Silymarin protects neurons from oxidative stress associated damages in focal cerebral ischemia: a behavioral, biochemical and immunohistological study in Wistar rats
Introduction

Stroke is the 3rd leading cause of death after heart attack and cancer according to the World Health Organisation (Mackay and Mensah, 2008). About 15 million people worldwide have a stroke annually, out of which approximately 33.3% die and among the survivors 50% are left with permanent disabilities, placing a burden on family and community. Approximately 87% of stroke cases are of ischemic (Lloyd-Jones et al., 2009), a consequence of the disruption of blood flow and oxygen in cerebral arteries thus damaging the physiological activity of the brain, leading to a complicated pathophysiological event involving the interplay between the nervous system and the immune system. The major pathobiological mechanisms of ischemia/reperfusion (IR) injury include excitotoxicity, oxidative stress, inflammation and apoptosis (Yousuf et al., 2009). The ischemic mediated brain damage might be a result of metabolic stress suffered by resident cells, in particular neurons which do not store ATP and are reliant on oxidative metabolism thus guiding improper functioning of mitochondria which in tum disrupt the delicate redox balance therefore triggering the chain of events that follows an ischemic cascade which leads to a combination of necrotic as well as apoptotic cell death pathway.

There has been a substantial body of evidence suggesting that oxidative stress is a fundamental mechanism of brain damage in occlusion and reperfusion ensuing stroke. Reactive oxygen species (ROS) has always been implicated in pathogenesis of ischemia-reperfusion insult, whereby they mediate damage to cell structures, including lipids, membranes, proteins, and DNA, disrupting cellular integrity. Ischemic damage is severe in those regions where cerebral blood flow is restored because reflow of the blood to the brain regions results in an increase in the oxygen level; therefore, severe oxidative injury occurs. ROS productions are more pronounced during reperfusion than ischemia (Courtois et al., 1998; Takemura et al., 1993). In relevance with the above point, many different radical scavengers have been used both in vivo and in vitro to target this critical moment of ROS burst (Hangaishi et al., 2001; Mc Donald et al., 1999).

Furthermore ROS mediated oxidative stress is well implicated in apoptosis. Several studies have revealed that many neurons in the ischemic pen62-69umbra may undergo apoptosis after several hours or days, and thus they are potentially recoverable after the onset of stroke (Racay et al., 2009). Apoptosis is believed to be responsible for up to 50% of cell death during ischemia (Choi, 1996). In general, there are three major mechanisms that have been
proposed to account for apoptosis: mitochondrial pathways, death receptors, and endoplasmic stress. Caspases, which are implicated in all the three mechanism, plays a key role in the execution phase of apoptosis by cleaving specific proteins resulting in an irreversible commitment to cell death.

Numerous experimental models of ischemic stroke have been developed to improve the understanding of detrimental mechanisms involved in the ischemic injury, and to swot up the potential efficiency of therapeutic strategies. Among all the animal models of ischemic stroke, filamentous reversible middle cerebral artery occlusion is one of the most widely used experimental paradigms as it closely mimics stroke in human, yielding trove of information regarding ischemia-reperfusion.

Despite considerable advances in our understanding of the pathophysiology of brain damage during ischemia, attempts to develop therapeutic options are in the progress. Since oxidative damage is implicated in the etiology of neurological complications, flooding the system with antioxidants maybe a sensible step (Ansari et al., 2004).

Silymarin, a flavonoid is a member of the Asteraceae family (Compositae), is extracted from the seeds of milk thistle (*Silybermammarianum*) and is known to own antioxidive(Singhal et al., 2010) and anti-apoptotic properties (Manna et al., 1999). Silymarin has been reported to decrease lipid peroxidation (Bosisio et al., 1992). Furthermore, it has been demonstrated that its anti-oxidative activity is related to the scavenging of free radicals (Raien et al., 1998) and activation of anti-oxidative defences: increases in cellular glutathione (GSH) content (Valenzuela et al., 1989) and superoxide dismutase activity (Muzes et al., 1991). However, less information is available about its effect on behavioral deficits, biochemical parameters and apoptotic changes associated with ischemia-reperfusion. To understand the complex mechanism of ischemia-reperfusion mediated oxidative stress and the above mention fact prompted us to explore its anti-oxidative and anti-apoptotic properties of silymarin on focal cerebral ischemic model in rats.

