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REVIEW ARTICLE

Antidotal effects of thymoquinone against neurotoxic agents

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ABSTRACT

Several plants which contain the active component thymoquinone (TQ) have been traditionally used in herbal medicine to treat various diseases. Several studies indicated the protective effects of TQ against neurotoxic agents. The present study was aimed to highlight the protective effects of TQ against neurotoxic agents. For this reason, the literature from 1998 to 2017 regarding the protective effects of TQ against neurotoxic agents and their involvement mechanisms has been studied. The present review suggests the protective effects of TQ against neurotoxic agents in experimental models. More clinical trial studies are however needed to confirm the antidotal effects of TQ in human intoxication.

KEY WORDS: thymoquinone; neurotoxic agents; antioxidant; antidote

ABBREVIATION

ACR: Acrylamide; **ALT:** Alanine transaminase; **CAT:** Catalase; **DM:** Diabetes mellitus; **FAS:** Fetal alcohol syndrome; **GSH:** Glutathione; **GSH-Px:** Glutathione peroxidase; **GST:** Glutathione S-transferase; **LDH:** Lactate dehydrogenase; **LPS:** Lipopolysaccharide; **MDA:** Malondialdehyde; **Met:** Metformin; **mRNA:** Messenger ribonucleic acid; **NE:** Norepinephrine; **NMDA:** N-methyl-D-aspartate; **NO:** Nitric oxide; **PA:** Passive avoidance; **PTZ:** Pentylenetetrazole; **ROS:** Reactive oxygen species; **SOD:** Superoxide dismutase; **STZ:** Streptozotocin; **TQ:** Thymoquinone

Introduction

Plants as an important source of active compounds have been used in traditional medicines (Samarghandian *et al.*, 2017; Samarghandian *et al.*, 2017). *Nigella sativa* (of the family ranunculaceae) and its seeds are commonly called black cumin, fennel flower, or nutmeg flower (Ahmad *et al.*, 2013). It is considered a medicinal herb with some religious usage, called the 'remedy for all diseases except death' (Prophetic hadith) and Habatul Baraka "the Blessed Seed" (Mohammad *et al.*, 2013). The black cumin oil consists of active components such as ocopherols, phytosterols, polyunsaturated fatty acids, thymoquinone, ρ-cymene, carvacrol, t-anethole and 4-terpineol. Thymoquinone (2-isopropyl-5-methylbenzo-1, 4-quinone) (TQ) has been found in many medicinal plants, as *e.g.* in several genera of the Lamiaceae family (Monarda) and the Cupressaceae family (Juniperus) (Farkhondeh *et al.*, 2017). TQ is the main ingredient of the *Nigella sativa*, which is effective for the treatment of various diseases, such as neurodegenerative disorders, coronary artery diseases, respiratory failures, and urinary system failures (Ahmad *et al.*, 2013). TQ has been indicated to possess antioxidant, anti-inflammation, anticancer, antimicrobial, anti-mutagenic and anti-genotoxic activities (Asaduzzaman Khan *et al.*, 2017). TQ may be considered a therapeutic agent for prevention of neurodegenerative diseases. However, the therapeutic effects of TQ against toxic agents remains nascent in the literature. The present review aimed to critically review studies from 1998 to 2017 regarding the protective effects of TQ against neurotoxic agents.

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Safety study

The LD50 value of TQ was found to be 10 mg/kg intraperitoneally (i.p.) in the rat. I.p. injection at doses of 4, 8, 12.5, 25 and 50 mg/kg TQ in mice has no effect on biochemical indices, such as serum alanine transaminase (ALT) and lactate dehydrogenase (LDH) (Mansour *et al.*, 2001). However, i.p. injection of TQ higher than 50 mg/kg was lethal in mice (Mansour *et al.*, 2001). Several toxicological studies indicated that oral administration of TQ in the range of 10–100 mg/kg has no toxic or lethal effects in mice (Kanter, 2008; Kanter, 2011a). The maximum tolerated dose of TQ was 22.5 mg/kg in male and 15 mg/kg in female rats when injected i.p., whereas in both male and female rats it was 250 mg/kg after oral administration (Kanter, 2011b). The difference in toxicity response between i.p. injection and oral ingestion of TQ can be related to the complete absorption of TQ into the systemic circulation after i.p. injection, whereas with oral administration, TQ is biotransformed in the gastrointestinal tract or metabolized in the liver.

Methods

Online literature resources were checked using different search engines such as Medline, PubMed, Iran Medex, Scopus, and Google Scholar from 1998 to 2017 to identify articles, editorials, and reviews about antidotal effects of TQ against neurotoxic agents. TQ, neurotoxicity, and neurotoxic agents were key words used to search the literature.

Protective effects of TQ against neurotoxic agents (Table 1)

Lead

Lead (Pb^{2+}) is one of the most hazardous heavy metals that threatens human health (Samarghandian *et al.*, 2013). Lead causes damage to the brain by disrupting ionic balance in neuronal cells, modifying normal brain function, interrupting neural signal transmission between neurons and inducing neurodegeneration and progressive neuronal cell death (Lidsky & Schneider, 2003). Chronic occupational exposure to low levels of lead may be a risk factor for some neurodegenerative diseases such as Parkinson's and Alzheimer's diseases (Coon *et al.*, 2006; Wu *et al.*, 2008). Chelation therapy is the most effective treatment for lead poisoning (Flora *et al.*, 2012). Natural antioxidants have been used to improve lead toxicity in experimental studies (Reckziegel *et al.*, 2011). Lead acetate exposure [0.5 g/l (500 ppm)] was found to cause degeneration of hippocampal and cerebellar neurons, endothelial lining of brain blood vessels with perivascular cuffing of mononuclear cells consistent to lymphocytes and chromatolysis and neuronal and also congestion of choroid plexus blood vessels, ischemic brain infarction, microglial reaction, neuronophagia, and axonal demyelination. TQ treatment (20 mg/kg in corn oil (0.5 ml/rat)) improved the lead-induced brain lesions in rats due to its antioxidant properties (Radad *et al.*, 2014), suggesting that TQ attenuated brain oxidative stress induced by lead. However more studies are needed to determine the underlying mechanisms of such protection of TQ against lead neurotoxicity.

