Chapter-VIII

Summary & Conclusion
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Stroke is one of the leading cause of morbidity and mortality and the first major cause of long term disability worldwide. WHO defined stroke as ‘rapidly developed clinical signs of focal disturbance of various cerebral functions, lasting more than 24h or leading to death, with no apparent cause other than vascular origin. Approximately 85% of stroke is ischemic in nature, characterized by the disruption or complete blockade of blood flow and lack of glucose and oxygen in cerebral arteries, resulting in energy failure that initiates a complex series of metabolic events, ultimately causing neuronal cell death.

Oxidative stress plays a fundamental role in cerebral ischemia reperfusion injury because of large consumption of oxygen by the brain. As brain is not well equipped with antioxidant defenses, and so free radicals or oxidants released by inflammatory cells threaten tissue viability in the vicinity of the ischemic core. Oxidative stress culminates due to an imbalance between prooxidants and antioxidants and consequent excessive production of reactive oxygen species. Reactive oxygen species are implicated in a number of disease processes, whereby they mediate damage to cell structures, including lipids, membranes, proteins, and DNA. The cerebral vasculature is a major target of oxidative stress playing a critical role in the pathogenesis of ischaemic brain injury following a cerebrovascular attack (ischemic stroke). Brain tissue is particularly susceptible to oxidative damage. Therefore, it is believed that pharmacological modification of oxidative damage is one of the most promising avenues for stroke therapy.

The use of appropriate animal models is essential to understanding of deleterious mechanisms involved in the brain ischemic damage, and to identify the potential efficiency of therapeutic strategies. There are many animal models available to investigate injury mechanisms and neuroprotective strategies; however the success lies only when we can reproduce the result. This has incited us to critically review the current animal models and discuss how these models may yield fresh insights into the pathogenesis associated to ischemia reperfusion, as well as new preventive or therapeutic strategies.

In recent years considerable attention has been devoted to the approach of neuroprotection by naturally occurring agents as a newer dimension in the management of stroke prevention. Many naturally occurring agents have shown preventive potential as a protective measure for cerebral stroke in a variety of bioassay systems and animal models. Neuroprotection might be provided by agents that interfere with factors involved in pathogenesis. To date, most basic and clinical trials have focused on antioxidants and anti-inflammatory agents.
Chapter IV

**S-allyl cysteine mitigates oxidative damage and improves neurologic deficit in a rat model of focal cerebral ischemia**

The present study examined the hypothesis that S-allyl cysteine (SAC), organosulfur compounds found in garlic extract, would reduce oxidative stress-associated brain injury after middle cerebral artery occlusion (MCAO). Rats were divided into four groups of eight animals in each group. Male Wistar rats were subjected to MCAO for 2 h and 22-h reperfusion. S-allyl cysteine was administered (100 mg/kg, b.wt.) intraperitoneally 30 minutes before the onset of ischemia and after the ischemia at the interval of 0, 6, and 12 h. After 24 h of reperfusion, rats were tested for neurobehavioral activities and were killed for the infarct volume, estimation of lipid peroxidation, glutathione content, and activity of antioxidant enzymes (glutathione peroxidase, glutathione reductase, catalase, and superoxide dismutase). This finding showed that MCAO cause behavioral deficits and oxidative stress due to abrupt generation of free radical. However S-allyl cysteine treatment significantly reduced ischemic lesion volume, improved neurologic deficits, combated oxidative loads, and suppressed neuronal loss. Behavioral and biochemical alterations observed after MCAO were further associated with an increase in glial fibrillary acidic protein and inducible nitric oxide expression and their significant protection by the treatment with SAC. Thus, this finding of SAC-induced adaptation to ischemic stress and inflammation exhibits exuberant neuroprotective potential in rat ischemia/reperfusion model.

