The Neuroprotective Effects of Thymoquinone: A Review

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Abstract

Thymoquinone (TQ), one of the main components active of *Nigella sativa*, exhibited very useful biomedical effects such as anti-inflammatory, antioxidant, antimicrobial, antiparasitic, anticancer, hypoglycemic, antihypertensive, and antiasthmatic effects. There are several studies about pharmacological activities of TQ but its neuroprotection effects are not fully described. The literature search has indicated many studies pertaining to the effects of TQ in neurological problems such as epilepsy, parkinsonism, anxiety, and improvement of learning and memory, and so on. In addition, TQ protected brain cells from various injuries due to its antioxidant, anti-inflammatory, and apoptotic effects in cell line and experimental animal models. The present study has been designed to review the scientific literature about the pharmacological activities of TQ to the neurological diseases. This study purposed that although experimental studies indicated the beneficial effects of TQ against nervous system problems, better designed clinical trials in humans are needed to confirm these effects.

Introduction

Plants as natural producers of chemical compounds are used as traditional medicines for human health.[1] *Nigella sativa* (of the family Ranunculaceae) is commonly called black cumin, fennel flower, or nutmeg flower. Kalonji seeds[2] and Ajaji, black caraway seed, and habbatu sawda are other names of *N sativa*.[3] It is considered as a medicinal herb with some religious usage, calling it the "remedy for all diseases except death" (Prophetic hadith)[4] and Habatul Baraka "the Blessed Seed."[3] The black cumin oil consists of main medicinal components such as tocopherols, phytosterols, polyunsaturated fatty acids, thymoquinone (TQ), ρ-cymene, carvacrol, *t*-anethole, and 4-terpineol. Thymoquinone (2-isopropyl-5- methyl benzo-1, 4-quinone), the main ingredients of the *N sativa* seeds, has been found in many medicinal plants such as several genera of the Lamiaceae family (Monarda) and the Cupressaceae family (Juniperus).[5] Thymoquinone is the main ingredient of the plant, which is effective for treatment of various diseases such as neurodegenerative disorders, coronary artery diseases, and respiratory and urinary system diseases.[6-13] Thymoquinone has also been indicated to possess antioxidant, anti-inflammation, anticancer, antibacterial, antimutagenic, and antigenotoxic activities.[9,14-27] Thymoquinone may be considered as a

therapeutic agent for the prevention of oral supplementation of chrysin (100 mg/kg body weight) to hyperammonemic rats, which considerably restored the levels of brain ammonia, water content, and the expressions of glutamine synthetase (GS), glial fibrillary acidic protein (GFAP), tumor necrosis factor α (TNF-α), interleukin (IL) 1β, IL-6, p65, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2). Our findings provided substantial evidence that the

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Table 1. A Summary of Neuroprotective Effects of Thymoquinone.

Ext./Cons.	Concentration	Experimental Model	Study Condition	Effects	References
TQ	2.5, 5, and 10 μM	BV2 mouse microglia cell line	LPS-induced neuroinflammation	Inhibition of NF-κB-mediated neuroinflammation by the activation of Nrf2/ARE signaling pathway	[19]
				Inhibition of inflammatory mediators (NO, PGE2, TNF-α, and IL-1β) production by blocking PI3K/Akt/NF-κB signaling pathway	[58]
				Attenuating neuroinflammation by decreasing IL-6, IL-1β, IL-12p40/70, CCL12/MCP-5, CCL2/MCP-1, GCSF, and Cxcl10/IP-10	[60]
	0-100 μM	Rat	LPS-induced depression-like behavior	Prevented depression behavior by decreasing immobility time and improving crossing number of animals in FST	[66]
	40 mg/kg		Lithium–pilocarpine model of SE	Prevented epilepsy by modulating Nrf2 signaling pathway involved in the activation of antioxidant defense system	[72]
	10 mg/kg		Lithium–pilocarpine model of SE	Prevented epilepsy by decreasing gene expression of NF-κB, which mediates inflammatory reactions	[20]
	10 mg/kg		Intrahippocampal kainate model of TLE	Prevented seizure activity and lipid peroxidation, hippocampal neuronal loss, and MFS and mitigate astrogliosis	[76]
	10 mg/kg		PTZ-induced seizure model	Prolonged the onset of seizures and decreased the duration of myoclonic seizures through an opioid receptor-mediated increase in GABAergic tone	[36]
	40 and 80 mg/kg		PTZ-induced seizure model	Prolonged the onset of seizures via ameliorating the decreased expression of GABAB1R, CaMKII, inhibition phosphorylation of CREB, decreased Bcl-2 expression, and activated caspase-3	[77]
	40 mg/kg	Human	Intractable seizure model	Has no effect on neurological function, laboratory variables, or vital signs, but was effective and tolerable	[78]
	1 mg/kg	Rat	Rotenone model of PD	Prevented motor defects via ameliorating oxidative stress	[85]
	7.5 and 15 mg/kg		MPP+-induced cell death	Protected mesencephalic dopaminergic neurons via preservation of mitochondrial function and inhibition of apoptotic cell death	[86]
	0.01, 0.1, 1, and 10 μM	Rat hippocampal and hiPSC	Alpha (SN)-induced synaptic toxicity in rat hippocampal and hiPSC-derived neurons	Protected neurons against inhibition of spontaneous firing activity and restoration of mutated P123H-induced inhibition of synaptic vesicle recycling	[89]
	0.01, 0.1, 1, 10 nM	Mice primary dopaminergic culture	MPP+- and rotenone-induced cell death	Protected primary dopaminergic neuron against MPP+- and rotenone-induced cell death	[90]
	0.1, 1, 10, 100 nM	Rat primary hippocampal and cortical neurons	$A\beta_{1-42}$-induced neurotoxicity in hippocampal and cortical neurons	Prevented neurotoxicity induced by $A\beta_{1-42}$ via ameliorating oxidative stress	[91]
	0.1 and 1 M	CGNs	$A\beta_{1-40}$-induced neuronal cell death	Prevented neurotoxicity induced by $A\beta_{1-40}$ via inhibiting apoptosis mediated by both extrinsic and intrinsic caspase pathways	[94]
	0.1 and 1 μM	Rat	LPS-mediated AD model	TQ plus PAM treatment in AD can be more effective than single drug treatment	[97]