Morphine

Morphine was indicated to induce oxidative stress in brain (Guzmán *et al.*, 2006; Özmen *et al.*, 2007; Ibi *et al.*, 2011). Reactive oxygen species (ROS), glutamate release, and nitric oxide (NO) have important roles in morphine tolerance, dependence and withdrawal symptoms (Sepulveda *et al.*, 1998; Özek *et al.*, 2003; Wen *et al.*, 2004; Mori *et al.*, 2007; 2011). Activation of the ionotropic N-methyl-D-aspartate (NMDA) subtype of glutamate receptors plays a crucial role in the development of morphine analgesic tolerance and dependence (Bajo *et al.*, 2006; Murray *et al.*, 2007; Wang *et al.*, 2007). Over-activation of the glutamatergic system increases ROS production (Alekseenko *et al.*, 2012). The protective effects of TQ against morphine induced tolerance and dependence have been indicated (Abdel-Zaher *et al.*, 2013). Repeated administration of TQ prevented the development of morphine tolerance and dependence in mice, decreased brain malondialdehyde (MDA) and NO levels, increased brain intracellular glutathione (GSH) level and glutathione peroxidase (GSH-Px) activity. TQ had no effect on the increased glutamate level in the brain induced by repeated administration of morphine. However, TQ inhibited morphine tolerance and dependence-induced increase in inducible NO synthase but not in neuronal NO synthase mRNA expression in mouse brain. It was indicated that TQ ameliorated the development of morphine tolerance and dependence via decreasing the brain glutamate level, oxidative stress, inducible NO synthase expression, and NO overproduction. The study suggested that the protective effect of TQ is very likely due to its strong antioxidant activities.

Ethanol

Ethanol exposure during brain development might cause neurodevelopmental defects referred to as fetal alcohol syndrome (FAS) (Jones *et al.*, 1973). Ethanol disturbs brain development by the dysregulation of neurogenesis, cell migration and cell survival (Miller, 1986; 1996; Naseer *et al.*, 2010). Ullah *et al.* (2012) suggested that TQ and metformin (Met) have neuroprotective effects against ethanol-induced apoptosis via regulating calcium (Ca^{2+}) homeostasis, mitochondrial function, cytochrome-c release, caspase activation and the Bcl-2 family of proteins. ROS generation is an important mediator of ethanol-induced apoptotic cell death (Ramachandran *et al.*, 2003; Young *et al.*, 2005; Antonio *et al.*, 2008). Exposure to ethanol accompanied with Met (10 mM), TQ (10, 15, 25 and 35 μM) or Met plus TQ inhibited ROS generation, which triggers apoptotic cell death pathways during early development of rat cortical and hippocampal neurons. TQ plus Met decreased the levels of Ca^{2+} induced by ethanol in the brain. Administration of TQ plus Met to rats reduced ethanol-induced apoptosis in cortical and hippocampal neurons by decreasing Bax/Bcl-2 ratio. Met plus TQ prevented cell death in primary rat cortical neurons induced by ethanol due to its antioxidant effect that

Table 1. Antidotal effects of TQ against neurotoxic agents.

Dose of TQ	Toxic agents	Experimental study	Mechanism	Ref
20 mg/kg	Lead acetate	Rat	Prevented neurotoxicity by modulating oxidative stress	Radad *et al.*, 2014
10 mg/kg	Morphine	Mice	Ameliorated the development of morphine tolerance and dependence via decreasing the brain glutamate level, oxidative stress, inducible NO synthase expression, and NO overproduction	Abdel-Zaher *et al.*, 2013
10, 15, 25 and 35 μM	Ethanol	Primary rat cortical neurons	Prevented neurotoxicity by decreasing elevated $[Ca^{2+}]c$ levels, decreasing Bax/Bcl-2 ratio in the mitochondrial of neurons, inhibition of ROS generation	Ullah *et al.*, 2012
50 mg/kg	Toluene	Rat	Prevented neurotoxicity by decreasing the immunoreactivity of degenerating neurons and apoptosis	Kanter, 2008 and 2011
0–100 μM	Glutamate	Human SH-SY5Y neuroblastoma cells	Prevented neurotoxicity by decreasing the ROS generation, mitochondrial dysfunction and apoptotic cascade	Al Mamun *et al.*, 2015
2.5, 5, 10 mg/kg	Acrylamide	Rat	Prevented neurotoxicity by inhibiting lipid peroxidation in cerebral cortex resulted in improved severe gait abnormalities	Mehri *et al.*, 2014
10 mg/kg	Streptozotocin	Rat	Prevented neuropathy diabetes via decreasing NO and MDA , increasing GSH, CAT and GST, and also decreasing norepinephrine	Hamdy & Taha, 2009
10 mg/kg	Lipopolysaccharide	Rat	Prevented neurotoxicity by decreasing hippocampal IL-6 and TNF-α level and increasing SOD and CAT activities.	Bargi *et al.*, 2017
5, 10 and 20 mg/kg	Pentylenetetrazole	Mice	Prevented neurotoxicity by decreasing glutamate, oxidative stress and NO production	Abdel-Zaher *et al.*, 2017

maintains mitochondrial integrity. The mechanism of TQ neuroprotection was similar to Met including stabilization of mitochondrial membrane potential, reducing Ca^{2+} overload and inhibition of apoptotic cascades.