Experimental procedures

Chemicals and reagents

As described in material and methods, chapter-II
Animals and treatment
As described in material and methods, chapter-II

Drug administration and dose selection
We examined the effects of different doses of silymarin on cerebral ischemia reperfusion injury in pilot studies to determine the optimal dose of silymarin that provides the most neuroprotection against degeneration. A dose of 200 mg/kg of silymarin was selected. This dose has also shown the maximal protection in different types of brain diseases (Lu et al., 2009; Nencini et al., 2007). On the basis of these findings, rats were pretreated systemically with 200 mg/kg silymarin p. o. dissolved in 0.3 % sodium carboxymethyl cellulose, once daily for 15 days. On day 16, MCAO was performed for 2h and reperfusion for 22h.

Middle cerebral artery occlusion (MCAO) to induce focal cerebral ischemia
As described in material and methods, chapter-II

Post-operative care
As described in material and methods, chapter-II

Experimental design
Animals were divided into four groups each having eight animals. The first group served as sham (sham) and received vehicle (0.3% sodium carboxymethyl cellulose) orally, second was middle cerebral artery occluded (MCAO) group and received vehicle only, third was MCAO group pretreated with silymarin (200 mg/kg in 0.3% sodium carboxymethyl cellulose, orally) (Sil+MCAO) and fourth was pretreated with drug alone (Sil+sham) i.e., silymarin group. After the completion of the reperfusion period, the animals were assessed for neurobehavioral activity and then sacrificed. The brains were taken out to dissect striatum and frontal cortex for biochemical estimations.

Neurological deficits
After 22 h of reperfusion, the neurological status of the animals was evaluated using spontaneous motor activity (SMA).
Spontaneous motor activity (SMA)
As described in material and methods, chapter-II

Behavioral studies
The behavioral test in each group was performed before and after occlusion and reperfusion. The experiment was performed between 9.00 A.M. to 4.00 P.M. at standard laboratory conditions. All tests were performed and analyzed by subject blind to the experiment.

Rota rod (muscular coordination)
As described in material and methods, chapter-II

Grip Strength
As described in material and methods, chapter-II

Biochemical studies
Tissue preparation for the assays
After behavioral study, the animals were sacrificed and their brains were taken out to dissect frontal cortex and striatum for the biochemical assays (TBARS, GSH, GPx, GR and SOD) as described in material and methods, chapter-II.

Assay for Na⁺ K⁺-ATPase
As described in material and methods, chapter-II

Histopathological examinations
After 24 h of MCAO, the animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and perfused as previously described by Nakayama et al. (1998). The brains were cut into 10-μm-thick coronal sections on cryostat (Leica, Germany). Every 10 sections of the cortex region, was mounted on glass slides, and processed for hematoxylin and eosin staining.

Immunohistochemistry for p53, Apaf-1 and caspase-9
As described in material and methods, chapter-II.
Caspase-3 activity
As described in material and methods, chapter-II.

Determination of protein
Protein was determined by the method of Lowry et al. (1951) using BSA as a standard.

Statistics
As described in material and methods, chapter-II.

Results
Effect of silymarin on behavioral output
Spontaneous motor activity (SMA)
The Spontaneous motor activity showed no neurological deficits in S group rats, while in MCAO group the neurological deficits was severe at 22 h after reperfusion. Silymarin alone did not show any change in the behavioral assessmentas compared to sham group. In spontaneous motor activity test, MCAO animals spend most of the time in the center of the cage with posture curved towards the paretic side. A marked decrease (p<0.001) in SMA efficiency was observed in MCAO group as compared to sham group. Silymarin pre-treated MCAO group (Sil+MCAO) group significantly (p<0.001) improved the neurological outcomes. Rats moved around in the cage and explored their environment more efficiently as compared to MCAO group (Fig. 1).

Rota Rod
Motor function impairment caused by cerebral ischemia was evident in the MCAO group. Silymarin was found to be effective insignificant (p<0.05) recovery of muscular in-
coordination in MCAO group (Sil + MCAO) as compared to MCAO group. A significant (p<0.001) depletion in muscles coordination was observed in MCAO group as compared to sham group (Fig. 2). No significant difference was found between sham and Silymarin arepretreated sham group.

Grip strength
The grip strength was significantly decreased (p<0.001) in MCAO group as compared to S group rats. The mean score in silymarin pre treated MCAO (Sil+MCAO) group was significantly high (p<0.001) as compared to the vehicle treated MCAO group (Fig. 3). However no significant alteration was observed in silymarin pretreated sham group (Sil+Sham) as compared to sham group.