(continued)

Table 1. (continued)

Ext./Cons.	Concentration	Experimental Model	Study Condition	Effects	References
	5 mg/kg		Transient forebrain ischemia by bilateral occlusion of carotid arteries	Prevented ischemia by decreasing oxidative stress-induced inflammation; increasing GSH, CAT, and SOD activities; preventing iNOS upregulation; inhibiting the formation of peroxynitrite	[47]
	2.5, 5, and 10 mg/kg		Global cerebral IRI	Prevented ischemia by decreasing oxidative stress	[101]
	5 mg/kg		TBI model	Prevented TBI by reducing the MDA levels in the neuronal nuclei and mitochondrial membranes of neurons	[106]
	10 and 20 mg/kg	Mice	Stressed condition by 6-hour immobilization	TQ prevented the feelings of anxiety and fear by modulating NO-cGMP and GABA-ergic pathways which play a main role in the unstressed condition	[107]
	1 mg/kg	Rat	EAE model	TQ prevented EAE by modulating oxidative stress	

Abbreviations: TQ, thymoquinone; hiPSC, human-induced pluripotent stem cell-derived neurons; CGNs, cerebellar granule neurons; LPS, lipopolysaccharides; SE, status epilepticus; TLE, temporal lobe epilepsy; PTZ, pentylenetetrazole; PD, Parkinson disease; MPP, 1-methyl-4-phenylpyridinium; Aβ, β-amyloid peptide; AD, Alzheimer disease; IRI, ischemia–reperfusion injury; TBI, traumatic brain injury; EAE, experimental allergic encephalomyelitis; NF-κB, nuclear factor kappa-activated B cells; Nrf2/ARE, nuclear factor (erythroid-derived 2)-like 2; NO, nitric oxide; PGE2, prostaglandin E2; TNF-α, tumor necrosis factor α; IL, interleukin; CCL/MCP, chemokine (C-C motif) ligand monocyte chemoattractant protein; GCSF, granulocyte colony-stimulating factor; Cxcl10/IP-10, C-X-C motif chemokine IFNγ-induced protein 10; PI3K/Akt, phosphoinositide 3-kinase; Cgmp, cyclic guanosine monophosphate; FST, forced swimming test; MFS, mossy fiber sprouting; GABAB1R, gamma-aminobutyric acid B1 receptor; CaMKII, calcium/calmodulin-dependent protein kinase II; CREB, response element-binding protein; GABAergic, gamma-aminobutyric acid; GSH, glutathione; CAT, catalase; SOD, superoxide dismutase; iNOS, inducible nitric oxide synthase nitric oxide; NO, nitric oxide.

chrysin synergistically attenuates the neuroinflammatory mechanism by repressing the expression of pro-inflammatory cytokines and upregulating the astrocytic protein expressions via ammonia-reducing strategies. These data suggest that TQ effectively acts as a therapeutic agent to treat hyperammonemia-mediated neuroinflammation.

However, the exact mechanism of TQ involved in the prevention of neurodegenerative diseases is still unclear. The present review aimed to critically review the recent study from 1997 to 2017 regarding the protective effects of TQ in the management of neurodegenerative diseases.

