Neuroprotective effects of thymoquinone against transient forebrain ischemia in the rat hippocampus

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Abstract

Increasing evidence demonstrates that oxidative stress plays an important role in brain injury in experimental models of brain ischemia. Thymoquinone, the main constituents of the volatile oil from Negella sativa seeds, is reported to possess strong antioxidant properties. Hence, the present study was undertaken to evaluate the neuroprotective effect of thymoquinone against transient forebrain ischemia-induced neuronal damage in the rat hippocampus. Rats were divided randomly into five groups: control, sham, ischemia, thymoquinone and ischemia+thymoquinone. Transient forebrain ischemia was induced with bilateral occlusion of both common carotid arteries for 10 min followed by 7 days of reperfusion. Thymoquinone was administered (5 mg/kg/day p.o.) 5 days before ischemia and continued during the reperfusion time. Animals were sacrificed, and brain tissues were isolated for histopathological examination. Hippocampal tissues were also used for determination of malondialdehyde levels, an end product of lipid peroxidation; glutathione (GSH) levels, a key antioxidant and the activities of the antioxidant enzymes catalase and superoxide dismutase (SOD). Thymoquinone and its metabolite thymohydroquinone were tested as inhibitors of the in vitro non-enzymatic lipid peroxidation induced by iron-ascorbate in the hippocampal homogenate. Forebrain ischemia–reperfusion neural injury in rats was demonstrated by histopathological observation, which revealed significant neural cell death in the hippocampus CA1 area 7 days post-ischemia (77% cell loss). Additionally, forebrain ischemia–reperfusion oxidative injury in rats was demonstrated by a significant increase in malondialdehyde and a significant decrease in GSH contents, catalase and SOD activities in the hippocampal tissue compared to the control or sham-operated groups. Pretreatment of thymoquinone attenuated forebrain ischemia-induced neuronal damage manifested by significantly decreasing the number of dead hippocampal neuronal cells (24% in thymoquinone-treated versus 77% for ischemia, P<0.001), which confirm the protective role of thymoquinone in ischemia–reperfusion injury. Also, pretreatment of ischemic rats with thymoquinone decreased the elevated levels of malondialdehyde and increased GSH contents, catalase and SOD activities to normal levels. Thymoquinone and thymohydroquinone inhibited the in vitro non-enzymatic lipid peroxidation in hippocampal homogenate induced by iron-ascorbate. The IC50 for thymoquinone and thymohydroquinone were found to be 12 and 3 μM respectively. This suggests that the protection of thymoquinone and its metabolite involve increased resistance to oxidative stress. In conclusion, thymoquinone is effective in protecting rats against transient forebrain ischemia-induced damage in the rat hippocampus. This spectacular protection makes thymoquinone a promising agent in pathologies implicating neurodegeneration such as cerebral ischemia.

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Keywords: Thymoquinone; Forebrain ischemia; Oxidative stress; Hippocampal damage

1. Introduction

Ischemic brain damage is a major cause of adult disability (Levy et al., 1985). Transient global cerebral ischemia (forebrain ischemia), occurring during cardiorespiratory arrest in patients or experimentally in animals, induces selective and delayed neuronal cell death (Petito et al., 1987). Pyramidal neurons in the CA1 region of the hippocampus are particularly vulnerable and die after global ischemia (Kirino, 1982; Pulsinelli et al., 1982; Kirino, 2000). Hippocampal CA1 injury is observed 3 to 7 days after untreated forebrain ischemia in the rat, gerbil, and human (Arai et al., 1986; Petito et al., 1987; Colbourne et al., 1999). Forebrain ischemia induces complete interruption of blood flow, producing inadequate delivery of oxygen to brain tissue and leading to a decrease in glucose utilization and adenosine

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Oxidative stress is one of the primary factors that exacerbate damage by cerebral ischemia (Chan, 2001). Several components of reactive oxygen species (superoxide, hydroxyl radical, hydrogen peroxide and peroxynitrite radical) that are generated after ischemia–reperfusion injury play an important role in neuronal loss after cerebral ischemia (Imaizumi et al., 1984; Oliver et al., 1990). Superoxide and hydroxyl radical are potent in producing destruction of the cell membrane by inducing lipid peroxidation (Bromont et al., 1989). Inducible nitric oxide synthase (iNOS) is upregulated after ischemia–reperfusion injury. This results in excessive nitric oxide (NO) production. This excess NO reacts with superoxide to form peroxynitrite, a powerful radical that produces neuronal death after cerebral ischemia. The brain is particularly vulnerable to oxidative stress injury because of its high rate of oxidative metabolic activity, intense production of reactive oxygen species metabolites, and high content of polyunsaturated fatty acids, relatively low antioxidant capacity, low repair mechanism activity and non-replicating nature of its neuronal cells (Evans, 1993).