Toluene

Toluene is an industrial aromatic solvent usually found in gasoline, paints, resins, cosmetic products, lacquers, inks, nail polish, paint thinners, and adhesives (Kurtzman *et al.*, 2001). Acute intoxication with toluene causes euphoria and disinhibition followed by hallucinations, tinnitus, ataxia, confusion, nausea and vomiting, an increased tendency to sleep, frequent headaches, and eye irritation in humans (Flanagan *et al.*, 1989; Echeverria *et al.*, 1991; Evans & Balster, 1991). Brain magnetic resonance imaging indicated cerebral and hippocampal atrophy with a loss in brain volume in toluene/solvent abusers (Flanagan *et al.*, 1989; Echeverria *et al.*, 1991; Evans & Balster, 1991; Yamanouch *et al.*, 1995; Kamran& Bakshi, 1998; Deleu & Hanssens, 2000). Toluene causes CNS depressant effects such as psychomotor impairment (Hester *et al.*, 2011), excitation, inhibition of locomotor activity (Shiotsuka *et al.*, 2000), and loss of righting reflex and sedation (Conti *et al.*, 2012). Furthermore, peripheral nerve dysfunction has been observed after toluene exposure (Hester *et al.*, 2011). I.p. injection of toluene disturbed the oxidant-antioxidant balance in the brain (Greenberg *et al.*, 1997; Zabedah *et al.*, 2001). It is suggested that increased lipid peroxidation and apoptosis with reduced antioxidant content in the brain are the important mechanisms involved in the neurotoxicity of toluene. The protective effect of TQ against neurodegeneration in the hippocampus after chronic toluene (3,000 ppm inhalation) exposure in rats has been shown (Kanter, 2008). TQ (50 mg/kg, p.o) treatment was found to decrease the immunoreactivity of degenerating neurons after chronic exposure to toluene. TQ treatment decreased the number of apoptotic neurons and prevented the deterioration of the hippocampal neuron, as well as memory and learning disabilities in animal models. TQ also improved morphological alteration in the hippocampus of rats after chronic exposure to toluene by ameliorating apoptosis. (Kanter, 2011) evaluated the protective effects of TQ on the neuronal injury in the frontal cortex of rats after chronic exposure to toluene (3,000 ppm inhalation). Chronic exposure to toluene caused severe degenerative changes, shrunken cytoplasm, slightly dilated cisternae of endoplasmic reticulum, and swollen mitochondria with degenerated cristae and nuclear membrane breakdown with chromatin disorganization in neurons of the frontal cortex. TQ treatment (50mg/kg p.o) was reported to ameliorate the severity of degenerative changes in the cytoplasm and especially in the cell nucleus of rats exposed to toluene. TQ treatment significantly decreased the immunoreactivity of degenerating neurons and the number of the apoptotic neurons (TUNEL positive neurons). The study also confirmed that TQ treatment improved morphologic neurodegeneration in frontal cortex tissues of rats exposed to toluene by modulating apoptotic pathways.

Glutamate

(Al Mamun *et al.*, 2015) investigated the protective effects of TQ against glutamate-induced (8 mM) cell death in SH-SY5Y neuronal cells. The findings indicated that TQ (0–100 μM) treatment had protective effects against glutamate induced viability loss, ROS generation, mitochondrial dysfunction and increased the apoptotic cascade via decreasing Bax/Bcl-2 ratio as well as caspase-9 expression. The study suggested that TQ protected against glutamate-induced cell death in SH-SY5Y neurons by inhibiting ROS production, mitochondrial dysfunction and intrinsic apoptotic cascade.

Acrylamide

Acrylamide (ACR) is a neurotoxic agent that target both the central and peripheral nervous system. ACR can lead to neurotoxicity characterized by ataxia, skeletal muscle weakness and body weight loss (Lopachin, 2005; Zhu *et al.*, 2008). Various mechanisms are responsible for neurotoxicity induced by ACR including disruption of presynaptic nitric oxide (NO) signaling, nerve-terminal degeneration, axonal degeneration, increment of lipid peroxidation, reduction of antioxidant capacity of the nervous system and induction of apoptosis signaling. The effects of TQ on ACR-induced (50 mg/kg/day i.p.) neurotoxicity on rats have been investigated (Mehri *et al.*, 2014). TQ (2.5, 5, 10 mg/kg i.p.) inhibited lipid peroxidation in cerebral cortex and improved the severe gait abnormalities in animals. A significant decrease in the number of apoptotic neurons was observed after TQ treatment in rats exposed to ACR. TQ treatment also ameliorated morphologic changes in the frontal cortex of rats exposed to ACR. The protective effects of TQ against ACR-induced toxicity may be due to its antioxidant effects (Mehri *et al.*, 2014).