Pharmacology Properties

Chemical Structure

Thymoquinone (2-isopropyl-5-methylbenzo-1,4-quinone) is the most bioactive ingredients of seeds with molecular formula $C_{10}H_{12}O_2$ and molar mass 164.20 g·mol^{-1}.[28] Thymoquinone consists of the enol, keto, and mixture forms. The keto form is the major form that is involved in the pharmacological effects of TQ.[29] The sensitivity to light of TQ was high and is deprecated in a short period of light exposure. Furthermore, it was unstable in aqueous solutions, especially at an alkaline pH.[30]

Pharmacokinetics

The hydrophobic property of TQ limits its bioavailability and drug formulation.[30] There are different routes for administration of TQ including intravenous (iv),[31,32] intraperitoneal (ip),[33-35] and oral subacute and subchronic administration.[33,36-39] After oral administration, TQ is metabolized via the liver metabolizing enzymes such as DT-diaphorase (a quinine reductase) that modifies TQ into a reduced form thymohydroquinone.[40] The information about the bioavailability and pharmacokinetic properties of TQ and formulation problems is not sufficient for usage in the clinical trial studies. The clearance rate of TQ after iv administration was 7.19 mL/kg/min, and the estimated volume of distribution at steady state (Vs) was 700.90 mL/kg in the animal model. Following oral exposure, the clearance rate was 12.30 mL/min/kg and Vs was 5109.46 mL/kg. The elimination half-life (T1/2) of TQ was about 217 minutes. In addition, the percentages of TQ protein binding in human and rabbit plasma were 98.99 and 99.19, respectively,[41] which indicates the quick elimination and slow absorption of TQ following oral exposure. It has been indicated that TQ causes complex formation with human serum albumin (HSA), bovine serum albumin (BSA), and α1-acid glycoprotein (AGP) in serum.[42,43] In addition, it was observed that the association between TQ and HSA as well as TQ and AGP does not affect the pharmacological properties of TQ.[42,43] However, the covalent binding of TQ to BSA prevented the TQ anticancer activity against cancer cells.[43] The estimated percentages of TQ-protein binding in human and rabbit plasma were 99.19 and 98.99, respectively.[41] In recent years, some analogs of TQ such as molecular micelle-modified poly (d, lactide-co-glycolide) nanoparticles, solid lipid nanoparticles (SLNs), TQ-encapsulated chitosan

nanoparticles, TQ-loaded liposomes, caryophyllene and germacryl conjugates, as well as fatty acid conjugates and TQ-loaded nanostructured lipid carriers have been synthesized that may affect its bioavailability and application in clinical phase.

Toxicological Evaluation

One toxicological study indicated that the lethal dose 50 (LD50) of TQ, when injected ip in rats, was 10 mg/kg. Another study indicated that 4, 8, 12.5, 25, and 50 mg/kg ip injection of TQ in mice has no change in the biochemical indices, such as serum alanine transaminase and lactate dehydrogenase (LDH).[44] However, ip injection of TQ higher than 50 mg/kg to mice was lethal and the LD50 was 90.3 mg/kg.[44] Several toxicological studies indicated that oral administration of TQ in the range of 10 to 100 mg/kg has no toxic or lethal effects in mice.[45-49] The maximum tolerated dose of TQ was 22.5 mg/kg in male and 15 mg/kg in female rats when injected ip, whereas in both male and female rats, the dose was 250 mg/kg after oral administration.[50]

Methods

Databases such as PubMed, Science Direct, Scopus, and Google Scholar were searched for the terms of N. sativa, TQ, neuroprotective effects, and different disorders between the years 1979 and 2017 to prepare this review. For validating the plant's scientific name, Plantlist.org and examine.com were used.

