Chapter VII

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

Stroke is a third commonest cause of morbidity and mortality after ischemic heart disease and cancer worldwide. It is single largest cause of long-term disability in millions of people in developed countries (Bhuiyan and Kim, 2010). Ischemic stroke produces severe reduction of blood flow to the affected brain region, which leads to neurological consequences. Minor changes in the cerebral oxygen and glucose supply may invoke damage that is irreversible because the brain has limited repair capabilities. The disadvantage of the neuronal repair is the inability of the neurons to divide in vivo. This implies that all neuronal loss caused by cerebral ischemia is irreversible and that it permanently affects the functioning of the brain. The major pathobiological mechanisms following ischemia/reperfusion (IR) injury include excitotoxicity, oxidative stress, inflammation and apoptosis (Lee et al., 2012; Buch et al., 2012; Lakhan et al., 2009; Broughton et al., 2009). These ensuing cascades associated with mitochondrial dysfunction and rapid decrease in ATP, which leads to the free radical generation and lipid peroxidation (Shah et al., 2010, Khan et al., 2010, Chen et al., 2011).

Accumulated evidence suggests that oxidative stress play a fundamental mechanism of brain damage in occlusion and reperfusion following cerebral ischemia. Reactive oxygen species (ROS) has always been implicated in pathogenesis of ischemia-reperfusion injury, whereby they mediate damage to cardinal cellular structures such as DNA, proteins, membranes, including lipids and disrupting cellular integrity leading to subsequent neuronal cell death (Yossi et al., 2002). Oxidative stress culminates due to an imbalance between prooxidants and antioxidants. Superoxide ($O_2^-$) is the key ROS and generates $H_2O_2$ by dismutation. $O_2^-$ is produced in tissues via a number of enzymatic reactions and common cellular sources of $O_2^-$ include auto-oxidation of small molecules, mitochondrial components and oxidative enzymes, e.g. nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase, cyclooxygenases, and oxidation of unsaturated fatty acids (Stamler et al., 1992).

However, enormous efforts have focused on the development of neuroprotective strategies to limit brain damage caused by acute ischemic stroke, there is as yet no routine, effective, generally accepted, specific cure for acute ischemic stroke, except for thrombolytic agent (Horn and Limburg, 2001). Therefore, it is essential to have an alternative line of therapy, which not only promotes symptomatic reinforcement but also has a neuroprotective activity. Therapy using free radical scavengers such as naturally derived antioxidants has the potential to prevent, delay, or ameliorate many neurologic disorders. However, the results of clinical trials using various naturally occurring compounds reported to powerful radical scavenging properties probably due its antioxidant activity.
Thymoquinone (TQ) is the bioactive constituent of the volatile oil of nigella sativa and has been shown to exert antioxidant and anti-inflammatory effects (Houghton et al., 1995; Abdel-Fattah et al., 2000). TQ acts as a scavenger of superoxide, hydroxyl radical and singlet molecular oxygen (Badary et al., 2003). TQ has also exerted neuroprotective effects against transient forebrain ischemia and MPP+ and rotenone model of Parkinson’s disease in rat (Al-Majed et al., 2006; Radad et al., 2009). Although TQ is a powerful antioxidant, its administration is limited due to poor water solubility (Gali-Muhtasib et al., 2006), leading to low absorptivity and bioavailability. In addition, administration of high dosages to rats has resulted in hypoactivity and difficulty in respiration associated with reduced glutathione in the liver and kidney (Badary et al., 1997). To overcome these disadvantages, biodegradable and biocompatible polymeric nanoparticles would be attractive alternatives for TQ delivery. Such nanoparticles would likely provide improved TQ solubility, controlled delivery, and enhanced therapeutic properties.

In recent years, nanoparticle-based controlled delivery of antioxidants has allowed a novel approach for disease prevention and treatment which possess excellent free radical scavenging activity and bioavailability (Ganea et al., 2010). We have polymeric nanoparticle based formulation of TQ-nanoparticles utilizing the micellar aggregates of cross-linked and random copolymers of Nisopropylacrylamide (NIPAAM), with N-vinyl-2-pyrrolidone (VP) and poly ethyleneglycol monoacrylate (PEG-A), which provide potent antioxidant activity and enhanced uptake by cell as compared to the native TQ (Bisht et al., 2007).