Streptozotocin

Neuropathy is one of the main complications of diabetes mellitus (DM) with ROS involved in its pathogenesis (Gaweł *et al.*, 2003). The effects of TQ on brain function in streptozotocin (STZ)-induced (60 mg/kg i.p.i) diabetes model have been investigated (Hamdy & Taha, 2009). It was suggested that TQ prevented the development of diabetes-mediated complications via decreasing the levels of NO and MDA with increasing the levels of GSH, catalase (CAT) and glutathione S-transferase (GST) enzymes. The levels of norepinephrine (NE) in the brain of diabetic rats decreased after TQ treatment (10 mg/kg p.o). Correlation analysis indicated that correction of the monoamine levels in the TQ treated diabetic rats was related to the increase in the levels of GST in these animals. The findings suggested that TQ protected the brain against STZ-induced diabetes by modulating oxidative stress.

Lipopolysaccharide

Lipopolysaccharide (LPS) is the major component of the outer membrane of gram-negative bacteria that is used in research for the evaluation of LPS structure, metabolism, immunology, toxicity, physiology, and biosynthesis (Wang & Quinn, 2010). It has also been used to induce inflammation in animal models. Inflammation has been considered a major mechanism involved in the disruption of learning and memory (Chesnokova *et al.*, 2016; Wolf *et al.*, 2016). Inflammation induced by LPS resulted in releasing pro-inflammatory cytokines and inducing ROS production (Valero *et al.*, 2014; Song *et al.*, 2016). It has been suggested that natural antioxidants with anti-inflammatory properties may be effective against memory impairment. It has been reported that TQ improved learning and memory impairments induced by LPS (1 mg/kg i.p.) in rats (Bargi *et al.*, 2017). The protective effects of TQ on memory function has been evaluated using water maze test.

The findings indicated that TQ treatment (2, 5, 10 mg/kg i.p.) decreased the time to find the platform and improved remembered location of the platform in rats. The protective effects of TQ on learning and memory task was also indicated in passive avoidance (PA) test, which was presented by a longer delay for entering to the dark room after the shock. TQ also increased the time spent in the light, while decreased the time spent in the dark compartment where they have previously received a shock. TQ decreased the levels of IL-6 and TNF-α and increased superoxide dismutase (SOD) and CAT activities in the hippocampus of rats. The study suggested antioxidative and anti-inflammatory effects of TQ against learning and memory impairments induced by LPS.

Pentylenetetrazole

Pentylenetetrazole (PTZ) is a drug used as a circulatory and respiratory stimulant; however, side-effects such as epilepsy were difficult to avoid (Dhir, 2012). Cognitive diseases are a group of mental health disorders that affect learning, memory, perception, and problem solving, and include amnesia, dementia, and delirium. It was confirmed that overproduction of ROS and RNS in the brain are involved in the pathogenesis of cognitive impairments (Qu *et al.*, 2012; Chindo *et al.*, 2015; Jain *et al.*, 2015; Singh& Kumar, 2015). PTZ-kindling is a known animal model which simulates epilepsy (Hassanzadeh *et al.*, 2014; Kaur *et al.*, 2015). One study indicated that administration of TQ (5, 10 and 20 mg/kg i.p.) inhibited PTZ-induced (35 mg/kg i.p) kindling and cognitive impairment in mice. According to the results of the study, TQ ameliorated PTZ-induced kindling in mice via decreasing the levels of glutamate, oxidative stress and NO production in the brain (Abdel-Zaher *et al.*, 2017).

Conclusion

The term "neurotoxicity" pointed to damage to the nervous system induced by exposure to natural or chemical toxic agents (Adewale *et al.*, 2015). These toxic agents may modify the nervous system function in ways that can destroy the neurons (Adewale *et al.*, 2015).

In the present study, several studies from 1998 to 2017 have been reviewed to identify the protective effects of TQ against neurotoxic agents. Based on the present findings, TQ acts as an antidote in neurotoxicity induced by toxic agents. Lead, ethanol, toluene, glutamate, ACR, LPS and STZ are some examples of chemical agents against which TQ could protect the brain. TQ showed protective effects against some chemical drugs such as morphine and PTZ which have organ toxicities, particularly in overdose.

The above mentioned agents are risk factor for causing neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, learning and memory deficiency, epilepsy, *etc.* Inhibition of oxidative stress, inflammation