Effect of silymarin on endogenous antioxidant system
Silymarin pretreatment decreased the TBARS contents in striatum and frontal cortex. The effect of Silymarin on TBARS content was measured to demonstrate the oxidative damage in frontal cortex and striatum of MCAO group. A significant increased (p<0.001) level of TBARS was observed in MCAO group animals as compared to sham group. Rats of Sil+MCAO group exhibited significant attenuation (p<0.05) in TBARS content as compared
to MCAO group (Fig. 4). Silymarin alone pre-treated group showed no significant changes in TBARS as compared to sham group.

Fig 4. The content Silymarin pretreated TBARS content was significantly increased in the MCAO group as compared to S group (\( p < 0.001 \) MCAO vs. S group). Silymarin pretreatment significantly decreased TBARS content in the Sil+MCAO group as compared with MCAO group (\( \# p < 0.05 \) MCAO vs. Sil+MCAO group). Values are expressed as mean±SEM of 8 animals.

Silymarin pretreatment restored the GSH level in striatum and frontal cortex

Protective effect of Silymarin on GSH level in frontal cortex and striatum was observed. The level of GSH was depleted significantly in frontal cortex (\( p < 0.01 \)) and striatum (\( p < 0.001 \)) in MCAO group as compared to sham group. Silymarin pretreatment increased its level significantly (\( p < 0.05, \# p < 0.01 \)) in Sil+MCAO group as compared to MCAO group. Silymarin alone pre-treated group exhibited no significant changes in GSH level as compared to sham group (Fig. 5).

Fig 5. GSH was significantly decreased in MCAO group rats compared to S groups (\( \# p < 0.001 \) MCAO vs. S). Silymarin pretreatment significantly increased the level of GSH in Sil+MCAO rats compared to MCAO rats (\( \# p < 0.05, \# \# p < 0.01 \) MCAO vs. Sil+MCAO group). Values are expressed as mean±SEM of 8 animals.

Effect of silymarin pretreatment on Na\(^{+}\)-K\(^{+}\) ATPase activity in striatum and frontal cortex

The activity of Na\(^{+}\)-K\(^{+}\) ATPase was significantly decreased (\( p < 0.001 \)) in MCAO group as compared to sham group. Silymarin supplementation significantly restored (\( p < 0.05 \& p < 0.01 \)) the activity of Na\(^{+}\)-K\(^{+}\) ATPase in Sil+MCAO group as compared to MCAO group. There was no significant alteration in the activity of Na\(^{+}\)-K\(^{+}\) ATPase in Sil+Sham group animals as compared to sham group (Fig. 6).
Fig. 6. The activity of Na\(^+-\) K\(^+\) ATPase was significantly decreased in the MCAO group rats as compared to S group rats (\(^*p<0.001\) MCAO vs. S). Silymarin pretreatment significantly increased the activity of Na\(^+-\) K\(^+\) ATPase in the Sil + MCAO group rats as compared to MCAO rats (\(^#p<0.05\), \(^##p<0.01\) MCAO vs. Sil + MCAO group) Values are expressed as mean ± SEM of 8 animals.

Silymarin pretreatment attenuated the activities of antioxidant enzymes in frontal cortex and striatum

The activities of antioxidant enzymes (GR, GPx and SOD) were decreased significantly in Table I. Protection on the activity of GPx, GR, SOD in cerebral ischemia by silymarin in frontal cortex of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GPx (nmol NADPH oxidized/min/mg protein)</th>
<th>GR (nmol NADPH oxidized/min/mg protein)</th>
<th>SOD (nmol epinephrine protected from oxidation/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>418.74±23.96</td>
<td>504.26±28.5</td>
<td>432.26±21.51</td>
</tr>
<tr>
<td>MCAO</td>
<td>243.65±28.15 (-41.81%)</td>
<td>297.76±49.4* (-40.95%)</td>
<td>219.36±6.72* (-49.25%)</td>
</tr>
<tr>
<td>Sil+MCAO</td>
<td>384.64±22.96* (57.86%)</td>
<td>428.85±8.39* (44.02%)</td>
<td>350.9±8.2* (59.96%)</td>
</tr>
<tr>
<td>Sil+Sham</td>
<td>421.32±33.43 (0.61%)</td>
<td>494.09±28.36 (2.01%)</td>
<td>425.18±61.75 (-1.63%)</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SE of 8 animals. The activity of GPx, GR, GST and SOD was decreased significantly in MCAO group as compared to S group (\(^*p<0.01\), \(^##p<0.001\)). The pretreatment with 200 mg/kg silymarin has protected their activity significantly in Sil+MCAO group as compared to MCAO group (\(^*p<0.05\), \(^##p<0.001\)). Values in parentheses show the percentage increase or decrease with respect to their control.