**Experimental Procedure**

**Chemicals and reagents**

As described in material and methods, chapter-II

**Preparation of polymeric nanoparticles**

**Synthesis and characterization of NIPAAM/ VP/ PEG-A copolymeric nanoparticles (DLS and TEM)**

The polymeric nanoparticles of thymoquinone were formulated in collaboration with the Department of Industrial Chemistry, Jamia Hamdard, New Delhi. The polymeric nanoparticle was synthesised by the method of Bisht et al., (2007). Random co-polymerization of NIPAAM with VP and PEGA was performed by free radical polymerization process of the micellar aggregates of the amphipilic monomers. The polymeric nanoparticles formed in this way also have an amphiphilic character with a hydrophobic core inside the micelles, and a hydrophilic outer shell composed of hydrated amides, pyrrolidone and PEG moieties that project from the monomeric units.
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The size and size distribution of the polymeric nanoparticles were measured by means of dynamic light scattering (DLS). In Fig. 1A, the typical graph of size distribution of the nanoparticles is illustrated, and the average size corresponds to less than 130 nm diameter at 25°C with a narrow size distribution. Photomicrograph of Transmission electron microscopy (TEM) of the polymeric nanoparticles is illustrated in Fig. 1B, and shows that the particles have spherical morphology and low polydispersity with an approximate size of around 115 nm diameter, which is comparable to the size obtained from DLS measurements.

**Fig. 1** Size characterization of the polymeric nanoparticles using dynamic laser light scattering (DLS) and transmission electron micrograph (TEM) studies. (A) DLS of the polymeric nanoparticles confirms a narrow size distribution in the 130 nm range. All the data analysis was performed in automatic mode. (B) TEM picture demonstrates particles with a spherical morphology, low polydispersity, and an average size of 115 nm, comparable to what is observed in the DLS studies.

**Animals and treatments**

As described in material and methods, chapter-II

**Drug administration and dose selection**

In a pilot study effects of different doses of nanoparticle of thymoquinone (0.25, 0.50 and 0.75 mg/kg b.wt.) and thymoquinone (5 mg/kg b.wt.) on cerebral ischemia reperfusion injury to determine the optimal dose that provides the most neuroprotection against degeneration. On the basis of biochemical estimation of TBARS and GSH, a dose of 5 mg/kg b.wt. of TQ and 0.25 mg/kg b.wt. for nanoparticles of TQ (NTQ) was found to be most protective. The dose of TQ (5 mg/kg b.wt.) has also shown the maximal protection in different types of diseases (El-Abhar, 2003; Al-Majed 2006). On the basis of these findings, rats were pretreated interaperitoneally (i.p.) with 5 mg/kg b.wt. of TQ and 0.25 mg/kg b.wt. of NTQ daily for 15 days. Additional injections of TQ (5 mg/kg) and NTQ (0.25 mg/kg) were administered 30
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minutes before the onset of ischemia and after the ischemia at the interval of 0 and 6 h. On day 16, MCAO was performed for 2 h and reperfusion for 22 h.

**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**

The MCAO model of Longa et al., (1989) was used to investigate the neuroprotective effects of TQ and NTQ. Animals were divided into six groups each group having eight animals. The first group served as sham (S) and vehicle was given i.p., 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 with TQ (5 mg/ kg b.wt., i.p., TQ+MCAO group), the fourth was pretreated with NTQ (0.25 mg/ kg b.wt., i.p., NTQ+MCAO group) respectively followed by MCAO for 2 h and reperfusion for 22 h, the fifth was pretreated with TQ alone (TQ+S group), and the sixth was pretreated with NTQ alone (NTQ+S group). Rats were pretreated interaperitoneally (i.p.) with 5 mg/ kg b.wt. of TQ and 0.25 mg/ kg b.wt. of NTQ daily for 15 days. Additional injections of TQ (5 mg/ kg) and NTQ (0.25 mg/ kg) were given 30 minutes before the onset of ischemia and after the ischemia at the interval of 0 and 6 h. After the completion of the reperfusion period, the animals were assessed for neurobehavioral activity and then sacrificed. The brains were taken out to dissect the frontal cortex for biochemical estimations.