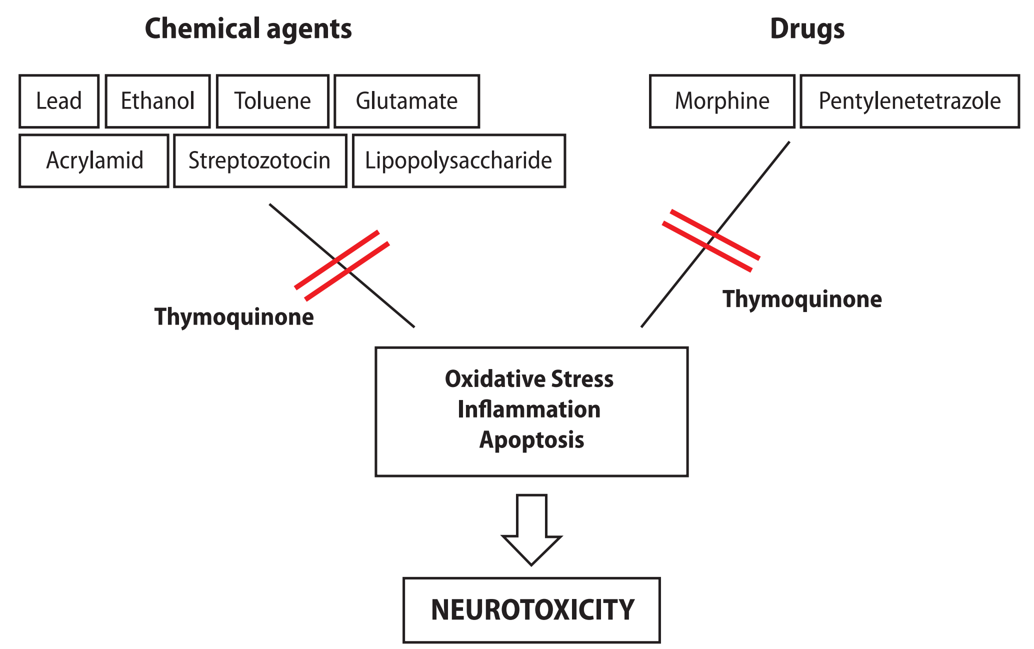

Figure 1. Protective effects of TQ against neurotoxic agents.

and apoptosis are responsible for antidotal effects of TQ (Figure 1). Oxidative stress is recognized as a main mechanism involved in the pathogenesis of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, anxiety disorders, depression, *etc.* (Salim, 2016). The present review indicated that TQ prevented CNS damage induced by lead, morphine, ethanol, glutamate, ACR, STZ, LPS and PTZ via modulating the oxidant-antioxidant system. TQ could balance between oxidant-antioxidant system via enhancing antioxidant contents and decreasing free radical production. Additionally, the strong antioxidant effects of TQ may be related to its free radical-scavenging activity (Badary *et al.*, 2003).

Neuronal apoptosis has an important role in the developing brain and also in neurodegenerative diseases (Esen *et al.*, 2017). However, there are main differences in the mechanisms by which apoptosis is initiated. Apaf-1 (apoptotic protease-activating factor 1), proteins of the Bcl-2 and caspase families are the key molecular components of apoptosis in neurons (Udhayabanu *et al.*, 2017). Neutrophils modulate neuronal apoptosis via activating main protein kinase cascades including phosphoinositide 3-kinase/Akt and mitogen-activated protein kinase. Similarly, abnormal protein structures such as amyloid fibrils activate the apoptosis pathways in Alzheimer's disease (Yan *et al.*, 2017). The present study observed that ethanol, toluene, glutamate and ACR caused neuronal apoptosis by elevating intracellular Ca^{2+}concentration and disrupting mitochondrial membrane potential. Activation of the caspase families might be the key factor in the neurodegenerative diseases induced by toxic agents. Selective caspase inhibition might be an effective approach against neurotoxic agents. The present review also confirmed that TQ prevented neuronal injuries by modulating the activation of caspase families.

Inflammation is recognized as a major effective factor against acute and chronic CNS diseases. Inflammatory mediators such as complement and adhesion molecules, cyclooxygenase enzymes, and cytokines are elevated in neurodegenerative disease. Inflammation may have beneficial and also detrimental effects in the CNS, especially in repair and recovery. Several anti-inflammatory targets have been triggered for CNS disorders treatment. It was observed that TQ prevented learning and memory problems induced by LPS via decreasing hippocampal IL-6 and TNF-α level. In conclusion, the present review confirmed the protective effects of TQ against neurotoxic agents in experimental models, however more clinical trials should be done to confirm the antidotal effects of TQ in human.

Authors' contributions

ARS provided most of the reference and drafted the first version of the paper, designed the table in the paper, SS designed the study and did the overall editing of the paper, TF helped with the design of the paper. All authors read and approved the final manuscript.

REFERENCES

Abdel-Zaher AO, Farghaly HS, Farrag MM, Abdel-Rahman MS, Abdel-Wahab BA. (2017). A potential mechanism for the ameliorative effect of thymoquinone on pentylenetetrazole-induced kindling and cognitive impairments in mice. *Biomed Pharmacother* **88**: 553–561.

Abdel-Zaher AO, Mostafa MG, Farghly HM, Hamdy MM, Omran GA, Al-Shaibani NK. (2013). Inhibition of brain oxidative stress and inducible nitric oxide synthase expression by thymoquinone attenuates the development of morphine tolerance and dependence in mice. *Eur J Pharmacol* **702**: 62–70.

Adewale OO, Brimson JM, Odunola OA, Gbadegesin MA, Owumi SE, Isidoro C, Tencomnao T. (2015). The Potential for Plant Derivatives against Acrylamide Neurotoxicity. *Phytother Res* **29**: 978–85.

Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, Damanhouri ZA, Anwar F. (2013). A review on therapeutic potential of Nigella sativa: A miracle herb. *Asian Pac J Trop Biomed* **3**: 337–352.

Akhtar M, Maikiyo AM, Najmi AK, Khanam R, Mujeeb M, Aqil M.. (2013). Neuroprotective effects of chloroform and petroleum ether extracts of Nigella sativa seeds in stroke model of rat. *J Pharm Bioallied Sci* **5**: 119–125.

Al Mamun A, Hashimoto M, Katakura M, Hossain S, Shido O. (2015). Neuroprotective effect of thymoquinone against glutamate-induced toxicity in SH-SY5Y cells. *Curr Top Nutraceut Res* **13**: 143.

Alekseenko AV, Lemeshchenko VV, Pekun TG, Waseem TV, Fedorovich SV. (2012). Glutamate-induced free radical formation in rat brain synaptosomes is not dependent on intrasynaptosomal mitochondria membrane potential. *Neurosci Lett* **513**: 238–242.