Neuroprotective Effects

Effect on Neuroinflammation

Neuroinflammation is the main factor involved in the pathogenesis of neurodegenerative diseases such as Alzheimer disease (AD) and Parkinson disease (PD). Microglia activation is the main factor involved in the ignition and progression of the neuroinflammation by the response to stimuli such as infection, traumatic brain injury (TBI), and so on. Nuclear factor kappa-light-chain-enhancer of activated B cells is a transcription factor that binds to DNA and activates gene transcription, and its activation is related to inflammation in microglia in the central nervous system (CNS).[51] Activated NF-κB induces the pro-inflammatory cytokines,[52-54] such as iNOS,[55] COX-2, and microsomal prostaglandin E synthase-1.[56] In addition, inflammation increases cellular reactive oxygen species production by releasing various NF-κB-mediated pro-inflammatory mediators.[57] Therefore, inhibition of microglial activation may be effective for neuronal cell survival. In this regard, one study[19] indicated that TQ treatment (2.5, 5, and 10 μM) inhibited the release of TNF-α, IL-6, and IL-1β. Thymoquinone also decreased the release and levels of messenger RNA (mRNA) of TNF-α, IL-6, IL-1β, and prostaglandin E2 (PGE2) in the microglia cells of rats exposed to lipopolysaccharides (LPS; 100 ng/mL). Results showed that TQ treatment (2.5, 5 and 10 μM) inhibited NF-κB-dependent neuroinflammation in BV2 microglia via decreasing iNOS protein levels, κB inhibitor phosphorylation, and binding of NF-κB to the DNA. It also increased nuclear accumulation of nuclear factor (erythroid-derived 2)-like 2 (NFE2L2 or Nrf2) protein, binding of Nrf2 to the antioxidant responsive element (ARE) consensus binding site, and ARE transcriptional activity. These findings suggested that activation of the Nrf2/ARE signaling pathway by TQ resulted in the inhibition of NF-κB-mediated neuroinflammation. Thymoquinone inhibited LPS-induced neuroinflammation through interference with NF-κB signaling in BV2 microglia. Thymoquinone also activated Nrf2/ARE signaling by increasing transcriptional activity of Nrf2, nuclear localization, and DNA binding, as well as increasing protein levels of NAD(P)H: quinone oxidoreductase 1 and Heme oxygenase 1. Suppression of Nrf2 activity through siRNA or with the use of trigonelline resulted in the loss of anti-inflammatory activity by TQ. Taken together, these studies show that TQ inhibits NF-κB-dependent neuroinflammation in BV2 microglia, by targeting antioxidant pathway involving activation of both Nrf2 and ARE. It seems that activation of Nrf2/ARE signaling pathways by TQ probably results in inhibition of NF-κB-mediated neuroinflammation.

Another study[58] also indicated that TQ (2.5, 5, and 10 μM) prevented neuroinflammation by inhibiting inflammatory mediators nitric oxide (NO), PGE2, TNF-α, and IL-1β production in BV2 microglial cells. It has been found that TQ inhibited LPS-induced inflammatory mediator production by blocking phosphoinositide 3-kinase (PI3K)/protein kinase B or Akt/NF-κB signaling pathway on LPS-stimulated BV2 microglial cells.[59]

Taka et al[60] also indicated that TQ (0-100 μM) reduced a set of cytokines including IL-6, IL-1β, IL-12p40/70, chemokine (C-C motif) ligand 12 (CCL12)/monocyte chemotactic protein 5 (MCP-5), CCL2/MCP-1, granulocyte colony-stimulating factor (GCSF), and C-X-C motif chemokine 10 (Cxcl 10)/IFN-γ-induced protein 10 (IP-10) in LPS-stimulated BV-2 murine microglia cells in rats.

Effect on Depression

Depression (major depressive disorder) is a serious mood disorder that disturbs normal feel, thinking, and handling daily activities, such as sleeping, eating, or working at least for 2 weeks.[61,62] The *Diagnostic and Statistical Manual of Mental Disorders* (*DSM*) is one of the 2 standard classification systems of mental disorders used by mental health professionals. The *DSM* originated in 1952 (*DSM-I*); the other widely used system—the *International Statistical Classification of Diseases and Related Health Problems* (*ICD*)—for the first time included a section on mental disorders in 1949 (*ICD-6*). Both the American Psychiatric Association and the World Health Organization are currently working on revisions of the respective classification systems. The inflammatory indices such as C-reactive protein, IL-6, and TNF-α may be involved in the pathogenesis of depression.[63,64] The recent documents indicate

that neuroinflammation is involved in depression.[65] It was indicated medicinal plants such as *Nigella sativa* might be effective against depression-like behavior in animal models. In this regard, Hosseini et al[66] studied the effects of hydroalcoholic extract of *Nigella sativa* and TQ in a model of LPS (100 μg/kg, ip)-induced depression-like behavior in rats. Fifty male rats placed in 5 groups: control, LPS + saline, LPS + *Nigella sativa* 200 mg/kg, LPS + *Nigella sativa* 400 mg/kg, and LPS + TQ. Forced swimming test (FST) was performed 3 times for all groups and immobility time was recorded. Separate administration of *Nigella sativa* and TQ decreased immobility times compared to LPS group; however, co-administration of *Nigella sativa* (400 mg/kg) and TQ induced lowest immobility times compared to others. In addition, *Nigella sativa* and TQ improved the crossing number of treated animals in FST. Taken together, *Nigella sativa* and TQ had protective effects on LPS-induced depression-like behavior in rats. Findings of these studies indicated that TQ improved LPS-induced learning and memory impairments induced by LPS in rats by attenuating the hippocampal cytokine levels and brain tissues oxidative damage.