**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

**Evaluation of ischemic damage**

**Infarct volume analysis**

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 for the biochemical assays (TBARS, GSH, GPx, GR, SOD and Catalase) 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 standard.

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

**Results**

**Effect of TQ and NTQ on behavioral output**

**Rota Rod**

The muscular coordination skill showed no neurological deficits in sham group rats, while in MCAO group the neurological deficits were severe at 22 h after reperfusion. TQ and NTQ alone did not show any change in the behavioral assessment as compared to sham group. In muscular coordination skill, a significant (P<0.05) improvement was observed in the TQ + M and NTQ + M (P<0.01) group in staying on the accelerating rod for a longer duration of time when compared with the MCAO group (Fig. 2A).

\((M = \text{MCAO}, \ S = \text{Sham})\)

![Fig. 2](image)

**Fig. 2.** TQ and NTQ pretreatment for 15 days improve performances in the muscular coordination skill and grip test after cerebral ischemia. A significant decrease in motor coordination in MCAO group as compared to sham group was observed and it was significantly more protected in NTQ and TQ as compared to MCAO group (Fig. 2A). Values are expressed as mean ± SEM for 8 animals. *p<0.001, MCAO vs sham; #p<0.05, TQ + M and ##p<0.01, NTQ + M vs MCAO. The grip strength was decreased significantly in MCAO group animals as compared to sham group animals. Pretreating the animals with TQ and NTQ followed by MCAO has protected motor deficit as compared to MCAO group (Fig. 2B). Values are expressed as mean ± SEM of 8 animals. *p<0.001, MCAO vs sham; #p<0.05, TQ + M and ##p<0.01, NTQ + M vs MCAO.
**Grip Test**

The grip strength was found to be significantly decreased ($p<0.001$) in MCAO group as compared to sham group. A noticeable improvement in grip strength was observed in TQ+MCAO ($p<0.05$) and NTQ + MCAO ($p<0.01$) pretreated group when compared with MCAO group animals (Fig. 2B). However, no significant alteration was observed in TQ and NTQ pretreated sham group (TQ + S, NTQ + S) as compared to sham group.

**Effect of TQ and NTQ on TTC stain and infarction volume**

Brain infarction is an important index for estimating neuronal damage with subsequent neurological impairment. TTC staining of the brain sections obtained from MCAO rats showed reproducible and readily detectable lesions in the infarct area at 24 h after MCAO (Fig. 3). The lesions were present in hippocampus, lateral striatum and the overlying cortex. We hypothesized that NTQ play a more protective role in cerebral ischemia than TQ. It was observed that TQ and NTQ pretreatment significantly decreased ($p<0.05; p<0.01$) the infarct volume as compared to the MCAO group. There was no significant difference in the infarct volume between sham and TQ + S and NTQ + S group (Data not shown).

![Fig. 3. Effect of TQ and NTQ pretreatment for 15 days on brain infarct size by TTC stain after middle cerebral artery occlusion for 2 h and reperfusion of 22 h. Representative photographs of the brain sections stained with 0.1% TTC, and measurement of infarct volumes of MCAO and TQ + MCAO and NTQ + MCAO group are presented. MCAO group produced a significant lesion over sham group (Data not shown). However, TQ + MCAO and NTQ + MCAO group showed a significant reduction in tissue damage as compared to MCAO.](image)
Effect of TQ and NTQ on endogenous antioxidant system

TQ and NTQ treatment decreased the TBARS Level in Frontal Cortex

The level of TBARS was elevated significantly (p<0.01) in MCAO group rats as compared to sham group rats. Rats of TQ + MCAO (p<0.05) and NTQ + MCAO (p<0.01) group has exhibited significant decrease in TBARS level in frontal cortex as compared to MCAO group rats (Fig. 4). NTQ + M1 group showing more protective effect as compared with NTQ + M2 and NTQ + M3 group rats. TQ and NTQ alone pre-treated sham group (TQ + S and NTQ +S) showed no significant changes in TBARS level as compared to sham group.