frontal cortex as well as in striatum (\(p<0.01 \& 0.001\)) of MCAO groups as compared to S groups and their activities were restored significantly in frontal cortex as well as in striatum (\(p<0.05 \& p<0.01\)) in MCAO group pretreated with silymarin (Sil+MCAO) as compared to MCAO groups. No significant change was observed in Sil+sham groups as compared to S groups (Tables I and II).
Table II. Protection on the activity of GPx, GR and SOD in cerebral ischemia by silymarin in striatum of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GPx(nmol NADPH oxidized/min/mg protein)</th>
<th>GR(nmol NADPH oxidized/min/mg protein)</th>
<th>SOD(nmol epinephrine protected from oxidation/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>458.07±22.55</td>
<td>474.52±30.66</td>
<td>430.15±30.55</td>
</tr>
<tr>
<td>MCAO</td>
<td>288.05±12.96** (-37.11%)</td>
<td>301.59±10.59* (-36.44%)</td>
<td>223.48±19.10** (-48.04%)</td>
</tr>
<tr>
<td>Sil+MCAO</td>
<td>378.05±15.54# (31.24%)</td>
<td>432.07±26# (43.26%)</td>
<td>380.27±12.76## (70.15%)</td>
</tr>
<tr>
<td>Sil+Sham</td>
<td>451.04±22.45 (-1.53%)</td>
<td>473.52±49 (-0.21%)</td>
<td>436.79±33.1 (1.54%)</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E of 8 animals. The activity of GPx, GR, GST and SOD was decreased significantly in MCAO group as compared to S group (*p<0.01, **p<0.001). The pretreatment with 200 mg/kg silymarin has protected their activity significantly in Sil+MCAO group as compared to MCAO group ( #p<0.05, ##p<0.01). Values in parentheses show the percentage increase or decrease with respect to their control.

Effect of silymarin pretreatment on histopathological changes in MCAO rats

Fig. 7 shows the histopathological changes after 2 h of occlusion and 22 h of reperfusion. Sections of the brain passing from frontal cortex of MCAO, Sil+MCAO and sham groups were examined. Sections of MCAO group showed neuronal loss and presence of numerous vacuolated spaces. Intact neurons were absent in that area. The corresponding areas in the

![Image](70)

Fig7. Cortical area of S group animal showing uniform distribution of neuron (A). Normal neurons with the characteristic conical outlines with no abnormal features are seen. Tissues around infarcted area in MCAO group showing a focal area of vacuolation and neuronal loss (B). Silymarin-pretreated group Sil + MCAO shows partial neuronal loss (C). Magnification at 20X.
sections of EA+MCAO group showed partial neuronal loss and the presence of intact neurons in between the vacuolated spaces. The section of the sham group showed normal neurons with no pathological change.

Effect of silymarin pretreatment on p53, Apaf-1 and caspases-9 expression in MCAO rats

The activation of p53 protein is associated with neuronal cell death in cerebral ischemia. p53 expression was found to be remarkably high in ischemic hemisphere of the vehicle treated MCAO group rats (Fig. 8B). A noticeable reduction in p53 expression was observed in silymarin pretreated group as compared to MCAO group (Fig. 8C). Expression of p53 was seen to be very scarce in sham group (Fig. 8A). Silymarin pre-treatment did not show any remarkable effects in the Sil + sham group compared with the sham group (data not shown).
The upregulation of Apaf-1 expression is associated with neuronal cell death in cerebral ischemia. Apaf-1 expression was increased strongly in the ischemic hemisphere of the vehicle control rats. In Sil + MCAO group Apaf-1 was decreased as compared to MCAO group (Fig. 8C). Silymarin pretreatment did not show any remarkable effects on Sil + S group as compared with S group (data not shown). Caspase-9 expression was significantly increased in MCAO group as compared to sham group. Caspase-9 activity was significantly attenuated by the administration of silymarin (Fig. 8C). Silymarin pretreatment did not show any remarkable effects in the Sil + sham group compared with the sham group (data not shown).

