Chapter-V

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

Stroke, a catastrophe which threatens life, is the third leading cause of death and the first major cause of long term disability in developing nations (Cherubini et al., 2008). Approximately 80–85% of stroke is ischemic in nature, characterized by the disruption/severe reduction of cerebral blood flow and lack of oxygen in cerebral arteries that leads to the ischemia-reperfusion (I/R) insult (Allen and Bayraktutan, 2009). Ischemia reperfusion injury following ischemic stroke results from the extremely interconnected multiple pathobiological mechanisms includes excitotoxicity, oxidative stress, inflammation and apoptosis (Doyle et al., 2008; Khan et al., 2009; Raza et al., 2011).

Oxidative stress plays a fundamental role in cerebral I/R injury because of large consumption of oxygen by brain. As brain is not well equipped with antioxidant defenses, and so free radicals/oxidants released by inflammatory cells threaten tissue viability in the vicinity of the ischemic core (Lakhan et al., 2009). At the time of cerebral I/R, robust reactive oxygen species (ROS) are generated that promotes damage to cellular macromolecules such as lipids, proteins and DNA that leads to the irreversible neuronal injury and cell death (Chan, 2001).

In addition to oxidative stress, an inflammatory mechanism has been recently proposed in acute neuronal cell death following cerebral ischemia. This response is necessary to remove cell debris and tissue remodeling after injury. After I/R, energy depletion and necrotic neuron death in the local ischemic area start the inflammatory cascades. The reperfusion generated ROS induces the upregulation of NF-kB that leads to production of cytokines and chemokines which participate in the inflammatory process. Cytokines along with iNOS are thought to be the major mediators behind these complex inflammatory responses. Astrocyte involvement have also been linked to proinflammatory cytokines and iNOS upregulation in many pathologies of the CNS including cerebral ischemia (Hu et al., 1995) which markedly upregulates the expression of glial fibrillary acidic protein (GFAP).

Experimental models of cerebral ischemia have been developed to improve the understanding of deleterious mechanisms involved in the brain ischemic damage, and to identify the potential efficiency of therapeutic strategies. Animal model of MCAO closely mimic the changes that occur during and after human ischemic stroke because most human ischemic strokes are caused by occlusion of the middle cerebral artery (MCA) and so animal models were developed to induce ischemia in this arterial territory.

In the present era, much attention has been given to the use of naturally occurring compounds as the alternative therapeutic agents and their formulations for the treatment of different neurodegenerative diseases including stroke. These effects are attributed to their antioxidant as
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well as anti-inflammatory potential. Catechin hydrate (CH) is an abundant the major flavonoid present in the grape seed and green tea (Yilmaz and Toledo, 2004). It exhibits biological and pharmacological properties and a number of studies have examined the antioxidant activity and anti-inflammatory properties of CH using a variety of assay systems (Li et al., 2009). Keeping this in view, the present study was primarily aimed to examine the neuroprotective effect of CH against MCAO induced neuronal damage in rats. Neuroprotective effects of CH were assessed by studying markers of oxidative damage and NF-kB mediated neuro inflammation in rat brain.

Experimental Procedure

Chemicals and reagents
As described in material and methods, chapter-II.

Animals and treatments
As described in material and methods, chapter-II.

Drug Administration
Catechin hydrate (20 mg/kg body weight in normal saline) was administered orally (p.o.) once daily for 21 days before MCAO. We examined the effects of different doses of catechin hydrate (CH) on cerebral ischemia reperfusion injury in pilot studies to determine the optimal dose of CH that provides the most neuroprotection against ischemia/reperfusion injury and also the CH dose (20 mg/kg) and treatment regimen used in this experiment was supported from previous studies showing that this amount provided the maximal protective effects in the treatment of different types of brain injury (Kalender et al., 2012; Daisy et al., 2010; Uzun et al., 2010)