Effects on Epilepsy

An epileptic seizure is produced by a temporally limited, synchronous electrical discharge of neurons in the brain. It presents as a variable combination of motor, somatosensory, special sensory, autonomic, and/or behavioral disturbances, which arises suddenly and may last for a few seconds or a few minutes. On rare occasions, seizure activity persists for more than 20 minutes and may go on for hours, or even longer, without interruption (status epilepticus [SE]). The epileptic event may affect a circumscribed area of the brain (partial or focal seizures) or both cerebral hemispheres at the same time (generalized seizures). An impairment of consciousness is found in generalized seizures and in the so-called complex focal seizures.[67] Status epilepticus is a type of seizures that last too long and the patient does not recover between seizures. It is indicated that oxidative stress plays a main role in the pathogenesis of SE.[67,68]

The protective effects of TQ on brain injury in a lithium–pilocarpine rat model of SE have been studied. Nrf2 is a key transcription factor involved in the antioxidant response and can thus protect cells from toxic substances and pathogens.[69-71] This study[72] indicated that TQ treatment (10 mg/kg ip) decreased brain injuries induced by SE via modulating the Nrf2 signaling pathway involved in the activation of the antioxidant defense system. In addition, the behavioral experiments indicated that TQ also improved learning and memory function.

Another study[20] indicated TQ (10 mg/kg ip) prevented epilepsy by decreasing gene expression of NF-κB, which mediates inflammatory reactions, in a lithium–pilocarpine model of SE. Thymoquinone improved electroencephalography profiles, lowered death rate, decreased seizure severity, and improved learning and memory functions.

Temporal lobe epilepsy (TLE) is another type of epilepsy in adults, characterized by neuronal loss,[73] reactive astrogliosis,[74] and enhanced oxidative stress.[75] One study[76] indicated that TQ has a protective effect in the intrahippocampal kainate model of TLE in rat. Thymoquinone pretreatment (10 mg/kg) decreased oxidative stress indices such as malondialdehyde (MDA) and nitrate in the hippocampal tissue and severe seizure activity. Thymoquinone also ameliorated astrogliosis and reduction in neurons in cornu ammonis-1 (CA1), CA3, the hilar regions, and mossy fiber sprouting (MFS) in the dentate gyrus of kainate-lesioned rats. This study indicated that the antiepileptogenic effect of TQ may be related to decreasing seizure activity and lipid peroxidation, hippocampal neuronal loss, and MFS and mitigated astrogliosis in the kainate model of TLE.

Thymoquinone administration (40 and 80 mg/kg, ip) prolonged the onset of seizures and decreased the duration of myoclonic seizures in pentylenetetrazole (PTZ)-induced seizure models in mice through opioid receptor-mediated increase in gamma-aminobutyric acid (GABA)ergic tone.[36]

Ullah et al[77] studied the effects of TQ and vitamin C against PTZ-induced generalized seizures in rats. Pretreatments with TQ (40 mg/kg, orally [po]) and vitamin C (250 mg/kg ip) or either alone of these drugs ameliorated PTZ-induced seizures and mortality in rats and neurodegeneration in the cells. Furthermore, TQ and vitamin C prolonged the onset of seizures and reduced the high-grade seizures. Both TQ and vitamin C administration ameliorated decreased expression of the gamma-aminobutyric acid B1 receptor, calcium/calmodulin-dependent protein kinase II, inhibition of phosphorylation of cyclic adenosine monophosphate response element-binding protein, decreased Bcl-2 expression, and activated caspase-3 in the cortex and hippocampus in rats. Treatment of mice with TQ (5, 10, and 20 mg/kg ip) along with alternate-day subconvulsive dose of PTZ produced dose-dependent protection against PTZ-induced kindling and learning and memory impairments. Moreover, treatment of mice with TQ (20 mg/kg) inhibited the biochemical alterations induced by PTZ in the brain except the elevation of brain glutamate level. The associated increase in brain inducible NO synthase mRNA and protein expressions was also inhibited. These results suggest that glutamate and subsequent oxidative stress and NO overproduction, via inducible NO synthase, play an important role in the pathophysiology of PTZ-induced kindling and cognitive impairments in mice. Thymoquinone dose dependently protects against PTZ-induced kindling and cognitive impairments. Inhibition of PTZ-induced brain oxidative stress and NO overproduction, via increase in the expression and activity of inducible NO synthase, may play an important role in the neuroprotective action of TQ brain inyurirsnkueryjury Ury action. Also in the stressed mice, TQ (20 mg/kg) showed anxiolytic effects, with a significant decrease in plasma nitrite and reversal of the decreased brain GABA content. Pretreatment with methylene blue enhanced the antianxiety effect of TQ in both unstressed and stressed mice.

For the first time, a pilot trail study[78] investigated the effects of oral administration of TQ (1 mg/kg po) on seizure frequency

in the on children with refractory epilepsy for 2 periods of 4 weeks with 2 weeks. The results indicated that TQ has no effect on neurological function, laboratory variables, or vital signs of children with refractory epilepsy compared with placebo group.