Effect of silymarin on Caspase-3 activity
Caspase-3 activity was significantly increased in MCAO group as compared to sham group. Caspase-3 activity was significantly protected by the administration of silymarin (Fig. 9). Silymarin pretreatment did not show any remarkable effects in the Sil+Sham group compared with the sham group (data not shown).

Discussion
In the present study we have critically evaluated the most abundant naturally occurring flavonoid silymarin isolated from milk thesilie as a potential new prophylactic anti-oxidative and anti-apoptotic target in cerebral stroke. Characteristically, ischemic-reperfusion induce brain injury is associated with biochemical, behavioural and histopathological alterations which is seen to be well ameliorated with the pretreatment of silymarin. Herein, we observed that silymarin partially inhibits ischemia reperfusion induce brain injury.
It has been proposed that oxidative stress plays a critical role in the development of pathogenesis of cerebral ischemia (Halliwell, 1992), which is associated with an increased production of free radicals, specifically hydroxyl radical, superoxide, higher lipid peroxidation and lower enzymatic antioxidant defences. The above damages can be prevented by detoxification of free radicals (Dringen, 2000). Beneficial effects of various antioxidants and free radical scavengers in ischemic stroke have been demonstrated in a number of studies (Andrabi et al., 2006). Neuroprotective effects of Silymarin suggest that it is a powerful antioxidant, corroborating previous studies (Bosisio et al., 1992). Silymarin seems to protect against oxidative stress by decreasing lipid peroxidation, a sensitive marker of oxidative damage. The mechanisms manifested to overcome the stress induce by pro-oxidants includes: enhanced protein synthesis (Sonnenbichler et al., 1999), scavenging free radicals (Locher et al., 1997), increase in glutathione (GSH) content (Valenzuela et al., 1989), and preservation of cell membrane integrity (Dehmlow et al., 1996). Recent studies also show that silymarin possesses anti-apoptotic activity (Li et al., 2007). Silymarin has also shown to reverse the deficits observed in spatial and social memory in rats. A less evidences are reports that silymarin has protective effects on the central nervous system against ethanol-induced brain injury (LaGrange et al., 1999) and lipopolysaccharide-induced neurotoxicity (Wang et al., 2010).

Experimental models of stroke have been developed in animals in an attempt to mimic the events of human cerebral ischemia and to investigate the pathology of cerebral ischemia. However the success behind these models lies only when they can reproduce the end results. The long term goal of animal model is to identify and develop treatments that may enhance recovery of stroke survivors. Here we have used a well-characterized filamentous MCAO rat model which physically resembles the most common form of human stroke i.e. thrombo-embolic infraction. Clinical characteristics of stroke such as movement disorders and muscle strength dysfunctions are known to well mimic in the aforesaid model.

Free radicals are known to play key role in neurobehavioral deficit in experimental models through oxidative stress (Fukui et al., 2002). The poor neurobehavioral outcome in ischemic group may be due to free radicals generation, which alters locomotion and motor coordination due to the necrosis induce in sensorimotor cortices and caudate-putamen (Hunter et al., 1998) which has a command on motor and sensorimotor activities. The rotarod and grip strength have indicated that vehicle injection did not cause deterioration of
motor performance in the rats, while the MCAO group showed depletion in locomotion and poor co-ordination. In addition, we also observed that the spontaneous motor activity was severely altered in the ischemic group. The pre-injection of silymarin protected these events significantly.

Oxidative stress induce biochemical alterations are well-known in ischemia reperfusion brain injury. Cells have evolved elaborated systems, including enzymatic and non-enzymatic systems to cope with various forms of oxidative stress. Surplus amount of free radicals generation is thought to be the key module of neuronal damage because of their high reactivity and capacity to produce cellular impairments (Demopoulos et al., 1980). ROS threaten to neuronal survival by their ability to propagate the initial attack on lipid rich membranes of the brain to cause LPO (Kale et al., 1993). However, the cell damage can be prevented by detoxification of free radicals, which eventually prevent the progress of LPO.

We have observed an elevated level of LPO in the form of TBARS accompanied by depleted content of glutathione (GSH) which are in agreement with the previous study (Zafar et al., 2003). This may be due to hyperexcitability of neurons in the early post-ischemic period caused by excessive accumulation of glutamate in the extracellular fluid which can induces excessive activation of N-methyl-D-aspartate resulting in accumulation of intracellular fluid and sodium and calcium ions which induced generation of lipid peroxide and free radicals. However, the above alteration was significantly ameliorated with the pretreatment of silymarin, which was in agreement with the previous observations where antioxidant was taken as a remedy in ischemia-reperfusion models (Imam and Ali, 2000).