Experimental Design
To investigate the neuroprotective effects of catechin hydrate in an experimental model of cerebral ischemia, we used rat MCAO model (Khan et al., 2009). Animals were divided into four groups each having eight animals. The first group served as sham (S) and saline was given orally, the second was middle cerebral artery occluded (MCAO), i.e., ischemia was induced for 2 h followed by reperfusion for 22 h, the third was pretreated for 21 days with Catechin hydrate (20 mg/kg, orally) followed by MCAO for 2 h and reperfusion for 22 h (i.e., CH + MCAO group) and the fourth was pretreated for 21 days with Catechin hydrate alone, i.e., Catechin hydrate group (20 mg/kg, orally). After the completion of the reperfusion period, animals were assessed for neurobehavioral activities and then sacrificed. The brains were taken out to dissect the frontal cortex and striatum for biochemical estimations.

Induction of transient focal cerebral ischemia (MCAO)
As described in material and methods, chapter-II.
Post-operative care
As described in material and methods, chapter-II.

Behavioral studies
Adhesive-Removal Test
As described in material and methods, chapter-II.
Rotarod (motor coordination skill) test
As described in material and methods, chapter-II.
Grip test
As described in material and methods, chapter-II.

Evaluation of Ischemic Damage
Extent of ischemic damage was assessed using TTC and cresyl violet staining.

Infarct Volume Analysis
As described in material and methods, chapter-II.

Assessment of Neuronal Damage Cresyl violet
After 2 h MCAO/22 h reperfusion, animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and perfused transcardially with 0.9% sodium chloride at 4 °C, followed by 4% paraformaldehyde in 0.1 M phosphate-buffer (PB, pH 7.4) as described in material and methods, chapter-II.

Biochemical studies
Tissue Preparation for Antioxidant Enzymes, TBARS, Protein Carbonyl and Glutathione Assays
After the behavioral studies, the animals were killed, and their brains were removed to dissect out hippocampus and frontal cortex for the biochemical assays (TBARS, Protein Carbonyl GSH, GPx, GR, SOD and catalase) as described in material and methods, chapter-II.

Expression of NF-kB by Western Blot
As described in material and methods, chapter-II

Measurement of Cytokines
Brains from Sham, MCAO, catechin hydrate treated MCAO (CH + MCAO) and catechin hydrate treated sham-control (CH + Sham) animals were removed without fixation after cervical dislocation 24 h following surgery and processed as describe in material and methods, chapter-II.

Immunohistochemistry for iNOS and GFAP
After 22 h of reperfusion the animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and perfused transcardially with 0.9% sodium chloride at 4 °C, followed by 4%
paraformaldehyde in 0.1 M phosphate-buffer (PB, pH 7.4). The brain was removed, and processed as describe in material and methods, chapter-II.

**Determination of Protein**
Protein was determined by the method of Lowry et al (1951) using Bovine Serum Albumin as standard.

**Statistical analyses**
As described in material and methods, chapter-II.

**Results**

**Effect of Catechin Hydrate on Behavioral Output**
Tape removal test is a technique that assesses sensory and motor impairments in forepaw function. After 2 h MCAO/22 h reperfusion, an increase in the time needed to remove adhesive tape from the contralateral and ipsilateral forepaws was observed in MCAO group as compared with sham group rats (Fig. 1A). Interestingly, catechin hydrate treated MCAO group (CH + MCAO) significantly shortened the time to remove the adhesive tapes from the forepaws compared with MCAO group (p<0.01). Catechin hydrate alone pre-treated group (CH + Sham) showed no significant changes as compared to sham group. A significant decrease in muscular coordination skill was observed in MCAO group as compared to the sham group animals (p<0.01) (Fig. 1B). Animals treated with catechin hydrate in CH + MCAO group afforded a significant protection in muscular coordination skill, as compared to MCAO group animals (p<0.01). No significant difference was observed between sham and catechin hydrate treated sham group (CH + Sham). The grip strength was found to be significantly decreased (p<0.01) in MCAO group as compared to sham group. Whereas catechins hydrate pretreatment significantly (p<0.05) improved the grip strength in CH + MCAO group as compared to MCAO group. However, no significant alteration was observed in catechin hydrate pretreated sham group (CH + Sham) as compared to sham group (Fig. 1C).
**Effect of Catechin Hydrate on TTC Stain and Infarct Volume**