(M, M1, M2, M3=MCAO, S, S1, S2, S3=Sham)

![Graph showing TBARS content](image)

Fig. 4. Effect of TQ and NTQ pretreatment on TBARS content in frontal cortex in the MCAO group rats. Thiobarbituric acid reactive substances content was significantly (*P<0.01) increased in the MCAO group as compared with sham group. TQ and NTQ treatment has decreased the content of TBARS significantly in the frontal cortex of the TQ + M (#P<0.05) and NTQ + M (##P<0.01) group as compared with the MCAO group. Values are expressed as means ± SEM (n = 8).

TQ and NTQ treatment restored the GSH level in frontal cortex

Protective effect of TQ and NTQ on GSH level in frontal cortex was observed. The level of GSH was depleted significantly (P<0.01) in frontal cortex of MCAO group as compared to sham group. TQ and NTQ pretreatment has restored its level significantly in TQ + MCAO (p<0.05) and NTQ + MCAO (p<0.01) group as compared to MCAO group. NTQ + M1 group exhibit more protective effect as compared with NTQ + M2 and NTQ + M3 group rats.
TQ and NTQ alone pretreated Sham group (TQ + S, NTQ + S) exhibited no significant changes in GSH level as compared to sham group (Fig. 5).

(M, M1, M2, M3=MCAO, S, S1, S2, S3=Sham)

![Graph showing GSH level in different groups]

**Fig. 5.** Effect of TQ and NTQ pretreatment on GSH level in the frontal cortex. Reduced glutathione level was significantly decreased in the frontal cortex (*p<0.01) of MCAO group rats as compared with sham group. TQ and NTQ pretreatment has significantly (#p<0.05) increased the level of GSH in the frontal cortex of TQ + M and NTQ + M group as compared with the MCAO group. Values are expressed as means ± SEM (n = 8).

**TQ and NTQ treatment attenuated the activities of antioxidant enzymes in frontal cortex**

The activities of antioxidant enzymes (GPx, GR, SOD and Catalase) were depleted significantly (p<0.01) in frontal cortex of the MCAO group animals as compared with the sham group animals. TQ and NTQ pretreatment significantly increase the activity of antioxidant enzyme as compared with MCAO group animals. No significant change was observed in TQ + S and NTQ + S group as compared to sham group (Tables 1).
Table 1. Effect of cerebral ischemia on the activity of various enzymes in frontal cortex of different groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sham</th>
<th>MCAO</th>
<th>TQ+MCAO</th>
<th>NTQ+MCAO</th>
<th>TQ+S</th>
<th>NTQ+S</th>
</tr>
</thead>
<tbody>
<tr>
<td>GR (nmol of NADPH oxidized/min/mg protein)</td>
<td>269.3±23.08</td>
<td>141.4±27.90</td>
<td>199.2±10.21</td>
<td>219.1±14.62</td>
<td>265.4±25.06</td>
<td>267.8±25.06</td>
</tr>
<tr>
<td></td>
<td>(−47.57%)</td>
<td>(−47.57%)</td>
<td>(40.87%)</td>
<td>(54.96%)</td>
<td>(−1.45%)</td>
<td>(−0.53%)</td>
</tr>
<tr>
<td>GPx (nmol of NADPH oxidized/min/mg protein)</td>
<td>219.6±18.23</td>
<td>131.1±8.68</td>
<td>181.2±10.33</td>
<td>193.1±9.02</td>
<td>217.6±22.34</td>
<td>221.3±22.34</td>
</tr>
<tr>
<td></td>
<td>(−40.30%)</td>
<td>(−40.30%)</td>
<td>(38.19%)</td>
<td>(47.84%)</td>
<td>(−0.90%)</td>
<td>(0.75%)</td>
</tr>
<tr>
<td>SOD (nmol of epinephrine protected from oxidation/min/mg protein)</td>
<td>419.6±19.22</td>
<td>220.4±12.90</td>
<td>332.4±32.41</td>
<td>345.3±22.08</td>
<td>422.2±26.70</td>
<td>420.5±26.70</td>
</tr>
<tr>
<td></td>
<td>(−47.47%)</td>
<td>(−47.47%)</td>
<td>(50.79%)</td>
<td>(56.66%)</td>
<td>(0.60%)</td>
<td>(0.21%)</td>
</tr>
<tr>
<td>CAT (nmol of H2O2 consumed/min/mg protein)</td>
<td>9.1±0.73</td>
<td>3.7±0.09</td>
<td>5.6±0.38</td>
<td>5.9±0.69</td>
<td>8.9±0.91</td>
<td>9.8±0.91</td>
</tr>
<tr>
<td></td>
<td>(−59.21%)</td>
<td>(−59.21%)</td>
<td>(52.68%)</td>
<td>(59.94%)</td>
<td>(−1.75%)</td>
<td>(0.84%)</td>
</tr>
</tbody>
</table>