Effect on PD

Parkinson disease is caused by the degeneration of dopaminergic neurons in the substantia nigra of the midbrain and aggregation of α-synuclein (α-SN) in the brain.[79] In addition, induction of inflammation and oxidative stress response has long been suggested to play the main role in the pathogenesis of PD.[80] The neuroprotective effects of some flavonoids against oxidative stress in mesencephalic dopamine neurons induced by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride have been previously indicated.[81-84]

The protective effects of TQ (7.5 and 15 mg/kg, po) in the management of PD in animal models exposed to rotenone have been investigated.[85] It was indicated that co-administration of TQ with rotenone prevented PD symptoms such as movement failure induced by rotenone during motor assessments in rotarod, rearing, and bar tests. These findings show that TQ effects on ameliorating the PD symptoms induced by rotenone might be associated with the neuroprotective and antioxidant effects of this compound. In addition, TQ decreased prealbumin serum concentration and oxidative stress indices. The results of this study indicated that TQ ameliorated the motor defects in the animal model of PD due to its antioxidant effects. Thymoquinone has been reported to have antioxidant and anti-inflammatory characteristics in vitro and in vivo. Thymoquinone scavenges free radicals, so prevents cell damage against oxidative agents. It was indicated[86] that TQ (0.01, 0.1, 1, and 10 μM) protected mesencephalic dopaminergic neurons against 1-methyl-4-phenylpyridinium (MPP+)-induced cell death through activation of enzymatic degradation, preservation of mitochondrial function, and inhibition of apoptotic cell death. The TQ significantly protected dopaminergic neurons, decreased the release of LDH, and increased the mitochondrial membrane potential. This study suggested that TQ activated a lysosomal degradative process in dopaminergic neurons and decreased mitochondria-mediated apoptotic cell death. Synapse degeneration is a common finding in patients with neurodegenerative diseases such as PD, AD, and dementia with Lewy bodies (DLB).[87] Oligomers of α-SN are the main mediators of neuropathology in PD and DLB.[88] The protective effects of TQ against α SN-induced synaptic toxicity in rat hippocampal and human-induced pluripotent stem cell (hiPSC)-derived neurons have been investigated.[89] It was observed that TQ (100 nM) protected cultured hippocampal neurons against α-SN-induced synapse damage and decreased synaptophysin level and inhibition of synaptic activity. In addition, TQ protected human hiPSC-derived neurons against inhibition of spontaneous firing activity and restored mutated P123H-induced inhibition of synaptic vesicle recycling in hippocampal neurons. This study suggested that TQ protected

human iPSC-derived neurons from α-SN-induced synapse damage in patients with PD or from those with other α-synucleinopathies. Another study[90] indicated the protective effects of TQ against MPP+- and rotenone-induced cell death in primary dopaminergic cultures. Thymoquinone (0.1 and 1 μM) protected the total number of their neurons against MPP+- and both short- and long-term rotenone toxicity. The other study reports that the SLNs encapsulated TQ (TQ-SLNs; 10 and 20 mg/kg) and TQ suspension (TQ-S; 80 mg/kg)-treated animals showed a significant ($P < .01$) improvement in the muscle strength, rigidity, movement, and memory performances on 7th- and 14th-day behavioral analysis than TQ-S (40 mg/kg)-treated group. Similarly, TQ-SLNs highly attenuated the levels of oxidative stress markers such as lipid peroxidation, NO, and protein carbonylsin 3-nitropropionic acid (3-NP)-induced animals. Further, TQ-SLNs significantly restores the antioxidant defense system, controls the mitochondrial succinate dehydrogenase inhibition, and alleviates anticholinergic effect upon (3-NP) induction. In addition, TQ-SLNs efficiently protected the striatal structural microelements against 3-NP toxicity, which was confirmed by light microscopic studies. Thus, the researchers collectively suggest that the low dose of TQ-SLNs supplementation is highly sufficient to attain the effect of TQ-S (80 mg/kg) to attenuate behavioral, biochemical, and histological modifications in 3-NP-exposed HD model.