Many antioxidants are reported to reduce reactive oxygen species-mediated reactions and rescue neurons from ischemia–reperfusion-induced neural loss in animal models of cerebral ischemia (Al-Omar et al., 2006; Clemens et al., 1991; Cuzzocrea et al., 2000; Fuson et al., 1999; Soehle et al., 1998; Thiyagarajan and Sharma, 2004). Thymoquinone, the main constituent of the volatile oil from *Nigella sativa* seeds is reported to possess a strong antioxidant property (Houghton et al., 1995). Thymoquinone (Fig. 1) protects organs against oxidative damage induced by a variety of free radical generating agents including doxorubicin-induced cardiotoxicity (Nagi and Mansour, 2000), carbon tetra-chloride evoked hepatotoxicity (Nagi et al., 1999), nephropathy produced by cisplatin (Badary et al., 1997), autoimmune as well as allergic encephalomyelitis (Mohamed et al., 2003; Mohamed et al., 2005) and gastric mucosal injury induced by ischemia reperfusion (El-Abhar et al., 2003). The present study was undertaken to test whether oral administration of thymoquinone could protect against transient forebrain ischemia-induced neuronal damage in the rat hippocampus.

## 2. Materials and methods

### 2.1. Chemicals

Thymoquinone, thiobarbituric acid, Ellman’s reagent (5,5′-dithiobis-(2-nitrobenzoic acid; DTNB) and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of the highest analytical grades commercially available.

### 2.2. Animals

Male Wister albino rats, weighing 230–250 g, were obtained from the Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia and were housed in metabolic cages under controlled environmental conditions (25 °C and a 12 h light/dark cycle). Animals had free access to pulverized standard rat pellet food and tap water unless otherwise indicated. The protocol of this study has been approved by Research Ethics Committee of College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

### 2.3. Experimental protocols

Rats were divided randomly into five groups of 15 animals each: control, sham, ischemia, thymoquinone alone and ischemia + thymoquinone. Transient forebrain ischemia was induced with bilateral occlusion of both common carotid arteries for 10 min followed by 7 days of reperfusion. Thymoquinone was administered (5 mg/kg/day p.o.; Badary et al., 1997; Badary, 1999; El-Abhar et al., 2003) 5 days before ischemia and continued during the reperfusion time. After the animals were sacrificed, brain and hippocampal tissue were isolated for biochemical studies and histopathological examination.

### 2.4. Induction of transient forebrain ischemia

Transient forebrain ischemia was induced in the rats under general anaesthesia (sodium pentobarbital; 30 mg/kg, i.p.) with 2-vessel occlusion combined with systemic hypotension according to the method of Smith et al. (1984) and Henrich-Noack et al. (1996). First, blood was gradually withdrawn from the jugular vein into a heparinized syringe to reduce mean arterial blood pressure to 45–50 mmHg. Reduction of blood pressure was measured by a non-invasive tail cuff plethysmography method (Muromachi BP-Monitor MK-2000, Muromachi Kihai CO. Ltd., Tokyo, Japan). The common carotid arteries were exposed by means of a ventral midline neck incision. Briefly, in the supine position, a midline ventral incision was made in the neck. Both common carotid arteries were exposed, separated from the vagus nerve and occluded for 10 min with microaneurysmal clips (Diefenbach Bulldog Clamp, 25 mm straight, Germany) which consistently resulted in delayed neuronal death in the CA1 region of the hippocampus (Kirino, 1982; Henrich-Noack et al., 1996). At the end of the

![Fig. 1. Chemical structures of thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone) and thymohydroquinone.](image-url)
occlusion period, the clamps were released; allowing restoration of
carotid blood flow. The shed blood was re-injected to restore blood
pressure to normal, and the incision was sutured with 2–0 silk sutures.
In sham-operated animals, the arteries were freed from connective
tissue but were not occluded. Body temperature was kept at 37 °C by
using controlled heating pad and heating lamps throughout the entire
period of ischemia and post-ischemic recovery under anesthesia. A
rectal thermometer was used to monitor body temperature (Aplex
Rectal Thermometer, Panlab, Bagneux, France).