TTC staining of MCAO brain sections showed reproducible and readily detectable lesions in the areas that are supplied by the MCAO after 22 h of reperfusion (Fig. 2A). The lesions were present in striatum and the overlying cortex. We hypothesized that catechin hydrate play a protective role in stroke. Indeed, MCAO group rats have shown a significantly increased infarct volume as compared with sham. Catechin hydrate pretreatment has reduced the infarct volume significantly (p<0.01) as compared to MCAO group.
Effect of Catechin Hydrate on Cresyl Violet Staining

To access the neuronal damage induced by ischemic reperfusion, we evaluated MCAO group against CH+MCAO group animals. Sections of the MCAO group showed significant neuronal loss, neuronal shrinkage and marked vascular changes throughout the cortex and striatum. Intact neurons were almost absent in that area. The corresponding area in the sections of CH+MCAO group showed partial loss of neuronal staining with presence of intact neurons in between the vacuolated spaces. The section of the sham group showed normal neuronal staining with no pathological change (Fig. 3).

Fig. 2 Effect of catechin hydrate pretreatment for 21 days on brain infarct size by TTC stain after middle cerebral artery occlusion for 2 h and reperfusion of 22 h. Fig. 2 Representative photographs of brain sections stained with 0.1 % TTC, and measurement of infarct volumes of MCAO and CH + MCAO group are presented. MCAO group produced a significant lesion over sham group (figure not shown). However, CH + MCAO group showed a significant (*p<0.01) reduction in tissue damage as compared to MCAO group.

Fig. 3 Cresyl violet staining shows the neuronal alterations in the ipsilateral brain after 2 h MCAO/22 h reperfusion. Normal morphologic features of neurons are present in sham (Fig. A). Characteristic features of hyperchromia, shrinkage of nucleus and cytoplasm in neurons present in MCAO groups (Fig. B). Administration of catechin hydrate clearly ameliorates ischemia-induced neuronal damaged in CH + MCAO (Fig. C). Magnification is 20×
Catechin hydrate Pretreatment Decreased the TBARS Level in Striatum and Frontal Cortex

The effect of catechin hydrate on TBARS level was measured to demonstrate the oxidative damage by lipid peroxidation (LPO) in frontal cortex and striatum of MCAO group rats. A significant increased (p<0.001) TBARS level was observed in MCAO group animals as compared to sham group. Rats of CH + MCAO group has exhibited significant attenuation in TBARS level in frontal cortex (p<0.05) and striatum (p<0.01) as compared to MCAO group rats (Fig. 4). Catechin hydrate alone pre-treated sham group (CH + Sham) showed no significant changes in TBARS level as compared to the sham group.

Fig. 4 Effect of catechin hydrate pretreatment on TBARS contents in the frontal cortex and striatum. TBARS contents were significantly increased in MCAO group as compared to sham group. Catechin hydrate pretreatment has decreased the TBARS significantly in frontal cortex and striatum. Values are expressed as mean ±SEM. (n = 8). *p<0.001 MCAO versus Sham, #p<0.05 ##p<0.01 CH + MCAO versus MCAO

Effect of Catechin Hydrate on the Indices of Protein Oxidation

Protein oxidation was assessed by the determination of protein carbonyl content in the samples of frontal cortex and striatum. MCAO induced a significant (p<0.01) increase in protein carbonyl content. Pre-treatment with CH, however reduced the MCAO-induced increase in protein carbonyl content significantly (p<0.05) in the frontal cortex and striatum. There was no statistical significant reduction in protein carbonyl content in rat pretreated with CH alone in CH + Sham group (Fig. 5).
Effect of Catechin Hydrate on Endogenous Antioxidant System

