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ORIGINAL PAPER

Nigella sativa and Derived Thymoquinone Prevents Hippocampal Neurodegeneration After Chronic Toluene Exposure in Rats

Mehmet Kanter

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Abstract The aim of this study was designed to investigate the possible beneficial effects of *Nigella sativa* (NS) and derived thymoquinone (TQ) on neurodegeneration in hippocampus after chronic toluene exposure in rats. The rats were randomly allotted into one of four experimental groups: A (control), B (toluene treated), C (toluene treated with NS) and D (toluene treated with TQ); each group contain 10 animals. Toluene treatment was performed by inhalation of 3,000 ppm toluene, in a 8 h/day and 6 day/week order for 12 weeks. Control group received 1 ml serum physiologic and the rats in NS and TQ treated groups (C and D) were given NS (in a dose of 400 mg/kg body weight) and TQ (50 mg/kg body weight) once a day orally by using intra gastric intubation for 12 weeks starting just after toluene exposure respectively. Tissue samples were obtained for histopathological investigation. To date, no histopathological changes of neurodegeneration in hippocampus after chronic toluene exposure in rats by NS and TQ treatment have been reported. In this study, chronic toluene exposure caused severe degenerative changes, shrunken cytoplasma, slightly dilated cisternae of endoplasmic reticulum, markedly swollen mitochondria with degenerated cristae and nuclear membrane breakdown with chromatin disorganization in neurons of the hippocampus. The distorted nerve cells were mainly absent in the TQ and NS-treated rats. We conclude that TQ and especially NS therapy causes morphologic improvement on neurodegeneration in hippocampus after chronic toluene exposure in rats. 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.

Keywords Toluene · *Nigella sativa* · Thymoquinone · Morphology · Hippocampus · Rats

M. Kanter (✉)
Department of Histology and Embryology, Faculty of Medicine, Trakya University, Edirne, Turkey
e-mail: mkanter65@yahoo.com

Introduction

Toluene, one of the most widely used industrial aromatic solvents, is used as an ingredient in gasoline, paints, lacquers, resins, nail polish, inks, cosmetic products, paint thinners, and adhesives. In addition to the industrial uses, toluene is abused via inhalation [1]. In humans, acute intoxication with toluene produces euphoria and disinhibition followed by hallucinations, tinnitus, ataxia, confusion, nausea and vomiting [2, 3]. In animal experiments, acute exposure to toluene at low to intermediate doses can increase locomotor activity, with an observable increase in the frequency of sniffing and rearing and at high doses induces ataxia [1, 4–7]. Subchronic toluene exposure at low concentrations also leads to adverse changes in neurobehavioral and neurochemical functions [8–12].

Toluene is distributed throughout the body, with accumulation in tissues with high lipid content [13]. In humans and animals, the primary effect associated with inhalation exposure to toluene is central nervous system (CNS) depression [14, 15]. Toluene's affinity for the lipid-rich structures of nervous tissue results in CNS toxic effects within minutes [13, 16]. Despite the widespread abuse of toluene and other solvents, the molecular sites of action of these compounds are largely unknown [17]. Mattia et al. [18] demonstrated that intraperitoneal injection of toluene caused a significant elevation in the rate of reactive oxygen species (ROS) generation and a reduction in glutathione

(GSH) levels in the brain. Long-term toluene abuse has led to neuropsychiatric and neurobehavioral disorders, which in many cases, but not all, were reversible. Some chronic toluene abusers have developed structural CNS damage [14, 19].

Apoptotic processes play an essential role during the development of the nervous system and hence for the final structure and function of the brain. The most critical time window for studying the influence of neurotoxicants on the level of apoptosis is probably when apoptosis is at its highest [20]. Recent studies have shown that prenatal exposure of rats to 1,800 ppm toluene induced neurobehavioral changes in the offspring [21] and increased apoptotic neurodegeneration at postnatal day 21 in the cerebellar granular cell layer [22]. In the control animals of that study, a specific peak of apoptotic activity in the granula cell layer of cerebellum was observed at postnatal day 21 compared to day 11 and 90. Exposure to toluene has been proposed to postpone the migration of granule cells to their final destination [22] resulting in a delay in the apoptotic peak. Prenatal stress produces learning deficits associated with inhibition of hippocampal neurogenesis [23, 24]. Prenatal stress has also been associated with an increased number of apoptotic neurones in the hypothalamic paraventricular nucleus [25].