2.5. Biochemical analysis

7 days after ischemia, 10 rats from each group were decapitated,
the brains were quickly removed and the hippocampi were har-
vested on a cold stage. Hippocampi were washed with saline,
blotted dry on a filter paper and weighed before 10% (w/v) homog-
genates were made in ice-cold saline.

2.5.1. Assay of lipid peroxidation and reduced glutathione

The degree of lipid peroxidation in the hippocampal neuronal
tissue was determined by measuring thiobarbituric acid reactive
substance in the tissue homogenate (Ohkawa et al., 1979). The absorbance was measured spectrophotometrically at
532 nm and the concentrations were expressed as nmol malon-
dialdehyde/mg protein. The tissue levels of the acid soluble
thiols, mainly GSH, were assayed colorimetrically at 412 nm
(Ellman, 1959). The contents of GSH were expressed as μmol/g
wet tissue.

Fig. 2. Protective effect of thymoquinone against ischemia-mediated cell loss in the CA1 hippocampal area 7 days after forebrain ischemia in rat. Photomicrographs
illustrate neurons within the CA1 region of the hippocampus stained with hematoxylin and eosin at a magnification of 20× after transient forebrain ischemia. A.
Coronal sections showing intact neurons in the hippocampal CA1 region of the control rats; B. coronal sections showing intact neurons in the hippocampal CA1 region
of the sham-operated rats; C. administration of thymoquinone (5 mg/kg/day p.o.) alone for 7 successive days showed no effect on the number of the intact neurons in
the hippocampal CA1 region; D. most pyramidal cell died in the CA1 subfield 7 days following reperfusion in rats subjected to 10 min forebrain ischemia. In contrast,
administration of thymoquinone (5 mg/kg/day p.o.) 5 days before ischemia and continued for 7 successive days conferred neuroprotection by markedly reduced
number of damaged pyramidal cells in the CA1 subfield E. (scale bar 50 μm).
2.5.2. Assay of catalase and superoxide dismutase

The catalase activity was determined spectrophotometrically by the method of Higgins et al. (1978). The decrease in absorbance of hydrogen peroxide (H$_2$O$_2$) at 240 nm was followed. The activity was expressed as $\mu$mol/min/mg protein using the molar absorbance of 43.6 for H$_2$O$_2$. The superoxide dismutase assay was a slight modification of the indirect inhibition assay developed by McCord and Fridovich (1969). In this method, xanthine-oxidase was utilized to generate a superoxide flux, which reacts with 2-(4-iodophenyl)-3(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The absorbance obtained from (4-iodophenyl)-3(4-nitrophenol)-5-phenyltetrazolium chloride reduction to red formazon by superoxide was measured at 505 nm.

2.5.3. Measurement of protein

Protein concentration was estimated by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

2.5.4. Assay of the in vitro non-enzymatic lipid peroxidation induced by iron-ascorbate in the hippocampus homogenate

Lipid peroxidation in the hippocampal tissue was determined by measuring thiobarbituric acid reactive substance induced by iron-ascorbate (Houghton et al., 1995). The reaction mixture contained varying amount of thymoquinone or thymohydroquinone, 0.75 ml phosphate buffer (50 mM, pH 7.4), 50 μl hippocampal homogenate, 0.1 ml of 1 mM ferric chloride and 0.1 ml of 1 mM ascorbic acid. The tubes were incubated at 37 °C for 30 min and the extent of peroxidation was measured by thiobarbituric acid test. To these test tubes, the following were added: 0.1 ml butylated hydroxytolouene (2% w/v) to stop further lipid peroxidation, 1.0 ml thiobarbituric acid (1% w/v in 0.05 M NaOH) and 1.0 ml TCA acid (2.8%). The tubes were then placed in a water bath at 90 °C for 20 min. At the end of the incubation time, the tubes were cooled and centrifuged for 5 min at 5000 g. The chromogen was extracted with 2.0 ml n-butanol and absorbance was read at 532 nm. Tubes without antioxidants were subjected to thiobarbituric acid test and served as control. Data were plotted as a percentage of inhibition.