Table 1. Values are expressed as mean±S.E in nmoles/min/mg protein. Significance was determined as *p<0.01, when MCAO compared with sham group; #p<0.05, ##p<0.01, when TQ+MCAO and NTQ+MCAO compared with MCAO group. Values in parentheses show the percentage increase or decrease with respect to their control.

Discussion

In the present study, we used thymoquinone (TQ) and polymer-based nanoparticles of thymoquinone (NTQ) to evaluate the best one on neuroprotection. We examined their abilities to ameliorate oxidative stress as a consequence of cerebral ischemia. The finding of present study suggest that the dose of NTQ was more effective as free TQ to improve the behavioral outcome, reduces oxidative damage, and suppresses the neuronal loss due to its potential antioxidant property and enhanced uptake by cell. It suppresses the early accumulation of lipid peroxidation products and raises the activity of antioxidant enzymes.

Even though their benefits, the approach of exogenous delivery of the native form of TQ to neutralize the deleterious effect of ROS has been less effective because TQ has poor water solubility, leading to low absorptivity and bioavailability across the BBB. Different alternatives have been investigated to resolve these issues. Recently, a novel antioxidant loaded drug delivery systems such as polymeric nanoparticles have been recognized as alternatives that should provide long term delivery at the therapeutic level, prevent antioxidant degradation, and increase pharmacological activity of such antioxidants (Kaur, et al., 2007, Ganea et al., 2010).
Disruption of the BBB as a consequence of cerebral ischemia can make the tight junction between the endothelial cells of the brain capillaries leaky. Therefore, it is quite possible that increased permeability of the BBB during reperfusion following transient focal brain ischemia might have allowed nanoparticles to cross the BBB to the brain parenchyma (Reddy and Labhasetwar, 2009; Jin et al., 2012). Earlier studies have reported that nanoparticles particles of approximately 200 nm in diameter, are able to cross the BBB after intravenous administration and act as drug carriers for CNS (Costantino et al., 2010; Gabathuler et al., 2010; Tsai et al., 2011).

In this framework, we have evaluated the neuroprotective activity of TQ and NTQ in MCAO rat model with reperfusion for the behavioral and biochemical parameters which are widely used to study neuroprotective effect of drugs because it recapitulates the biochemical and pathological features of stroke in humans (Khan et al., 2009; Longa et al., 1989). It is well recognized that MCAO results in behavioral and neurochemical modifications in rat brain and excessive generation of free radicals has been implicated in brain damage through different cellular and molecular mechanisms, and it is further aggravated by impaired cellular antioxidant defense systems under ischemic conditions to be one of the major causative factors (Chan, 2001; Khan et al., 2010).

The neurobehavioral parameter is related to the degree of neuronal dysfunction (Schwarting et al., 1991). Our study revealed that NTQ was more potent than free TQ treatment; it has improved neurobehavioral activity and reduced infarct volume, especially in the cerebral cortex as compared with MCAO group animals by scavenging free radicals, which are thought to cause behavioral deficits in experimental model of cerebral ischemia (Beckman et al., 1990). Previous studies have shown an improvement in various behavioral outputs like motor coordination skill as a result of antioxidant treatment and antioxidant loaded drug delivery systems such as polymeric nanoparticles (Zafar et al., 2003; Kovalenko et al., 2006; Takamiya et al., 2011 ). In the present study, the motor function was found to be disturbed in rota rod and grip strength task. We observed impaired motor functions after ischemic insults in rat and was improved significantly improved by supplementation of NTQ and TQ.