Antonio AM, Druse MJ. (2008). Antioxidants prevent ethanol-associated apoptosis in fetal rhombencephalic neurons. *Brain Res* **1204**: 16–23.

Asaduzzaman Khan M, Tania M, Fu S, Fu J. (2017). Thymoquinone, as an anticancer molecule: from basic research to clinical investigation. *Oncotarget* **8**: 51907–51919.

Badary OA, Taha RA, Gamal el-Din AM, Abdel-Wahab MH. (2003). Thymoquinone is a potent superoxide anion scavenger. *Drug Chem Toxicol* **26**: 87–98.

Bajo M, Crawford EF, Roberto M, Madamba SG, Siggins GR. (2006). Chronic morphine treatment alters expression of N-methyl-D-aspartate receptor subunits in the extended amygdala. *J Neurosci Res* **83**: 532–537.

Bargi R, Asgharzadeh F, Beheshti F, Hosseini M, Sadeghnia HR, Khazaei M. (2017). The effects of thymoquinone on hippocampal cytokine level, brain oxidative stress status and memory deficits induced by lipopolysaccharide in rats. *Cytokine* **96**: 173–184.

Chesnokova V, Pechnick RN, Wawrowsky K. (2016). Chronic peripheral inflammation, hippocampal neurogenesis, and behavior. *Brain Behav Immun* **58**: 1–8.

Chindo BA, Schröder H, Becker A. (2015). Methanol extract of Ficus platyphylla ameliorates seizure severity, cognitive deficit and neuronal cell loss in pentylenetetrazole-kindled mice. *Phytomed* **22**: 86–93.

Conti AC, Lowing JL, Susick LL, Bowen SE. (2012). Investigation of calcium-stimulated adenylyl cyclases 1 and 8 on toluene and ethanol neurobehavioral actions. *Neurotoxicol Teratol* **34**: 481–8.

Coon S, Stark A, Peterson E, Gloi A, Kortsha G, Pounds J, Chettle D, Gorell J. (2006). Whole-body lifetime occupational lead exposure and risk of Parkinson's disease. *Environ Health Perspect* **114**: 1872.

Deleu D, Hanssens Y. (2000). Cerebellar dysfunction in chronic toluene abuse: beneficial response to amantadine hydrochloride. *J Toxicol: ClinToxicol* **38**: 37–41.

Dhir A. (2012). Pentylenetetrazol (PTZ) kindling model of epilepsy. *Curr Protoc Neurosci* **9**: Unit9.37.

Echeverria D, Fine L, Langolf G, Schork T, Sampaio C. (1991). Acute behavioural comparisons of toluene and ethanol in human subjects. *Occup Environ Med* **48**: 750–761.

Esen F, Orhun G, Ozcan PE, Senturk E, Kucukerden M, Giris M, Akcan U, Yilmaz CU, Orhan N, Arican N, Kaya M, Gazioglu SB, Tuzun E. (2017). Neuroprotective effects of intravenous immunoglobulin are mediated through inhibition of complement activation and apoptosis in a rat model of sepsis. *Intensive Care Med Exp* **5**: 1.

Evans EB, Balster RL. (1991). CNS depressant effects of volatile organic solvents. *Neurosci Biobehav Rev* **15**: 233–241.

Farkhondeh T, Samarghandian S, Borji A. (2017). An overview on cardioprotective and anti-diabetic effects of thymoquinone. *Asian Pac J Trop Med* **10**: 849–854.

Farkhondeh T, Samarghandian S, Sadighara P. (2015). Lead exposure and asthma: an overview of observational and experimental studies. *Toxin Rev* **34**: 6–10.

Flanagan R, Ruprah M, Meredith T, Ramsey J. (1989). An introduction to the clinical toxicology of volatile substances. *Drug Safety* **5**: 359–383.

Flora G, Gupta D, Tiwari A. (2012). Toxicity of lead: a review with recent updates. *Interdiscip Toxicol* **5**: 47–58.

Gaweł S, Wardas M, Niedworok E, Wardas P. (2003). Malondialdehyde (MDA) as a lipid peroxidation marker. *Wiadomosci lekarskie* **57**: 453–455.

Greenberg MM. (1997). The central nervous system and exposure to toluene: a risk characterization. *Environ Res* **72**: 1–7.

Guzmán GR, Ortiz-Acevedo A, Ricardo A, Rojas LV, Lasalde-Dominicci JA. (2006). The polarity of lipid-exposed residues contributes to the functional differences between Torpedo and muscle-type nicotinic receptors. *J Membr Biol* **214**: 131–138.

Hamdy NM, Taha RA. (2009). Effects of Nigella sativa oil and thymoquinone on oxidative stress and neuropathy in streptozotocin-induced diabetic rats. *Pharmacol* **84**: 127–134.

Hassanzadeh P, Arbabi E, Rostami F. (2014). The ameliorative effects of sesamol against seizures, cognitive impairment and oxidative stress in the experimental model of epilepsy. *Iran J Basic Med Sci* **17**: 100.

Hester SD, Johnstone AF, Boyes WK, Bushnell PJ, Shafer TJ. (2011). Acute toluene exposure alters expression of genes in the central nervous system associated with synaptic structure and function. *Neurotoxicol Teratol* **33**: 521–9.