Protective effect of catechin hydrate on endogenous antioxidant defense system in frontal cortex and striatum was observed. The level of GSH or total free SH group was depleted significantly in frontal cortex and striatum in MCAO group as compared to sham group. Catechin hydrate pretreatment has protected its level significantly \((p < 0.05)\) in CH+MCAO group as compared to MCAO group. Catechin hydrate alone pre-treated group (CH+Sham) exhibited no significant changes in GSH level total free SH group as compared to sham group (Fig. 6). The activities of antioxidant enzymes (GPx, GR, SOD and catalase) were decreased significantly in frontal cortex and striatum of MCAO group animals as compared to sham group animals and their activities were protected significantly in frontal cortex as well as in striatum of catechin hydrate pretreated MCAO group (CH+MCAO) animals as compared to MCAO group animals. No significant change in the activity of these enzymes was observed in CH+Sham group as compared to sham group (Tables 1, 2).

Fig. 5 Effect of catechin hydrate pretreatment on protein carbonyl contents in the frontal cortex and striatum. Protein carbonyl contents were significantly increased in MCAO group as compared to sham group. Catechin hydrate pretreatment has decreased the protein carbonyl contents significantly in frontal cortex and striatum. Values are expressed as mean ± SEM. \((n = 8)\). *\(p<0.001\) MCAO versus S, #\(p<0.05\) CH + MCAO versus MCAO.
Table 1 Activities of antioxidant enzymes (GPx, GR, SOD and catalase) in frontal cortex of MCAO rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sham</th>
<th>MCAO</th>
<th>CH+MCAO</th>
<th>CH+Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>GR (nmol of NADPH oxidized/ min/ mg protein)</td>
<td>282.68 ± 11.78</td>
<td>168.66 ± 7.03*</td>
<td>248.87 ± 6.87#</td>
<td>280.80 ± 10.86</td>
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<tr>
<td>(40.33 %)</td>
<td></td>
<td>(-49.08 %)</td>
<td>(47.55 %)</td>
<td>(-0.66 %)</td>
</tr>
<tr>
<td>GPx (nmol of NADPH oxidized/ min/ mg protein)</td>
<td>206.71 ± 7.81</td>
<td>126.66 ± 6.48*</td>
<td>165.86 ± 9.39#</td>
<td>207.25 ± 8.23</td>
</tr>
<tr>
<td>(39.20 %)</td>
<td></td>
<td>(-39.20 %)</td>
<td>(30.94 %)</td>
<td>(-0.26 %)</td>
</tr>
<tr>
<td>SOD (nmol of epinephrine protected from oxidation/ min/ mg protein)</td>
<td>474.51 ± 32.43</td>
<td>41.58 ± 15.29*</td>
<td>362.31 ± 21.09#</td>
<td>474.95 ± 30.74</td>
</tr>
<tr>
<td>(49.08 %)</td>
<td></td>
<td>(-49.08 %)</td>
<td>(33.32 %)</td>
<td>(0.33 %)</td>
</tr>
<tr>
<td>CAT (nmol of H2O2 consumed/ min/ mg/ protein)</td>
<td>9.85 ± 0.67</td>
<td>3.19 ± 0.98*</td>
<td>6.56 ± 0.78#</td>
<td>9.41 ± 0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-67.61 %)</td>
<td>(105.64 %)</td>
<td>(-4.46 %)</td>
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</table>