The black seed, *Nigella sativa* (NS), family Ranunculaceae has been shown to contain >30% of fixed oil and 0.4–0.45% wt/wt of volatile oil. The volatile oil has been shown to contain 18.4–24% thymoquinone (TQ = 2-isopropyl-5-methyl-1,4-benzoquinone) and 46% monoterpenes such as *p*-cymene and α-piene [26]. Recently, clinical and animal studies have shown that the extracts of the black seeds have many therapeutic effects such as bronchodilatation, immunomodilative [27], antibacterial [28], hypotensive [29], antidiabetic [30, 31], hepatoprotective [30, 32], gastroprotective [33], antihistaminic and antioxidative [34] and neuroprotective [35]. TQ was isolated as the principal active ingredient from the volatile oil of NS [36]. TQ has been shown to attenuate eicosanoid generation [37], cisplatin nephrotoxicity [38], tetrachloride hepatotoxicity [39], rheumatoid arthritis [40] and gastric mucosal damage [34].

In the present study, it was aimed to investigate the possible beneficial effects of NS and derived TQ on neurodegeneration in hippocampus after chronic toluene exposure in rats.

Experimental procedure

Plant material and extraction procedure

The NS seeds were purchased from a local herb store in Van, Turkey. Sample specimens have been kept at the Department of Histology and Embryology, Yuzuncu Yil University, Van, Turkey for future reference. The seeds of NS were powdered in a mixer. 20 g of the powdered seeds were added to 400 ml of distilled water and extraction was carried out by steam distillation. The distillation process was continued until about 200 ml of distillate was collected. The distillate was extracted three times with chloroform. Moisture was removed by anhydrous sodium sulphate and the resultant extract was evaporated using a 40°C water bath leading to the appearance of the volatile oil. 500 mg of the volatile oil were dissolved in 1 ml of dimethyl sulphoxide (DMSO) then 9 ml of normal saline was added to yield a concentration of 50 mg volatile oil per 1 ml solution. The oil was given once daily orally in a dose of 400 mg/kg body weight by using intra-gastric intubation for up to 12 weeks [41]. TQ was obtained from Sigma Chemical Co. (St. Louis, MO). It was dissolved by the initial addition of DMSO, followed by the addition of normal saline (the final concentration of DMSO was less than 0.5%). The solution was administered at a dose of 50 mg/kg body weight once daily by using intra-gastric intubation for up to 12 weeks [42].

Animals

Forty healthy male Wistar albino rats, weighing 200–250 g and averaging 16 weeks old were utilized in this study. The animals were purchased from a local supplier (Ankara Laboratories), and housed in individual cages (360 × 200 × 190 mm) 1 month before the start of the experiments. Food and tap water were available ad libitum. In the windowless animal quarter automatic temperature (22 ± 2°C) and lighting controls (light on at 07 AM and off at 09 PM: 14 h light/10 h dark cycle) was performed. Humidity ranged from 50 to 55%. All animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

Experimental design

The rats were randomly allotted into one of four experimental groups: A (control), B (toluene treated), C (toluene treated with NS) and D (toluene treated with TQ); each group contain 10 animals.

Solvent exposures

Animals in group B, C and D were exposed to 3,000 ppm toluene (Merck, 99.5%) in inhalation chambers equipped

with a trap, and designed to sustain dynamic and adjustable airflow (11,250 mg/m^3). These animals were housed in individual cages within an inhalation chamber for 8 h/day (from 08 AM to 04 PM). This application was continued 6 day/week for a total of 12 weeks. Toluene was vaporized by bubbling additional airflow through a flask containing the test compound. The solvent concentration in the chambers was continuously monitored using a gas chromatography (GC). Control group (A) animals were housed in identical chambers ventilated with fresh air. The initial and final body weight changes of the various groups were recorded.

Drug preparation and sample collection

Group A and B received 1 ml serum physiologic and the rats in NS and TQ treated groups (C and D) were given NS (in a dose of 400 mg/kg body weight) and TQ (50 mg/kg body weight) once a day orally by using intra gastric intubation for 12 weeks starting just after toluene exposure respectively. At the end of the solvent exposure, all animals were anesthetized with i.p. injection of sodium thiopenthal (100 mg/kg, Sigma, St. Louis, MO, USA). Twenty minutes later, the anesthetized rats were sacrificed. Animals were pinned onto a dissection board and the peritoneal cavity was opened. Thus, immediately the hippocampal tissues were removed for histopathological investigation.

Histopathological procedures

Biopsies from the hippocampal tissues of the rats were harvested and tissue fragments were fixed in 10% neutral buffered formalin solution, embedded in paraffin, sectioned at 5 μm thickness and then, stained with hematoxylin and eosin (H&E). The preparations were evaluated by means of a bright-field microscope and photographed (Optiphot 2; Nikon, Tokyo, Japan).