Large quantities of ROS are generated after cerebral ischemia which leads to accumulation of lipid peroxidation products causing cellular disintegrity and neuronal loss (Kontos et al., 1992; Chen et al., 2011). Oxidative stress is a major factor in cerebral ischemic reperusions injury because the brain consumes a large quantity of oxygen and less equipped with antioxidant defenses, and so free radicals/ oxidants released by inflammatory cells threaten tissue viability in the surrounding area of the ischemic core. Excessive reactive oxygen species (ROS) have been...
indicated as one of the earliest and most important components of tissue injury after reperfusion of the ischemic organ through different cellular and molecular mechanisms (Wu et al., 2008; Pandey et al., 2012). ROS threaten neuronal endurance by their ability to propagate the initial attack on lipid rich membranes of the brain to cause lipid peroxidation. Lipid peroxides cause secondary damage by further generating relatively more stable and diffusible cytotoxic agents like malondialdehyde (MDA) and 4-hydroxy-trans-2-nonenal (4-HNE), respectively (Halliwell, 2007). They react with cellular nucleophiles such as GSH, and causes constant decrease in its level through increased oxidant content or protein modification. GSH is a well-known endogenous anti-oxidant that is synthesized in the cytoplasm and is present in higher concentrations in the mitochondrial matrix. The low levels of GSH may be directly related to increased ROS, lipid peroxides, and highly reactive hydroxyl radicals (Ishrat et al., 2009; Ahmad et al., 2012). The neurons are extremely vulnerable to oxidative insults due to low level of GSH (Dringen, 2000) which provides protection to the cells from oxidative damage by scavenging free radicals and active oxygen species (McLennan et al., 1991). Thus, GSH inhibition in cerebral ischemia would increase the susceptibility of plasma membranes towards peroxide 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 dysfunctioning. The present study shows that an elevated level of TBARS accompanied by depleted GSH level in MCAO rat brain was significantly attenuated the elevated level of TBARS and depleted level of GSH by administration of TQ and NTQ. These results are in conformity with earlier studies (Kiray et al., 2008; Yousuf et al., 2009; Khan et al., 2010), the present study of MCAO causes neural damage by generating overproduction of free radicals, which might have caused oxidative damage to membrane lipid and protein and ultimately lead to a decrease in GSH content.

Endogenous cellular antioxidant enzymes determine the cellular sensitivity to oxidative damage. The decreased activity of glutathione dependent enzymes, GPx and GR in the MCAO group resulted from the imbalance between ROS production and the endogenous scavenging system (Haung and Philbert, 1996). GPx plays a principal role in removing excess free radicals and hydroperoxides and is a major defence system against oxidative stress in the brain. GPx and GR are known to be inactivated by oxidant radicals, as observed in the present study and some earlier studies (Khan et al., 2010; Raza et al., 2011). ROS are usually scavenged by antioxidant enzymes, primarily by superoxide dismutase (SOD), by catalyzing the dismutation reaction of the superoxide anion to hydrogen peroxide. Catalase and glutathione peroxidase, on the other hand, protect cells from the toxic effects of hydrogen peroxide by catalyzing its decomposition.
into water (Freeman and Crapo, 1982). Under ischemic conditions, the imbalance between the two processes results in oxidative stress and contributes to several pathophysiological conditions. During reperfusion, oxidative stress causes damage as a result of the excess production of ROS and insufficiency of the free-radical scavenging process. Ultimately the imbalance may form a vicious circle which results in the collapse of the endogenous system. We observed that pretreatment with NTQ exhibit more potent efficacy than free TQ, which might be due to the restoration of the imbalance in the production, excellent free radical scavenging activity and enhanced bioavailability. Our results are in conformity with previous study (Shah and Vohora, 2002; Salim et al., 2003; Khan et al., 2009; Ahmad et al., 2011) that have shown that the treatment of rats with natural antioxidant resulted the increased activity of these enzymes because of the ROS scavenging activity of these conventional drugs which might lead to the restoration of the depleted enzymes.

**Conclusion**

Overall our finding demonstrate that the encapsulated TQ nanoparticles is a new potential treatment regime for improving neurobehavioral outcomes and shielding neuron from oxidative damages through strong antioxidative mechanisms in a rat cerebral ischemia-reperfusion model. The mechanism of efficacy appears to be due to enhanced antioxidant properties and sustained delivery of the encapsulated TQ, thus neutralizing the detrimental effect of ROS. Further studies to understand the neuroprotective potential and mechanisms of antioxidant loaded drug delivery systems and action of NTQ is essential to resolve, whether it can be a successful medication for ischemic stroke.

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