of rats treated with S-146, further AR-14 treated rats showed a reduction by 40.18%. Treatment with MLE showed antioxidant activity and also reversed the oxidative stress produced by rotenone. The upregulated super oxide dismutase level in the oxidative stress model is also an indication that the brain's antioxidant machinery is activated in response to excessive generation of free radicals. The extracts, MLE-AR-14 and MLE-S-146 were found to possess antioxidant activity as it was capable of attenuating the oxidative stress markers, SOD and MDA. So these two extracts were taken up further to study their neuroprotective effect on ischemia/reperfusion injury induced brain damage.

Further, these two extracts were subjected to outline its neuroprotective activity in focal cerebral ischemia model. Rats were subjected to MCA occlusion for 2 hr followed by 24 hr reperfusion. At 24 hr of I/R injury, the extent of cerebral damage was assessed by using TTC and CV staining, whereas the nature of cell death was assessed by HE and TUNEL staining in different brain regions. The staining was co related with the neurobehavioural deficit scoring. Oxidative stress was measured by estimating the brain levels of GSH, MDA. Rats were pretreated (1 hr) and post treated (6 hr) with extracts of MLE at doses of 50 mg and 100 mg/kg p.o at 2 hr ischemia and 24 hr reperfusion to study the role of oxidative stress in the I/R injury-induced cerebral damage.

One hour pre treatment with MLE-AR-14 at 50 mg oral improved ND significantly by 37% whereas, the 100 mg dose resulted further improvement by about 50% in neurological deficit scores in ischemic rats post 24 hrs of reperfusion. On the other hand, the average ND score was about 3.8 and 2.4 in groups received with MLE-S-146 at a dose of 50 mg/kg and 100 mg/kg at 2/24 h post I/R injury respectively. Six hour post treatment also had a good effect on lowering the ND score. When the ischemic animals were administered with a dose of 50 mg and 100 mg extract, six hour post reperfusion

injury, it was observed that the neurobehavioral alterations were commendable. The ND was found to be significantly increased with an average score of 7 in ischemic subjects at 2/24 hour of I/R injury. But the treatment with MLE-S-146 at 50 mg and 100 mg doses, lowered the ND score to 3.2 and 2.0 respectively.

Similar effect was seen in the ability of the extracts to reduce the infarct size in the MCAO induced brain of the subject animal. 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%. However, the pre treatment with the extract MLE-S-146 at doses of 50 mg and 100 mg proved to be effective in reducing the cerebral infarction by 40.05% and 59.32% respectively. The reduction in infarct size in 50 mg and 100 mg of extract which was administered animals six hr post reperfusion was about 48.14% and 64.07% respectively.

The MLE extract exhibited neuroprotection with reduction of infarct size, neurological deficit scores and lipid peroxidation as well as restoration of endogenous antioxidants. Glutathione acts both to directly detoxify ROS and as a substrate for various peroxidases. Pre treatment and post treatment with MLE significantly elevated brain glutathione content. Also, it was observed that the treatment with mulberry leaf extracts showed comparatively far less cellular alterations in brain when stained with HE and Cresyl violet as compared to other treatments. The treated rat brains had very few cells showing apoptotic and necrotic features in striatal and cortical regions.

Hence it can be suggestive that the extracts of mulberry leaf used in the experiments that constitute this thesis have neuroprotective effect in the transient cerebral ischemia model because of the presence of bioactive compound ie. the polyohenol.

In the next set of study, preventive treatment (1 hr pre) was assessed for CLE at a dose of 150 mg and 300 mg/kg p.o. The pretreatment of CLE at 300 mg/kg p.o. one hour prior to ischemic insult resulted in 60% improvement in neurological deficit in MCAO rats. There was a similar trend of equal magnitude of reversal of ND (55%) with pretreatment with 150 mg/kg p.o. dose as observed post 24 hrs of I/R injury. The treatment with CLE was able to illicit a significant decrease in infarct size by 42.69% and 68.32% at a dose of 150 mg/kg and 300 mg/kg. The levels of MDA, and glutathione were also found to be attenuated with the treatment of CLE in MCAO induced rats. Similarly, the appearance of apoptotic cell death following I/R injury as revealed by the TUNEL-positive cells in striatum was significantly reduced by CLE pretreatment at 300mg/kg. A moderate decrease in the TUNEL positive cells was also observed in rats, which were treated with CLE at a dose of 150 mg/kg. The neuroprotective effects of *Curcuma longa* against cerebral ischemia/reperfusion injury may be attributed to its ability to reduce through its antioxidant property.

The neuroprotection observed in ischemic rats treated with CLE is likely due to the presence of polyphenols. The free radical scavenging, increased expression of anti-oxidant enzymes and chelation of metal ions like Fe^{+2} leads to a decrease in oxidative stress. Interestingly, oxidative stress can regulate inflammation too. Therefore, polyphenol rich mulberry leaf extract and CLE can reduce post-ischemic inflammation by decreasing oxidative stress. The cumulative effect of a reduction in oxidative stress, inflammation and excitotoxicity is to limit brain tissue damage in stroke.

The studies in this thesis show that treatment of ischemic rats with mulberry leaf extract MLE-AR-14, MLE-S-146 and CLE decreased the oxidative stress and afforded neuroprotection. These effects are observed with both pretreatment and post treatment dosage schedule indicating the potential of these herbal preparations in attenuating cerebral ischemic injury.

In conclusion, these findings offer beneficial references for the application of natural antioxidants in clinical treatment of stroke. These novel medicinal targets and mechanisms provide innovative clues and can help researchers to screen drugs for therapeutic intervention in ischemic brain injury mediated by natural antioxidants. Overall, mulberry and *Curcuma longa* appears to carry promise in the treatment of ischemic cerebral injury and related neurodegenerative diseases.