Immunohistochemical procedures

Harvested hippocampal tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 5 μm thickness. Immunocytochemical reactions were performed according to the ABC technique described by Hsu et al. [43]. The procedure involved the following steps: (1) endogenous peroxidase activity was inhibited by 3% H_2O_2 in distilled water for 30 min; (2) the sections were washed in distilled water for 10 min; (3) nonspecific binding of antibodies was blocked by incubation with normal goat serum (DAKO X 0907, Carpinteria, CA) with PBS, diluted 1:4; (4) the sections were incubated with specific a rabbit polyclonal anti-caspase-3 antibody (Cat. # RB-1197-P, Neomarkers, USA), diluted 1:50 for 1 h, and then kept at room temperature; (5) the sections were washed in PBS for 3×3 min; (6) the sections were incubated with ABC complex (DAKO LSAB 2 Kit); (7) the sections were washed in PBS for 3×3 min; (8) peroxidase was detected with diaminobenzidine as substrate; (9) the sections were washed in tap water for 10 min and then dehydrated; (10) nuclei were stained with hematoxylin; and (11) the sections were mounted in DAKO paramount.

Electron microscopy

For electron microscopy, hippocampal tissue specimens were fixed with 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for 3 h at 4°C, washed in the same buffer for 1 h at 4°C, and post-fixed with 1% osmium tetraoxide in sodium phosphate buffer for 1 h at 4°C. The tissues were then dehydrated in graded series of ethanol starting at 50% each step for 10 min and, after two changes in propylene oxide. The tissue specimens were embedded in araldite. Ultrathin sections were prepared with Mg-uranyl acetate and lead citrate for the electron microscopic (Jeol JEM 1010) evaluation.

Microscopic examination

Histological specimens were examined under light microscopy, with the examination carried out at a magnification of 400 and the counts of neurons determined per square millimeter with the use of a standardized ocular grid. Intact or partially degenerated neurons were counted. The density and distribution of neurons was examined in the sections stained with hematoxylin–eosin. Tissue sections were examined under light microscopy (×400) and the number of the neurons counted within random high-power fields using a Nikon Optiphot 2 light microscope incorporating a square graticule in the eyepiece (eyepiece × 10, objective × 40, a total side length of 0.225 mm). Neuron density was assessed by counting the number of cells in 200 high power fields amongst the hippocampal tissue preparations of each group. The neuron density in each site was calculated and recorded as the number of neurons/mm^2. The tissue compartments were used to record neuron distribution in the hippocampal tissue.

Statistical analysis

All statistical analyses were carried out using SPSS statistical software (SPSS for windows, version 11.0). All data

were presented in mean (±) standard deviations (S.D.). Differences in measured parameters among the four groups were analyzed with a nonparametric test (Kruskal-Wallis). Dual comparisons between groups exhibiting significant values were evaluated with a Mann–Whitney *U*-test. These differences were considered significant when probability was less than 0.05.

Results

Initial of study there were 12 rats in the toluene group. Toluene dose was very high, so two animals died from toluene group during experiment and the rest of rats had loss of righting reflex and psychomotor impairment.

Histopathological findings

In the control group, the morphology of neurons in the CA1, CA2, CA3 and CA4 subfields of the hippocampus was normal (Fig. 1a). In the toluene treated group, the most consistent findings occurring in the histological tissue sections stained with hematoxylin-eosin were those indicating severe degenerative changes, shrunken cytoplasma and extensively dark picnotic nuclei in neurons of CA2,