Effects on AD

Alzheimer disease is one of the serious neurodegenerative diseases that leads to brain cells death and causes memory loss and cognitive decline. It seems that the mechanisms for induction of AD are related to the induction of oxidative stress and inflammation.[91] Several studies indicated that treatment with flavonoids may be effective against AD due to their antioxidant effects.[91] Several studies showed that β-amyloid peptides have a major role in the pathogenesis of AD. The protective effect of TQ (0.1, 1, 10, 100 nM) against amyloid β peptide ($A\beta_{1-42}$)-induced neurotoxicity has been investigated in rat hippocampal and cortical neurons.[91] Thymoquinone ameliorated $A\beta_{1-42}$-induced neurotoxicity and prevented the mitochondrial membrane potential depolarization and finally reduced the oxidative stress. Thymoquinone improved synaptic vesicle recycling inhibition in primary hippocampal and cortical neurons. Thymoquinone also reversed the loss of spontaneous firing activity and inhibited $A\beta_{1-42}$ aggregation in vitro. These beneficial effects may contribute to the protection against Aβ-induced neurotoxicity. Therefore, it seems that TQ has neuroprotection potential against $A\beta_{1-42}$ in rat hippocampal by ameliorating oxidative stress. The several evidences showed that natural agents that can inhibit the pathways related to Aβ-induced neurotoxicity may be effective in the treatment of AD.[92,93] The neuroprotective effects of TQ against β-amyloid peptide 1 to 40 sequence ($A\beta_{1-40}$)-induced neuronal cell death have been investigated in primary cultured cerebellar granule neurons (CGNs).[94] The pretreatment of CGNs with TQ (0.1 and 1 M) inhibited $A\beta_{1-40}$-induced apoptosis of CGNs via both extrinsic

and intrinsic caspase pathways. The pretreatment of TQ also decreased LDH release, maintained cell bodies, activated neurite network, improved condensed chromatin, increased free radical production, and inhibited caspase-3, -8, and -9 activation compared to those exposed to $A\beta_{1-40}$ alone. These findings confirmed that TQ may be an effective treatment in AD.

The nicotinic acetylcholine receptors (nAChRs) are ion channels distributed in the central or peripheral nervous system. They are receptors of the neurotransmitter acetylcholine and activation of them by agonists mediates synaptic transmission in the neuron and muscle contraction in the neuromuscular junction.[95] Current studies reveal the relationship between the nAChRs and the learning and memory as well as cognition deficit in various neurological disorders such as AD. There are various subtypes in the nAChR family, and the α7 nAChR is one of the most abundant subtypes in the brain. The α7 nAChR is significantly reduced in the patients with AD and is believed to interact with the Aβ amyloid. Aβ amyloid is co-localized with α7 nAChR in the senile plaque and the interaction between them induces neuron apoptosis and reduction in the α7 nAChR expression. Treatment with α7 agonist in vivo shows its neuron protective and procognition properties and significantly improves the learning and memory ability of the animal models.[96] PNU-282987 has been shown to be a potent and most specific α7 nAChR agonist. Moreover, PNU had significant effects on memory, thus improving performance. An alternative treatment strategy via compounds known as nicotinic "positive allosteric modulators" (PAMs) has been reported. The PAM of α7 nAChRs is known as PNU-120596.[97] Recently, studies aimed at investigating the combination of PAM of α7 nAChRs with PNU-282987 (α7 nAChR agonist) or with TQ as a possible treatment for AD in an animal model using histological, histochemical, immunohistochemical, and morphometric methods.

The present study aimed at investigating the combination of PAM of α7 nAChRs with PNU-282987 (α7 nAChR agonist) or with TQ as a possible treatment for AD in an animal model using histological, histochemical, immunohistochemical, and morphometric methods. These findings indicated that the early combined treatment in AD can be more effective than single-drug treatment to improve histological changes. Thymoquinone or α7 nAChR agonist combined with PAM plays more effective in the treatment of AD than TQ alone.

Effect on Ischemia

Transient global cerebral ischemia (forebrain ischemia) causes selective and delayed neuronal cell death.[98] Oxidative stress is one of the main factors involved in the pathogenesis of cerebral ischemia.[99] The iNOS is upregulated after ischemia–reperfusion injury (IRI) that causes overproduction of NO. The interaction between NO and superoxide leads to form the peroxynitrite radical that induces neuronal death after cerebral ischemia.[100] One study[47] investigated whether oral administration of TQ protected rat hippocampus neuron against transient forebrain ischemia. Thymoquinone was administered (5 mg/

kg/day po) 5 days before ischemia and continued during the reperfusion time. Thymoquinone decreased the neuronal cell death in the hippocampal CA1 region and MDA level and increased glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) activities after forebrain ischemia. Thymoquinone also decreased oxidative stress-induced inflammation, prevented iNOS upregulation, and inhibited the formation of peroxynitrite. Another study[101] also investigated the effect of TQ (2.5, 5 and 10 mg/kg) and *N sativa* oil (NSO; 0.048, 0.192, and 0.384 mg/kg) on lipid peroxidation level following global cerebral IRI in rat hippocampus. The results indicated that NSO and TQ protected against IRI by modulating oxidative stress in rat hippocampus.

