Summary

"No great discovery was ever made without a bold guess"

- Isaac Newton -

"Father of the modern science", (1642-1727)
The thesis describes the investigations carried out to explore the effect of curcumin and C.oil in experimental cerebral stroke produced by the transient middle cerebral artery occlusion model in the rat, and probed into the efficacy and the therapeutic time window. The study is broadly divides into the effects of curcumin and C.oil.

7.4 **Effect of curcumin**

In the first set of experiments, the dose response relationship and the behavioral outcome in the rats challenged with ischemic were studied. The rats were evaluated 24 hrs after MCAo for neurobehavioral deficits score, rota rod and up to 7 days for water maze test. A dose response assay showed that curcumin gave the highest level of neuroprotection. The extent of infarction caused by MCAo was quantified in control and curcumin treated rats. Administration of curcumin (2mg/kg body weight, i.v.) after 6hrs of MCA occlusion reduces the volume of ischemic brain infarct that develops subsequently. Curcumin treatment reduced the failure in the spatial memory function in the water maze test, suggesting that curcumin is capable of exerting a persistent effect on learning and memory function or of preventing the loss of spatial navigation ability due to ischemic damage from days 1 to 7 in animals. (Chapter 1)

In the second set of experiments, curcumin was administered at a dose of 2mg/kg, i.v. after 6 hrs of MCAo. The effect of curcumin was evaluated on the nitric oxide system and on the apoptotic death pathway. After 24hrs of MCAo, the expression of nNOS, eNOS and iNOS were significantly increased as seen in brain cryosections and in western blot analysis. TUNEL labeling detected the apoptotic cells, which were confirmed in neuronal-rich cell samples and in immunohistochemical experiments in brain cryosections. Western blot analysis in brain tissue and immunohistochemical investigations in brain cryosections demonstrated that the expression of cytochrome c and Bax /Bcl-2 were altered after the insult, and antagonized by treatment with curcumin. Curcumin significantly reduced nitrosative stress, tended to correct the decreased mitochondrial membrane potential, and also affected caspase-3 activation and finally, the neuronal apoptosis. Here it was seen that iNOS-derived NO produced during ischemic injury was crucial for the up-regulation of ischemic injury targets. Curcumin down-regulates these targets this coincided with an increased survival rate of neurons. (Chapter 2)
In the last set in these series of experiments, the effect of curcumin on the neurogenesis, gliogenesis and angiogenesis was explored. Rats were challenged with focal ischemia, after 6hrs of MCAo they were treated with curcumin, followed by the injection of BrdU. New cells were labeled with the thymidine-analogue 5-bromo-2V-deoxyuridine (BrdU). DNA synthesis in nascent neurons during the S-phase of the cell cycle and their identity was determined immunocytochemically with various phenotypic markers. These experiments provided the first evidence that the adult brain can use neuronal replacement from endogenous precursors to repair itself after stroke and this process was stimulated by curcumin. In order to determine the cell types that were protected by curcumin, immunofluorescence studies were performed for identification of markers for neuronally committed progenitors (DCX) and neurons (NeuN). A significant reduction was observed on day 1, 2 and 7 on latency time and neurological scores. The density of BrdU cells labeled with doublecortin, glial fibrillary acidic protein was increased after 8, 24, 48hrs and day 7 of MCAo. Curcumin increased the number of BrdU labeled new cells of all lineages. Much still needs to be learned about how extracellular signaling pathways coordinate the intricate balance of neurogenesis, gliogenesis and stem cell renewal in the adult sub ventricular zone (SVZ). Concomitant with this neurological benefit is a significant induction of angiogenesis, cell proliferation in neurogenic zones within brain. The data demonstrate that a low-dose of curcumin enhance recovery from cerebral ischemia through beneficial modulation of the brain vascular system and the promotion of angiogenesis. The data also demonstrate that curcumin significantly improve neurological function with treatment initiated after the onset of injury and concomitantly promote brain plasticity. (Chapter 3)