Ibi M, Matsuno K, Matsumoto M, Sasaki M, Nakagawa T, Katsuyama M, Iwata K, Zhang J, Kaneko S, Yabe-Nishimura C. (2011). Involvement of NOX1/NADPH oxidase in morphine-induced analgesia and tolerance. *J Neurosci* **31**: 18094–18103.

Jain S, Sangma T, Shukla SK, Mediratta PK. (2015). Effect of Cinnamomum zeylanicum extract on scopolamine-induced cognitive impairment and oxidative stress in rats. *Nutr Neurosci* **18**: 210–216.

Jones K, Smith D, Ulleland C, Streissguth A. (1973). Pattern of malformation in offspring of chronic alcoholic mothers. *Lancet* **301**: 1267–1271.

Kamran S, Bakshi R. (1998). MRI in chronic toluene abuse: low signal in the cerebral cortex on T2-weighted images. *Neuroradiol* **40**: 519–521.

Kanter M. (2011a). Protective effects of thymoquinone on the neuronal injury in frontal cortex after chronic toluene exposure. *J Mol Histol* **42**: 39–46.

Kanter M. (2011b). Thymoquinone attenuates lung injury induced by chronic toluene exposure in rats. *Toxicol Ind Health* **27**: 387–395.

Kanter, M. (2008). Nigella sativa and derived thymoquinone prevents hippocampal neurodegeneration after chronic toluene exposure in rats. *Neurochem Res* **33**: 579–588.

Kaur H, Onsare JG, Sharma V, Arora DS. (2015). Isolation, purification and characterization of novel antimicrobial compound 7-methoxy-2, 2-dimethyl-4-octa-4', 6'-dienyl-2 H-napthalene-1-one from Penicillium sp. and its cytotoxicity studies. *AMB Express* **5**: 40.

Kurtzman TL, Otsuka KN, Wahl RA. (2001). Inhalant abuse by adolescents. *J Adolesc Health* **28**: 170–180.

Lidsky TI, Schneider JS. (2003). Lead neurotoxicity in children: basic mechanisms and clinical correlates. *Brain* **126**: 5–19.

Lopachin RM. (2005). Acrylamide neurotoxicity: neurological, morhological and molecular endpoints in animal models. *Adv Exp Med Biol* **561**: 21–37.

Mansour M, Ginawi O, El-Hadiyah T, El-Khatib A, Al-Shabanah O, Al-Sawaf H. (2001). Effects of volatile oil constituents of Nigella sativa on carbon tetrachloride-induced hepatotoxicity in mice: evidence for antioxidant effects of thymoquinone. *Res Commun Mol Pathol Pharmacol* **110**: 239–252.

Mehri S, Shahi M, Razavi BM, Hassani FV, Hosseinzadeh H. (2014). Neuroprotective effect of thymoquinone in acrylamide-induced neurotoxicity in Wistar rats. *Iran J Basic Med Sci* **17**: 1007.

Miller MW. (1986). Effects of alcohol on the generation and migration of cerebral cortical neurons. *Science* **233**: 1308–1312.

Miller MW. (1996). Mechanisms of ethanol induced neuronal death during development: from the molecule to behavior. *Alcohol Clin Exp Res* **20**: 128A–132A.

Mori E, Bagcivan I, Durmus N, Altun A, Gursoy S. (2011). The nitric oxide–cGMP signaling pathway plays a significant role in tolerance to the analgesic effect of morphine. *Can J Physiol Pharmacol* **89**: 89–95.

Mori T, Ito S, Matsubayashi K, Sawaguchi T. (2007). Comparison of nitric oxide synthase inhibitors, phospholipase A2 inhibitor and free radical scavengers as attenuators of opioid withdrawal syndrome. *Behav Pharmacol* **18**: 725–729.

Murray F, Harrison NJ, Grimwood S, Bristow LJ, Hutson PH. (2007). Nucleus accumbens NMDA receptor subunit expression and function is enhanced in morphine-dependent rats. Eur J Pharmacol **562**: 191–197.

Naseer M, Lee H, Ullah N, Ullah I, Park M, Kim S, Kim M. (2010). Ethanol and PTZ effects on siRNA-mediated GABAB1 receptor: Down regulation of intracellular signaling pathway in prenatal rat cortical and hippocampal neurons. *Synapse* **64**: 181–190.

Özek M, Üresin Y, Güngör M. (2003). Comparison of the effects of specific and nonspecific inhibition of nitric oxide synthase on morphine analgesia, tolerance and dependence in mice. *Life Sci* **72**: 1943–1951.

Özmen İ, Nazıroğlu M, Alici HA, Şahin F, Cengiz M, Eren I. (2007). Spinal morphine administration reduces the fatty acid contents in spinal cord and brain by increasing oxidative stress. *Neurochem Res* **32**: 19–25.

Qu X, Xu C, Wang H, Xu J, Liu W, Wang Y, Jia X, Xie Z, Xu Z, Ji C. (2012). Hippocampal glutamate level and glutamate aspartate transporter (GLAST) are up-regulated in senior rat associated with isoflurane-induced spatial learning/memory impairment. *Neurochem Res* **38**: 59–73.

Radad K, Hassanein K, Al-Shraim M, Moldzio R, Rausch WD. (2014). Thymoquinone ameliorates lead-induced brain damage in Sprague Dawley rats. *Exp Toxicol Pathol* **66**: 13–17.

Ramachandran V, Watts LT, Maffi SK, Chen J, Schenker S, Henderson G. (2003). Ethanol-induced oxidative stress precedes mitochondrially mediated apoptotic death of cultured fetal cortical neurons. *J Neurosci Res* **74**: 577–588.