2.6. Histological analysis of hippocampal CA1 subregion

7 days after ischemia, 5 rats from each group were anesthetized with pentobarbital (100 mg/kg). Rats were then transectually perfused with cold saline followed by 4% formalin in phosphate-buffered saline (0.1 M; pH 7.4). The brains were removed from the skull and fixed in the same fixative for 24 h. Thereafter, the brains were embedded in paraffin and 5 μm thick sections were coronally cut at the level of the dorsal hippocampus by a rotatory microtome (Leica CM3050S, Leica Microsystems, Bensheim, Germany). The segments of the hippocampal CA1 region per 1000 μm lengths from bregma $-3.3$, $-3.8$, and $-4.3$ were counted for viable cells. Tissue sections were stained with hematoxylin and eosin. The hippocampal damage was determined by counting the number of intact neurons in the stratum pyramidal within the CA1 subfield at a magnification of 20× (Nikon E 600, digital camera DXM1200F, Nikon Corporation, Tokyo, Japan). Only neurons with normal visible nuclei were counted. The mean number of CA1 neurons per millimeter linear length for both hemispheres in sections of dorsal hippocampus was calculated for each group of animals. An observer who was unaware of the drug treatment for each rat made all the assessments of the histological sections.

Fig. 3. Effect of thymoquinone on hippocampal cell death induced by transient forebrain ischemia. Rats were divided randomly into five groups of 5 animals each: control, sham, ischemia, thymoquinone and ischemia+thymoquinone. Transient forebrain ischemia was induced with bilateral occlusion of both common carotid arteries for 10 min followed by 7 days of reperfusion. Thymoquinone was administered (5 mg/kg/day p.o.) 5 days before ischemia and continued during the reperfusion time. Animals were sacrificed, and hippocampal tissues were isolated for neuron counting. Results are expressed as mean±S.D. of 5 rats and data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparisons test. * Significantly different from the control, sham, thymoquinone and ischemia+thymoquinone groups.

Fig. 4. Effect thymoquinone on malondialdehyde levels a. and GSH contents b. in rat hippocampus after forebrain ischemia. Results are expressed as mean±SD of 10 rats and data were analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparisons test. * Significantly different from the control, sham, TQ and ischemia + TQ groups.
2.7. Statistical analysis

Differences between obtained values (mean±S.D.) were carried out by one way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test. A $P$ value of 0.05 or less was taken as a criterion for a statistically significant difference.

3. Results

The effects of 10 min of forebrain ischemia, thymoquinone and their combination on the number of intact neurons in CA1 subregion of rat hippocampus are shown in Figs. 2 and 3. Histopathological examination of the hippocampal CA1 subregion from the control (Fig. 2A), sham-operated (Fig. 2B) and thymoquinone-treated (Fig. 2C) groups revealed normal intact neurons. However, 7 days after the forebrain ischemia, there was widespread damage to the CA1 region of the hippocampus, as demonstrated by a highly significant decrease (77%) in the number of intact neurons (38.0±5.4 neurons for ischemia versus 164.5±3.5 neurons for control) (Figs. 2D, 3). Neuronal death in the CA1 hippocampal sector was significantly reduced by administration of thymoquinone (Fig. 2E) 5 days before ischemia and continued for 7 successive days. This was manifested as a significant increase (3.2 fold) in the number of intact neurons as compared to the ischemia group (Fig. 3).

7 days after 10 min of forebrain ischemia, malondialdehyde content, an index of lipid peroxidation, was significantly elevated in ischemia-subjected rats to reach 202% of the control and sham-operated groups (Fig. 4A; $P<0.001$). Ischemia-mediated lipid peroxidation was decreased significantly in thymoquinone -administered rats as shown in Fig. 4. The administration of thymoquinone in the non-ischemic groups did not alter malondialdehyde levels. Thymoquinone and its metabolite thymohydroquinone inhibited the in vitro non-enzymatic lipid peroxidation in hippocampus homogenate induced by iron-ascorbate. The IC50 for thymoquinone and thymohydroquinone were found to be 12 and 3 μM respectively (Fig. 5).

7 days after 10 min of forebrain ischemia, there was a 33% decrease in hippocampal GSH levels in comparison to the control and sham-operated groups. Treatment with thymoquinone (5 mg/kg, p.o.) significantly and dramatically increased GSH levels of ischemic rats ($P<0.001$; Fig. 4B). The administration of thymoquinone to the non-ischemic group did not alter GSH levels.