7.5 **Effect of Curcuma oil**

This part of study deals with effect of curcuma oil. In the first set of experiments the dose response and behavioral outcome of pretreatment by C.oil was studied. Ischemia was induced in rats by middle cerebral artery occlusion for 2hrs followed by 24 hrs of reflow. C.oil was given as pretreatment. C.oil showed a dose dependent effect on infarct and edema volume. A compound MK-801, an NMDA receptor antagonist was used as reference. The ischemic lesion volume visualized and estimated on diffusion-weighted magnetic resonance imaging was significantly attenuated by C.oil treatment. Rats with 24 hrs of MCAo showed significant infarct, behavioral deficit, impaired learning and memory of the tasks. C.oil produced a significant improvement in escape latency to find the platform in the Morris water
maze along with impaired learning and memory of the tasks, behavior scores and reduction in volume of infarct. (Chapter 4)

The second part of study deals with the effects of pretreatments with C.oil (500mg/kg body weight) pretreatment. In the present study, the decrease in functional capacity of the neuronal mitochondria produced by ischemia was ascertained by probing the mitochondrial membrane potential (ΔΨm) and oxidative stress markers such as the levels of SOD, CAT, GSH-Px and MDA were estimated at 24 hours to look for correlation of neurological deficit with infarct volumes. In the present study, a significant reduction in (ΔΨm) depolarization and ROS generation was observed in the C.oil-treated group when compared against the untreated ischemic reperfusion group. The MDA levels and the activities of CAT, GSH-Px and SOD, were markedly reversed and restored to near normal levels in the groups pre-treated with C.oil.

In an attempt to further clarify the mechanisms underlying the neuroprotection against experimental cerebral ischemia by Curcuma oil (C.oil; 250 mg/kg body weight) a low dose and occlusion of middle cerebral artery for 1 hr (a lower level of ischemia) was planned. Ischemia, leads to elevation in [Ca²⁺] which sets into motion a cascade of ischemic injury, which was attenuated by C.oil. C.oil reduced post ischemic brain neutrophil infiltration in the ischemic area, controlled tissue NOx levels and the neuronal levels of nitric oxide, peroxynitrite and reactive oxygen species when measured after 24hrs of reflow. Double immunofluorescence staining analysis and western immunoblot analysis with C.oil treatment showed that the expression of nitric oxide synthase (NOS) isoforms was decreased significantly compared to the untreated ischemia group. Ischemia is associated with increased in TUNEL (TdT-mediated dUTP nick-end labeling) positive cells in brain sections indicating DNA fragmentation were significantly decreased by C.oil treatment. The immunofluorescence staining and Western blot indicate that C.oil suppressed the elevated protein level of Bax, and aided mitochondrial translocation and activation of Bcl-2 by altered mitochondrial membrane potential. It also inhibits the cytosolic release of apoptogenic molecules like cytochrome c, inhibits the activation of caspase-3 and the expression of p53, ultimately inhibiting apoptosis. These observations suggest that high levels of NO generated by NOS isoforms are at least partially responsible for exacerbating the neuronal damage caused by MCAo by intraluminal filament model of stroke (chapter 5).

The last part of the study focus on the effect of C.oil on neurogenesis, gliogenesis and angiogenesis. It shows that cerebral ischemia, caused by transient middle cerebral artery
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occlusion in adult rats, leads to a marked increase of cell proliferation in the subventricular zone. Stroke-generated new neurons migrate into the severely damaged area of the striatum. C.oil promotes differentiation of new neurons into the phenotype of most of the neurons destroyed by the ischemic lesion. Evidence is produced to show that the adult brain has the capacity for self-repair after insults and C.oil treatment stimulated the angiogenesis, endogenous cell proliferation and neurogenesis in the damaged brain area. (Chapter 6)

Treatment with both components of Turmeric from *Curcuma longa* the curcumin and curcuma oil, improves the recovery from experimentally produced cerebral stroke of both sensorimotor and cognitive functions, and provides a good scientific base for future studies of the underlying molecular mechanisms of experience-induced plasticity, that can possibly be used to identify new therapeutic targets. If the new neurons are functional and their formation can be stimulated, a novel therapeutic strategy might be developed for cerebral stroke in humans. The relevance of present data for human stroke patients is quite significant. Safety evaluation studies indicate that both turmeric and curcumin are well tolerated at a very high dose without any toxic effects. Thus, both turmeric and curcumin have the potential for the development of modern medicine for the treatment of various diseases. Council of Scientific and Industrial Research (CSIR) has an agreement through the agency of the Central Drug Research Institute (CDRI), Lucknow, with M/S Themis Medicare, Mumbai, India for cerebral stroke and the programme has reached an advance stage of drug development.