Effect on TBI

Traumatic brain injury is caused after external force injuries on the brain that is the main cause of morbidity and mortality worldwide.[102] After injury, a series of biochemical processes, such as parenchymal inflammation, free radical production, increased intracellular calcium, and lipid peroxidation, as well as NO production induces neurological impairment.[103-105] The neuroprotective effects of TQ in a rat model of TBI have been investigated using biochemical and histopathological methods.[106] The researchers indicated that TQ (5 mg/kg ip) had healing effects on neural cells after TBI by reducing MDA levels in the neuronal nuclei and mitochondrial membranes of neurons. Neuron density in contralateral hippocampal regions (CA1, CA2-3, and CA4) 7 days after the trauma decreased significantly in the trauma and TQ-treated groups, compared with that in the control group. Neuron densities in contralateral hippocampal regions (CA1, CA2-3, and CA4) were greater in the TQ-treated group than in the trauma group. Thymoquinone did not increase SOD or GSH peroxidase antioxidant levels. However, TQ decreased the MDA levels.

These results indicate that TQ has a healing effect on neural cells after head injury and this effect is mediated by decreasing MDA levels in the nuclei and mitochondrial membrane of neurons.

Effect on Encephalomyelitis

Encephalomyelitis (EAE) is an autoimmune demyelinating disease of the CNS. It is accepted as an animal model for the human multiple sclerosis.[107] Oxidative stress plays a main role in the pathogenesis of EAE.[108] Based on these, Mohamed et al[109] studied this hypothesis that decreasing oxidative stress might ameliorate symptoms and signs of EAE in animal models. Therefore, TQ (1 mg/kg, injected at tail vein) administration was done for evaluating EAE symptoms in 2 groups of EAE rats (1 group injected at day 1-5 and other group injected at day 7-12). The results indicated that TQ ameliorated hind limb weakness and/or paralysis, tail weakness, perivascular inflammation, and low spinal cord GSH level. However, animals received TQ at day 12 to 17 had higher GSH level, no perivascular inflammation, and no symptoms compared with other groups. This study

suggested that TQ improved EAE animals by modulating oxidative stress. Summary of the neuroprotective effects of TQ are shown in Table 1.

Conclusion

Recent studies have been focused on the natural neuroprotective agents due to its low adverse effects with the increase in neurodegenerative diseases. Polyphenols have been considered as the main target for drug design due to the growing evidence that suggests that flavonoids possess beneficial effects on mental diseases. Thymoquinone is an important natural neuroprotective agent that is widely seen in *N sativa* seeds. The present review indicated the protective effects of TQ in the control of depression, epilepsy, PD, AD, ischemia, TBI, anxiety, encephalomyelitis, and brain cancer that have been found in many experiments and a few clinical trials. The present review suggests an involvement of NO-cGMP and GABAergic pathways in the anxiolytic-like activity of TQ. Thymoquinone also has potential to protect primary dopaminergic neurons against MPP (+) and rotenone relevant to PD. Thymoquinone pretreatment could attenuate seizure activity and lipid peroxidation, lower hippocampal neuronal loss, and mitigate astrogliosis in epilepsy model. Thymoquinone may prevent neurotoxicity and $A\beta_{1-40}$-induced apoptosis. Thymoquinone is, therefore, worth studying further for its potential to reduce the risks of developing AD. The neuroprotective effects of TQ may be related to modulatory effects on inflammation, apoptosis, and oxidative stress. The activation of the Nrf2/ARE signaling pathway by TQ resulted in the inhibition of NF-κB-mediated neuroinflammation. In addition, TQ inhibited inflammatory mediator production by blocking PI3K/Akt/NF-κB signaling pathway. Thymoquinone exhibited anti-inflammatory effects by decreasing several cytokines, including TNF-α, NF-κB, IL-6, IL-1β, IL-12p40/70, (CCL12)/MCP-5, (CCL2)/MCP-1, GCSF, and Cxcl 10/IP-10 of, NO, PGE2, and iNOS. Thymoquinone modulates oxidant–antioxidant system by increasing antioxidant content, including GSH, CAT, glutathione *S*-transferase, and SOD, and decreasing lipid peroxidation in brain tissue. The antianxiety effects of TQ may be related to the modulating effects on NO-cGMP and GABA-ergic pathways. Several studies have pointed out the use of TQ in the management of PD via reducing lack of climbing ability, oxidative stress, and apoptosis in the brain and also AD by decreasing the expression of β-amyloid. The neuroprotective effects of TQ have been shown by experimental studies, but not yet in clinical trials, and more safety studies should be performed to indicate possible toxic effects of TQ in long-term administration in humans. We believe that further preclinical research into the utility of NS and TQ may indicate its usefulness as a potential treatment on neurodegeneration after chronic toluene exposure in rats. In conclusion, this review suggests that the neuroprotective effects of TQ are associated with the antioxidant and anti-inflammatory activities. Although experimental studies indicated the beneficial effects of TQ

against nervous system problems, better designed clinical trials in humans are needed to confirm these effects.

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