Values are expressed as mean±SE in nmoles/ min/ mg protein. Significance was determined as *p<0.05 when compared with sham group; #p<0.01 when compared with MCAO group. Values in parentheses show the percentage increase or decrease with respect to their control.
Table 2 Activities of antioxidant enzymes (GPx, GR, SOD and catalase) in striatum of MCAO rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sham</th>
<th>MCAO</th>
<th>CH+MCAO</th>
<th>CH+Sham</th>
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<tbody>
<tr>
<td></td>
<td>(nmol of NADPH oxidized/min/mg protein)</td>
<td></td>
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<tr>
<td>GR</td>
<td>286.45 ± 7.90</td>
<td>169.07 ± 5.84*</td>
<td>251.64 ± 6.91*</td>
<td>284.85 ± 9.58</td>
</tr>
<tr>
<td></td>
<td>(-40.99%)</td>
<td>(48.83%)</td>
<td>(-0.55%)</td>
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<tr>
<td>GPx</td>
<td>211.09 ± 7.55</td>
<td>128.37 ± 3.36*</td>
<td>168.78 ± 9.52*</td>
<td>211.64 ± 6.22</td>
</tr>
<tr>
<td></td>
<td>(-39.18%)</td>
<td>(31.74%)</td>
<td>(48.83%)</td>
<td>(0.26%)</td>
</tr>
<tr>
<td>SOD</td>
<td>490.77 ± 20.90</td>
<td>251.06 ± 18.99*</td>
<td>366.01 ± 21.55*</td>
<td>493.01 ± 16.57</td>
</tr>
<tr>
<td></td>
<td>(-48.84%)</td>
<td>(45.08%)</td>
<td>(45.08%)</td>
<td>(0.45%)</td>
</tr>
<tr>
<td>CAT</td>
<td>10.18 ± 0.98</td>
<td>3.64 ± 0.54*</td>
<td>6.62 ± 0.63*</td>
<td>9.95 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>(-64.24%)</td>
<td>(18.86%)</td>
<td>(18.86%)</td>
<td>(0.22%)</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E in nmoles/min/mg protein. Significance was determined as *p<0.05 when compared with sham group; #p<0.01 when compared with MCAO group. Values in parentheses show the percentage increase or decrease with respect to their control.

Effect of Catechin Hydrate on NF-kB Expression

A significant increased expression level of NF-kB was observed in MCAO group animals as compared to sham group. Catechin hydrate supplementation has significantly decreased the expression levels of NF-kB in catechin hydrate pretreated MCAO group (CH+MCAO) when compared with MCAO group (Figs. 7A). However no significant alteration was observed in catechin hydrate treated sham group (CH+Sham) as compared to sham group (Data not shown).

Effect of Catechin Hydrate on IL-1β and TNF-α

Quantification of IL-1β and TNF-α in frontal cortex and striatum by ELISA showed a significant increased level of IL-1β (p<0.01) and TNF-α (p<0.001) in MCAO group as compared to sham group. Interestingly, catechin hydrate supplementation has significantly decreased the levels of IL-1β (p<0.01) and TNF-α (p<0.01) in catechin hydrate pretreated MCAO group (CH+MCAO) when compared with MCAO group (Fig. 7B). However, no significant alteration was observed in catechin hydrate treated sham group (CH+Sham) as compared to the sham group.
Effect of Catechin Hydrate Pretreatment on GFAP and iNOS Expression in MCAO Rats

The activation of astrocyte up regulation is associated with neuronal cell death in cerebral ischemia. GFAP expression was found to be remarkably high in ischemic hemisphere of MCAO group. A noticeable reduction in GFAP expression was observed in catechin hydrate pretreated group as compared to MCAO group. Expression of GFAP was seen to be very scarce in sham group animals (Fig. 8A). Catechin hydrate pre-treatment did not show any remarkable effects in the CH + Sham group as compared to sham group (data not shown). Numbers of iNOS positive cells are remarkably high in ischemic hemisphere of MCAO group, which was significantly attenuated with the pretreatment of catechin hydrate. However, iNOS expression was found to be almost negligible in sham group (Fig. 8B). Catechin hydrate pre-treatment did not show any remarkable effects in the CH + Sham group as compared to sham group (data not shown).
Discussion

The present study provides the compelling evidence that catechin hydrate (CH) protects the rat cortex and striatum from ischemia reperfusion (I/R) injury. I/R tissue damage may be a consequence of oxidative stress and could be partially ameliorated by herbal supplementation. In this context, we have evaluated middle cerebral artery occlusion (MCAO) in rats, a model widely used to study neuroprotective effect of drugs because of its recapitulation of biochemical and pathological features of stroke in humans (Khan et al., 2009; Raza et al., 2011; Lakhan et al., 2009; Chan, 2001; Hu et al., 1995).