Table 1
Effect of thymoquinone on catalase and superoxide dismutase activities in the hippocampal tissue

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Catalase (μmol/min/mg)</th>
<th>SOD (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.28±0.15</td>
<td>4.51±0.5</td>
</tr>
<tr>
<td>Sham</td>
<td>1.27±0.38</td>
<td>4.52±0.4</td>
</tr>
<tr>
<td>Ischemia</td>
<td>0.79±0.06 a</td>
<td>2.59±0.4 b</td>
</tr>
<tr>
<td>Thymoquinone</td>
<td>1.28±0.07</td>
<td>4.63±0.5</td>
</tr>
<tr>
<td>Ischemia+thymoquinone</td>
<td>1.27±0.11</td>
<td>4.67±0.22</td>
</tr>
</tbody>
</table>

a Significantly different from the control, sham, thymoquinone and ischemia + thymoquinone groups.

b Significantly different from the control, sham, thymoquinone and ischemia + thymoquinone groups.

The results of SOD and catalase activities were summarized in Table 1. The hippocampal activity of both SOD and catalase was significantly decreased in the ischemic rats as compared to the control and sham-operated groups by 43 and 37%, respectively ($P<0.001$). Treatment with thymoquinone, however, elevated the activity of the two enzymes to nearly control values.

4. Discussion

The results of this study demonstrated that thymoquinone protects rats from ischemia-induced brain injury. This protection was evident from the significant reduction in neuronal cell death in the hippocampal CA1 region, the significant decrease in the elevated levels of malondialdehyde, and reversal of the decreased GSH contents, catalase and SOD activities to normal levels after forebrain ischemia. Additionally, thymoquinone and thymohydroquinone inhibited the in vitro non-enzymatic lipid peroxidation in hippocampal homogenate induced by iron-ascorbate.

It is well documented that transient forebrain ischemia results in death of the neurons in the CA1 subregion of the hippocampus. Our results indicated that 10 min of forebrain ischemia induced selective neuronal damage. About 77% of the hippocampal CA1 neurons were lost (Figs. 2, 3). These results are in a harmony with other studies (Al-Omar et al., 2006; Kirino, 1982; Knuckey et al., 1995; Al Nita et al., 2001; Candelario-Jalil et al., 2001). Treatment with thymoquinone offered protection against hippocampal CA1 neuronal damage induced by 10 min of forebrain ischemia as evidenced by the fact that thymoquinone rescued most of CA1 pyramidal neurons from ischemic death.

The biochemical mechanism involved in the development of ischemia-induced brain injury is well studied (Evans, 1993; Petito et al., 1987; Kirino, 1982; Kirino, 2000). Reactive oxygen species are critical determinants in brain injury (Chan, 2001; Flamm et al., 1978). We found that forebrain ischemia caused a significant increase in lipid peroxides. These results are in agreement with other studies (Al-Omar et al., 2006; Chan, 2001; Evans, 1993; Candelario-Jalil et al., 2001).

The overproduction of reactive oxygen species can be detoxified by endogenous antioxidants, causing their cellular stores to be depleted (Candelario-Jalil et al., 2001). GSH, which is considered the most prevalent and important intracellular non-protein thiol, has a crucial role as a reactive oxygen species scavenger. In the current work, GSH content was significantly reduced due to ischemic insult. This could be explained by the consumption of...
GSH due to scavenging of the rapidly generating reactive oxygen species due to ischemia. The current work also showed that there was a significant decline in forebrain activity of the endogenous antioxidant enzymes SOD and catalase in ischemic rats. These results are in harmony with other studies (Such as Al-Omar et al., 2006; Homi et al., 2002).

Pretreatment of rats with thymoquinone were found to produce significantly less lipid peroxides than in ischemic rats. In the present study, both thymoquinone and thymohydroquinone also produced significant reduction of iron-ascorbate induced oxidative stress as indicated by the reduction in malondialdehyde production in hippocampal tissue. Quinone reductase, first described as DT-Diaphorase (Ernster et al., 1962), catalyses the two electron reduction of quinones to hydroquinones, preventing their participation in redox cycling, and subsequent generation of reactive oxygen species (Brunmark et al., 1988; Lind et al., 1982). Compounds that increase quinone reductase expression, including hippocampus (Schultzberg et al., 1988; Stringer et al., 2004). Quinone reductase activity was found in all rat brain regions, including hippocampus (Schultzberg et al., 1988; Stringer et al., 2004). Compounds that increase quinone reductase expression, such as tert-butyl-hydroquinone, significantly decrease the cytotoxic effects of glutamate, H2O2, and dopamine, which are known to generate oxidative stress-induced neurotoxicity (Murphy et al., 1991). Thymoquinone is a substrate for quinone reductase that forms dihydrothymoquinone (Nagi et al., 1999). Thymoquinone administration showed a significant induction in the enzyme activity of hepatic quinone reductase (Badary and Gamal El-Din, 2001). Whether quinone reductase is induced in the brain tissue by administration of thymoquinone or its metabolite dihydrothymoquinone is not known. We hypothesize that up-regulation of quinone reductase in hippocampal pyramidal cell neurons is a cellular neuroprotective response to dihydrothymoquinone based on the similarity between dihydrothymoquinone and tert-butyl-hydroquinone.