Reckziegel P, Dias VT, Benvegnú D, Boufleur N, Silva Barcelos RC, Segat HJ. (2011). Locomotor damage and brain oxidative stress induced by lead exposure are attenuated by gallic acid treatment. *Toxicol Lett* **203**: 74–81.

Salim S. (2017). Oxidative Stress and the Central Nervous System. *J Pharmacol Exp Ther* **360**: 201–205.

Samarghandian S, Azimi-Nezhad M, Borji A, Samini M, Farkhondeh T. (2017). Protective effects of carnosol against oxidative stress induced brain damage by chronic stress in rats. *BMC Complement Altern Med* **17**: 249.

Samarghandian S, Samini F, Azimi-Nezhad M, Farkhondeh T. (2017). Anti-oxidative effects of safranal on immobilization-induced oxidative damage in rat brain. *Neurosci Lett* **659**: 26–32.

Sepulveda MJ, Hernandez L, Rada P, Tucci S, Contreras E. (1998). Effect of precipitated withdrawal on extracellular glutamate and aspartate in the nucleus accumbens of chronically morphine-treated rats: an in vivo microdialysis study. *Pharmacol Biochem Behav* **60**: 255–262.

Shiotsuka RN, Warren DL, Halliburton AT, Sturdivant DW. (2000). A comparative respiratory sensitization study of 2,4- and 2,6-toluene diisocyanate using guinea pigs. Inhal Toxicol **12**: 605–15.

Singh A, Kumar A. (2015). Microglial inhibitory mechanism of coenzyme Q10 against Aβ (1-42) induced cognitive dysfunctions: possible behavioral, biochemical, cellular, and histopathological alterations. *Front Pharmacol* **6**: 268.

Song X, Zhou B, Zhang P, Lei D, Wang Y, Yao G, Hayashi T, Xia M, Tashiro SI, Onodera S. (2016). Protective Effect of Silibinin on Learning and Memory Impairment in LPS-Treated Rats via ROS-BDNF-TrkB Pathway. *Neurochem Res* **41**: 1662–1672.

Udhayabanu T, Manole A, Rajeshwari M, Varalakshmi P, Houlden H, Ashokkumar B. (2017). Riboflavin Responsive Mitochondrial Dysfunction in Neurodegenerative Diseases. *J Clin Med* **6**: pii: E52.

Ullah I, Ullah N, Naseer MI, Lee HY, Kim MO. (2012). Neuroprotection with metformin and thymoquinone against ethanol-induced apoptotic neurodegeneration in prenatal rat cortical neurons. BMC Neurosci **13**: 11.

Valero J, Mastrella G, Neiva I, Sánchez S, Malva JO. (2014). Long-term effects of an acute and systemic administration of LPS on adult neurogenesis and spatial memory. *Front Neurosci* **8**: 83.

Wang L, Xu J, Tian Y, Wu H, Liu Y. (2007). Protective effect of N-acetylcysteine against lipopolysaccharide injury in hepatocytes of neonatal mice. *Zhonghua Er Ke Za Zhi* **45**: 30–33.

Wang X, Quinn PJ. (2010). Endotoxins: lipopolysaccharides of gram-negative bacteria. *Endotoxins: Structure, Function and Recognition*. Springer 3–25.

Wen ZH, Chang YC, Cherng CH, Wang JJ, Tao PL, Wong CS. (2004). Increasing of intrathecal CSF excitatory amino acids concentration following morphine challenge in morphine-tolerant rats. *Brain Res* **995**: 253–259.

Wolf O, Atsak P, Quervain D, Roozendaal B, Wingenfeld K. (2016). Stress and memory: a selective review on recent developments in the understanding of stress hormone effects on memory and their clinical relevance. *J Neuro-Endocrinol* **28**(8). doi: 10.1111/jne.12353.

Wu J, Basha MR, Brock B, Cox DP, Cardozo-Pelaez F, Mcpherson CA, Harry J, Rice DC, Maloney B, Chen D. (2008). Alzheimer's disease (AD)-like pathology in aged monkeys after infantile exposure to environmental metal lead (Pb): evidence for a developmental origin and environmental link for AD. *J Neurosci* **28**: 3–9.

Yamanouchi N, Okada SI, Kodama K, Hirai S, Sekine H, Murakami A, Komatsu N, Sakamoto T, Sato T. (1995). White matter changes caused by chronic solvent abuse. *Am J Neuroradiol* **16**: 1643–1649.

Yan X, Huang G, Liu Q, Zheng J, Chen H, Huang Q, Chen J, Huang H. (2017). Withaferin A protects against spinal cord injury by inhibiting apoptosis and inflammation in mice. *Pharm Biol* **55**: 1171–1176.

Young C, Roth KA, Klocke BJ, West T, Holtzman DM, Labruyere J, Qin YQ, Dikranian K, Olney JW. (2005). Role of caspase-3 in ethanol-induced developmental neurodegeneration. *Neurobiol Dis* **20**: 608–614.

Zabedah M, Razak M, Zakiah I, Zuraidah A. (2001). Profile of solvent abusers (glue sniffers) in East Malaysia. *Malays J Pathol* **23**: 105–109.

Zhu YJ, Zeng T, Zhu YB, Yu SF, Wang QS, Zhang LP, Guo X, Xie KQ. (2008). Effects of acrylamide on the nervous tissue antioxidant system and sciatic nerve electrophysiology in the rat. *Neurochem Res* **33**: 2310–2317.