Oxylradical-induced damage is considered an important factor in the functional deficit in ischemia reperfusion. The behavioral deficits are the common neurological sequel in patients of
cerebral ischemic (Yousuf et al., 2009) and its assessment is a more powerful endpoint in evaluating neuroprotection. The behavioral testing data in the current study provides a sensitive evaluation of the ability of CH to provide protection in this MCAO model. The present study suggested that CH, a well known free radical scavenger has reduced neurobehavioral deficits by scavenging free radicals, which are thought to cause behavioral deficits in experimental animals (Hunter et al., 1998). The poor neurobehavioral outcome in MCAO group rats might be attributed to I/R induce necrosis in the frontal sensorimotor cortices and caudate-putamen, which comprise a wide range of motor and sensorimotor deficits including partial paralysis, poor locomotor activity and lack of coordination (Yousuf et al., 2005; Khan et al., 2010). We report here an appreciable increase in muscular coordination skill by Rotarod and motor strength by grip and tape adhesive remove test following pretreatment with CH. The restoration of CH induce behavioral deficits confirm its protective role against I/R injury, which was in corroborative with the previous similar findings where exogenous antioxidants supplementation have been used to treat cerebral ischemia (Khan et al., 2009; Raza et al., 2011; Chen et al., 2008).

Overproduction of free radicals cause an imbalance in the redox environment of cells, and react with proteins and nucleic acids to alter their functions, or induce lipid peroxidation (LPO) and protein oxidation, leading to eventual cell death. Therefore, scavenging free radicals and preventing lipid and protein peroxidation, which are the main effects of CH, can directly suppress oxidative damage and inflammatory response. In the line of previous findings, we observed different oxidative damage parameters, the critical determinant in I/R brain injury (Saleem et al., 2006) causes a significant increase in LPO and protein oxidation accompanied by significant depletion in brain GSH (Candelario-Jalil, 2009). The above state can be reverted back by nullifying the overproduction of ROS by endogenous antioxidants, especially by GSH (Verbunt et al., 1995). GSH is the major endogenous antioxidant in the brain (Keelan et al., 2001), which scavenges free radicals, reduces peroxides and can be conjugated with electrophilic compounds, thereby providing cells with multiple defenses against both ROS and their byproducts (Shukla et al., 2009). GSH level was found to be low in MCAO group thus often increase the susceptibility of plasma membranes towards peroxide and other free radicals attacks. The main cause of GSH loss during oxidative stress in brain ischemia is the formation of protein glutathione mixed disulphide (PrSSG) and loss of thiol proteins resulting in various membrane defunctioning (Reed,1990). We have observed an elevated level of TBARS accompanied by depleted GSH level in MCAO rat brain and pre-treatment with CH partially attenuates the elevated level of TBARS and depleted level of GSH which is in concomitant with the previous observations where antioxidants were used as a remedy in experimental stroke models (Khan et
GSH along with GPx forms glutathione disulphide in a hydroperoxides reaction. GPx uses GSH as a proton donor, converts H2O2 to water and molecular oxygen; in this process GSH is oxidized to GSSG, which is reconverted to GSH by enzyme GR, thus maintaining the pool. A significant decrease in GPx and GR activity could suggest inactivation by ROS (Huang and Philbert, 1996), which are increased in I/R injury. Another critical enzyme which provides the first line of defenses is SOD, which with GPx and catalase is thought to be the critical enzyme acting as free radical scavengers that could prevent tissue damage caused by peroxidase reactions. A decrease in the activity of the above antioxidant enzymes can lead to an excess availability of superoxide anion (O2•-) and hydrogen peroxide (H2O2), which in turn generate hydroxyl radicals (•OH), resulting in initiation and propagation of lipid peroxidation. SOD can catalyze dismutation of O2 •- into H2O2, which is then deactivated to H2O by GPx or catalase (Aebi, 1984; Kumuhekar and Katyane, 1992). In the present study, pretreatment of CH significantly elevated the GSH and its related enzymes activities along with an increase in activities of SOD and catalase, which is in corroboration with the former reports where antioxidants have been used in experimental I/R models (Cherubini et al., 2008; Khan et al., 2009; Raza et al., 2011; Wang et al., 2010).