Thymoquinone treatment was able to confer protection against hippocampal glutathione depletion. In this model, treatment with thymoquinone was expected to protect the rat brain against oxidative damage, revealed as normalization of the inhibited antioxidant enzymatic systems. Indeed, thymoquinone proved to be beneficial in restoring declined SOD and catalase due to ischemia insult.

The antioxidant activities of thymoquinone have been previously reported (Houghton et al., 1995; Nagi and Mansour, 2000). The inhibition of lipid peroxidation obtained with thymoquinone as well as its metabolite thymohydroquinone (Nagi et al., 1999) could account for the marked anti-oxidative stress effect of thymoquinone. Moreover thymoquinone acts as a scavenger of superoxide, hydroxyl radical and singlet molecular oxygen (Kruk et al., 2000; Badary et al., 2003). Thymoquinone can also reduce reactive oxygen species production indirectly. The inhibitory effect of thymoquinone against NO production has been reported. Thymoquinone inhibited the iNOS protein synthesis and iNOS mRNA expression in rat lipopolysaccharide-stimulated peritoneal macrophage cells (El-Mahmoudy et al., 2002). It is likely that ischemia-induced oxidative stress may lead to inflammation, resulting in the upregulation of iNOS and overproduction of NO that lead to the formation of peroxynitrite, a potent oxidizing molecule capable of eliciting lipid peroxidation and cellular damage. Thus, thymoquinone may reduce oxidative stress-induced inflammation leading to the prevention of iNOS upregulation and therefore thymoquinone will inhibit the formation of peroxynitrite. Thymoquinone was found to inhibit arachidonic acid metabolism (Houghton et al., 1995). Marsik et al. (2005) demonstrated that thymoquinone acts as an inhibitor of cyclooxygenase-1 and -2. Thymoquinone also inhibited synthesis of 5-lipoxygenase products in polymorphonuclear leukocytes from rats (El-Dakhakhny et al., 2002). The literature suggests that a marked generation of reactive oxygen species derived from the metabolism of arachidonic acid can cause organ oxidative damage (Reilly et al., 2001). Indeed, it has been suggested that COX-2 contributes specifically to the delayed progression of ischemic brain damage (Chan, 2001). The possible multi-inhibition of reactive oxygen species obtained with thymoquinone could account for its marked anti-oxidative stress effect on rat hippocampus. The results of the protective effects of thymoquinone against oxidative stress-induced hippocampal damage are in agreement with previous observations that thymoquinone protects against doxorubicin-induced cardiotoxicity (Nagi and Mansour, 2000), and cisplatin-induced nephrotoxicity (Badary et al., 1997), and carbon tetrachloride-induced hepatotoxicity (Nagi et al., 1999). These are all models of toxicity where oxidative stress is a common denominator. Also these findings are in accordance with several publications using the same ischemia model. Many antioxidants proved efficient as neuroprotectors, restoring deranged enzymes and parameters of oxidative damage, such as aminoguanidine (Al-Majed, 2004), ginkgo (Seif-El-Nasr and Abd-El-Fattah, 1995), curcumin (Al-Omar et al., 2006; Thiyagarajan and Sharma, 2004) and N-acetylcysteine (Knuckey et al., 1995).

In summary, oral administration of thymoquinone protected rats from ischemia-induced brain injury. The protection may be due to the reduction of oxidative stress. These observations suggest that thymoquinone may be a clinically viable protective agent against a variety of conditions where cellular damage is a consequence of oxidative stress. In addition, thymoquinone may have the potential to be used in the prevention of neurodegenerative diseases such as forebrain ischemia.

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