The protection of TBARS and GSH level suggests that silymarin not only functions as a simple antioxidant to inhibit lipid peroxidation but also as a modulator of cellular antioxidant potential by the restoration of GSH, which is an oxy-radical scavenger. GSH is the main non-enzymatic antioxidant in defending against electrophiles along with its important role in defending against oxygen free radicals; in addition it helps to maintain other low molecular weights antioxidants in their biological active forms (Meister, 1994). A reduction in the level of GSH may impair H$_2$O$_2$ clearance and promote formation of hydroxyl radical (·OH), the most toxic molecule of the cell, leading to oxidative insult and so results in various membrane dysfunction such as increase membrane permeability to ions by inactivating membrane bound enzymes (Rossier et al., 1987) like Na$^+$/K$^+$ ATPase, which is essential for cellular excitability and is very susceptible to free radical attack and LPO because of its
requirement for phospholipids for its activity and finally lead to cell death (Yu et al., 1994). Our data has shown that silymarin supplementation significantly reversed the depleted level of GSH, as well as the activity of Na⁺/K⁺ ATPase in the cerebral cortex and striatum of MCAO group rats. The above observations are in concordance with the early reports, where silymarin was used to ameliorate neurodegenerative diseases (Lu et al., 2009; Nencini et al., 2007).

As all antioxidant defences are interconnected (Sun et al., 2009), hence disruption of one would disrupt the whole microenvironment. The ischemia-reperfusion causes an overproduction of free radicals which, in turn, causes oxidative damages to membrane's lipid and protein levels, and ultimately leads to a decrease in the content of GSH and activity of its dependent enzymes (GPx and GR) activity along with SOD. This oxidative neuronal damage in ischemic brain insult is consistent with previous reports (Wang et al., 2002). However, pretreatment of silymarin significantly counteracted all the alteration in the markers of oxidative damage; this occurs often due to scavenging and neuroprotective properties of silymarin which was consistent with the previous findings (Baluchnejadmojarad et al., 2010; Chtourou et al., 2010).

Superoxide or its derivatives have been shown to damage or destroy cells in a variety of ways in ischemic-reperfusion insult. It is well appreciated that, under ischemia, free radical burden is preliminarily contained by SOD. The role of superoxide anion in the present model of brain injury is supported by the observation that SOD activity was significantly reduced in ischemic brain. These findings are in agreement with previous findings on ischemic brain injury (Chan, 1996). Prophylactic administration of silymarin prior to ischemia reperfusion injury significantly increased SOD activity, probably by utilising the production of superoxide radicals (O₂⁻) which was produced during ischemia-reperfusion. Similar effects were observed by other authors in a variety of experimental models where the superoxide anion has been implicated as one of the factor involved in deleterious effects of ischemia-reperfusion (Xu et al., 2009; Jung et al., 2009).

It has been well known that ischemia-reperfusion induce brain injury in rodents induces neuronal loss by apoptosis (Wang et al., 2009). Within minutes after focal cerebral ischemia, the cell in core region of the brain tissue exposed to dramatically reduced blood flow undergoes necrosis. This necrotic core is surrounded by a highly susceptible apoptosis zone, penumbra. Various signalling pathways are known to be involved in apoptotic cell death.
Induction of pro-apoptotic protein p53 signalling pathway is among one of them (Wang et al., 2010). MCAO induce focal ischemia causes DNA damage, inducing p53-controlled cytochrome c release from mitochondria, which then binds to apoptosis-activating factor-1 (Apaf-1), resulting in the activation of caspases 9, followed by the activation of effector caspases-3 leading to apoptotic cell death. Induction of p53, Apaf-1 and caspase-3 activation is associated with stroke-induced apoptotic neuronal cell death (Ji et al., 2007) and inhibition of p53, Apaf-1 expression and caspase-inhibition reduced ischemic injury (Fortin et al., 2001; Khan et al., 2010). These data are consistent with our findings that silymarin reduced the expression of p53, Apaf-1 and caspase-9 positive cells accompanied with activation of caspase-3. These findings were in agreement with the previous findings were antioxidants were used to ameliorate the neurodegenerative diseases (Diaz-Ruiz et al., 2007; Chong et al., 2003). In the present study, we showed that caspase-3 activity, p53, caspases-9 and Apaf-1 expressions arisen after ischemic injury; however these expressions were significantly suppressed by silymarin pre-treatment.