In the brain ischemia, NF-κB is involved in excitotoxic, oxidative and inflammatory events associated with neurodegeneration (Zhang et al., 2005; Schwaninger et al., 2006; Zhang et al., 2011; Chen, et al., 2012). Activation of NF-κB also induces pro-inflammatory genes encoding enzymes, cytokines, and other adhesion molecules, all of which are known to promote inflammatory tissue injury (Schwaninger et al., 2006; Harari and Liao, 2010). Consisting with this mechanism, we also found enhanced level of TNF-a and IL-1b, along with increased expression of proinflammatory enzyme iNOS in MCAO group animals. CH treatment downregulated the NF-κB expression and inhibited the upregulation of cytokines level and iNOS expression.

In the present study, we found that two important markers involved in the ischemic inflammatory cascade, GFAP and iNOS, were up-regulated. Increasing evidence points to a correlation between cerebral ischemia and GFAP over activation (Karin et al., 2007). On the other hand flavonoids have been reported to act on glial cells (Vafeiadou et al., 2009). A putative flavonoid binding sites for flavonoid have been described, such as adenosine, glutamate and GABA receptors present in post-mitotic neurons. Astrocytes are believed to be responsible for most glutamate uptake in synaptic areas and consequently are the major regulators of glutamate homeostasis (Matute et al., 2006). An over expression of GFAP in MCAO rats was observed as compared to CH pre-treated rats where GFAP expression has been significantly reduced. The
above protection offered by CH may be attributed to its regulatory mechanism of glutamate upregulation and thus reducing glial mediated ischemic injury.

Further astrocytes elaborate iNOS in response to a series of proinflammatory mediators, including cytokines such as IL-1b and TNF-a (Vaughan and Delanty, et al., 1999). Nitric oxide (NO) derived from iNOS in astrocytes and its oxidative by-product peroxynitrite are thought to contribute to neuronal death due to oxidation of structural neuronal proteins during ischemia (Tunon et al., 2009). It has been demonstrated that flavonoids inhibit the expression of isoforms of inducible nitric oxide synthase which are responsible for the production of nitric oxide, as well as inflammatory mediators such as cytokines, chemokines or adhesion molecules (Sun et al., 2009). Our finding shows that iNOS overexpression in MCAO rats were significantly attenuated with the supplementation of CH. These findings are in harmony with the earlier studies carried out by others (Zhang et al., 2005; Shohami et al., 1996).

To conclude our findings, we sought to observe the changes in the levels of IL-1b, and TNF-a with reference to cerebral ischemia. Together all represents crucial mediator of neurodegeneration induced by ischemic brain injury (Touzani et al., 1999). The increased content of IL-1b and TNF-a in MCAO group was significantly protected in CH+MCAO group rats suggesting the neuroprotective effect of CH following cerebral ischemia may be in part, mediated through modulation of the injury caused by proinflammatory cascades. Pretreatment of CH significantly counteracted the NF-kB associated upregulation of proinflammatory cytokines; a piece of evidence attributed to its anti-inflammatory property of CH which was consistent with the previous finding (Zhang et al., 2011; Tu et al., 2010). Taken together, the present study demonstrates that CH has neuroprotective effects against MCAO insult and its effect might be associated with inhibition of oxidative stress and NF-kB mediated downregulation of inflammatory response.

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