Chapter V

**Catechin hydrate ameliorates redox imbalance and limits inflammatory response in focal cerebral ischemia**

In this study, we investigate the pretreatment effect of catechin hydrate (CH) on functional outcome, neuronal damage and on secondary injuries in the ischemic brain of rats. Rats were divided into four groups each group having eight animals. Male Wistar rats were pretreated with CH (20 mg/kg b.wt) for 21 days and then subjected to 2 h middle cerebral artery occlusion (MCAO) followed by 22 h of reperfusion. After 22 h of reperfusion, neurological deficit, infarct sizes, activities of antioxidant enzymes and cytokines level were measured. Immunohistochemistry and western blot were used to analyse the expression of glial fibrillary acidic protein (GFAP), inducible nitric oxide (iNOS) and NF-kB in ischemic brain. The administration of CH showed marked reduction in infarct size, reduced the neurological deficits, suppressed neuronal loss and down regulated the iNOS, GFAP and NF-kB expression in MCAO rats. A significantly depleted activity of antioxidant enzymes and content of glutathione
in MCAO group were protected significantly in MCAO group pretreated with CH. Conversely, the elevated level of thiobarbituric acid reactive species and cytokines in MCAO group was attenuated significantly in CH pretreated group when compared with MCAO group.

The results indicated that CH protected the brain from damage caused by MCAO, and this effect may be through downregulation of NF-kB expression.

Chapter VI

Phloretin attenuates neuronal damage associated with transient focal cerebral ischemia in rats

We investigated the effects of phloretin against ischemia reperfusion induced brain insult in MCAO model of cerebral stroke. Rats were divided into four groups each group having eight animals. A dose of 10 mg/kg b. wt. of phloretin was administered orally (p.o.) once daily for 10 days before MCAO. On day 11, MCAO was performed for 2h and reperfusion for 22h. After 22h of ischemia the animals were assessed for neurobehavioral activity and then sacrificed for the estimation of lipid peroxidation, glutathione content, glucose-6-phosphate dehydrogenate, superoxide dismutase and to assess the anti-apoptotic potential of phloretin. This finding indicated that MCAO caused behavioral deficits, detectable infarct volume and oxidative stress due to abrupt generation of free radical. However phloretin pretreatment offered significant protection against transient focal cerebral ischemic induced characteristic behavioral and biochemical alteration, which was further corroborated by increased endogenous antioxidant defence system and suppress expression of Apaf-1, Caspase-3 and 9 and DNA damage which may be attributed to its anti-oxidative and anti-apoptotic property.

Thus findings of this study demonstrated that phloretin protected neurons against ischemia reperfusion induce brain injury.

Chapter VII

Polymeric nanoparticles of thymoquinone attenuates oxidative damage and neurological deficits following focal cerebral ischemia/ reperfusion injury in rats

The aim of this study was to identify the effect of thymoquinone and its nanoparticles on ischemia reperfusion induced oxidative damage in an experimental model of focal cerebral ischemia. Rats were divided into four groups each group having eight animals. Male Wistar rats were pretreated with thymoquinone (5 mg/kg b.wt) and nanoparticles of thymoquinone (0.01mg/kg b.wt) for 15 days and then subjected to 2 h middle cerebral artery occlusion (MCAO) followed by 22 h of reperfusion. After 22 h of reperfusion animals were assessed for neurobehavioral activity and then sacrificed for evaluation of the infarct volume and biochemical
parameters. This finding showed that MCAO cause behavioral deficits and oxidative stress due to rapid generation of free radical. The present study provided evidence that the pre-treatment with thymoquinone and its nanoparticles resulted a better performances in behavioral tests and reduced infarct volume. Furthermore, pre-treating rats with nanoparticles of thymoquinone as compared to TQ has shown a better restoration of the various enzymatic and non-enzymatic markers of lipid peroxidation and GSH. Thus findings of this study demonstrated that nanoparticles of thymoquinone offer better protection as compared with thymoquinone suplimentaion due to enhanced antioxidant properties and sustained delivery of the encapsulated thymoquinone, thus neutralizing the detrimental effect of ROS.

So these selected neuroprotective agents: S-allyl cysteine, catechin hydrate, phloretin, thymoquinone and nanoparticles of thymoquinone can be used as favoured remedies in ischemia reperfusion brain injury pending elucidation of proper molecular mechanisms and deciphering appropriate genetic pathways.

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