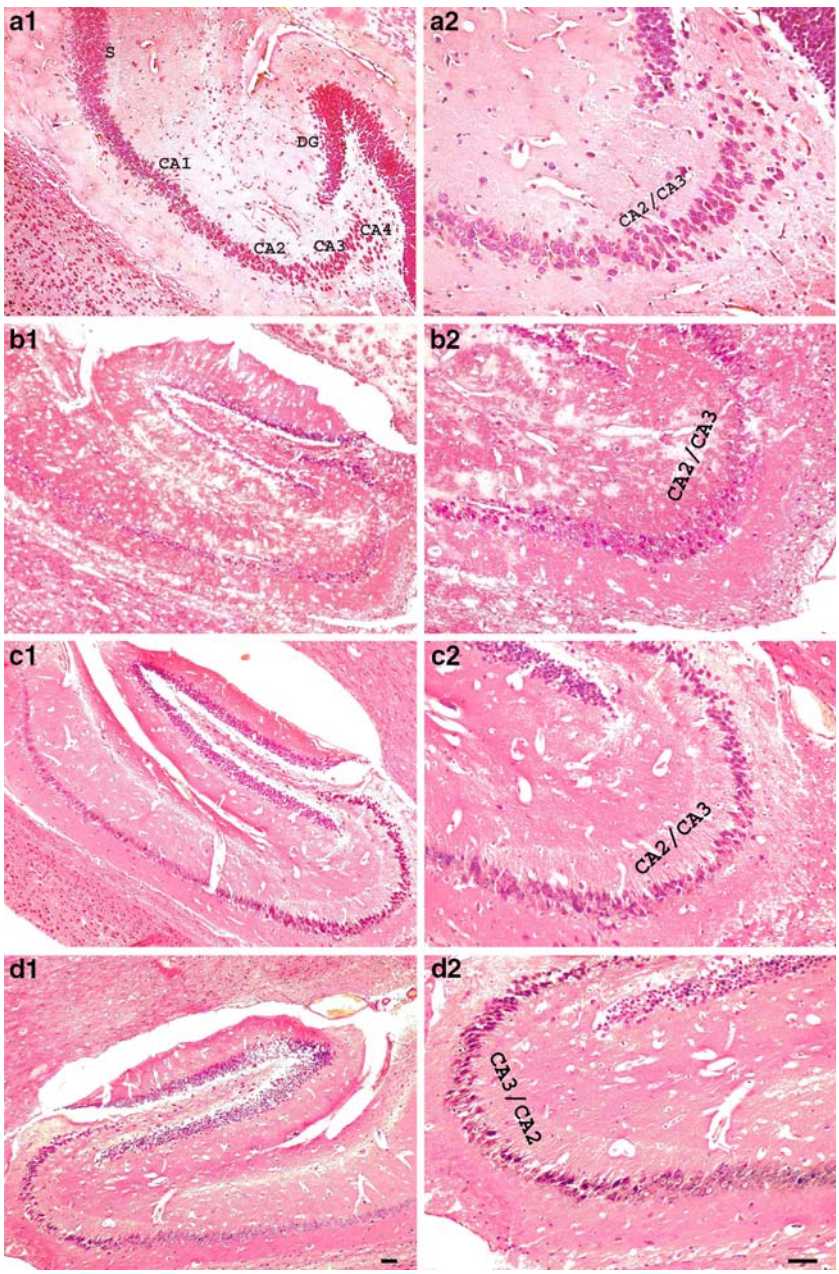

Fig. 1 Representative light microphotographs showing the histology of neurons in the hippocampus including the different subfields after chronic toluene exposure. (**a**–**d**). (**a** 1–2) Control rats: Normal hippocampal tissue histology is seen. (**b** 1–2) Toluene treated rats: Severe degenerative changes, shrinkage cytoplasma and extensively dark picnotic nuclei in neurons of CA2, CA3 and CA4 region of the hippocampus are seen. (**c** 1–2) Toluene treated rats with NS: Effective preventing of degenerative changes and shrunken in cytoplasma and nuclei are prominent in neuronal cells. (**d** 1–2) Toluene treated rats with TQ: Degenerative changes was less than that in the only toluene treated group (H&E, scale bars, 50 μm)

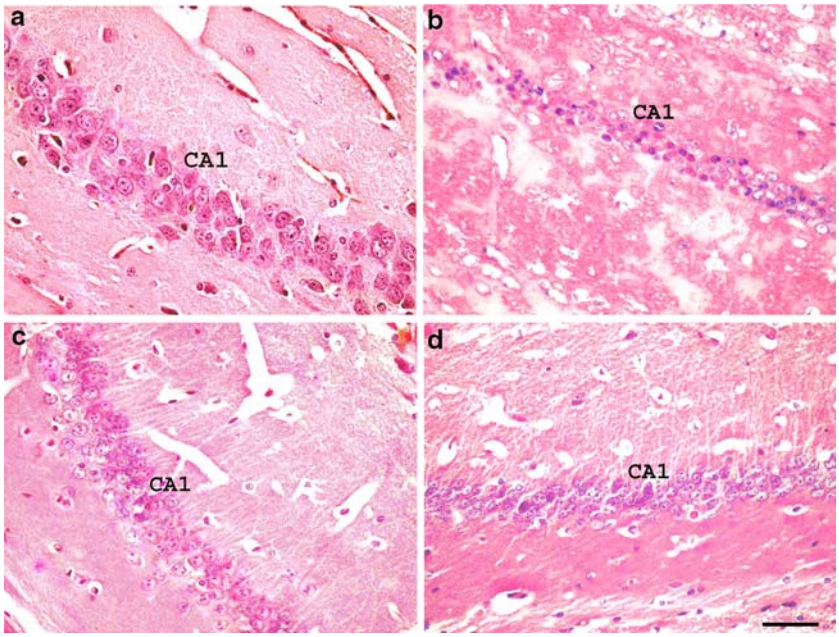

Fig. 2 Representative light microphotographs showing the histology of CA1 hippocampal neurons after chronic toluene exposure (**a**–**d**). (**a**) Control rats: Normal CA1 hippocampal neurons are seen. (**b**) Toluene treated rats: The severity of degenerative changes in the cytoplasma and especially in the nuclei of cells are seen, but the neuronal damage in the CA1 region of the hippocampus was not widespread as compared to the CA2, CA3 and CA4 region. (**c**) Toluene treated rats with NS: The dark stained nucleus and the distorted nerve cells were mainly absent in the NS treated rats. (**d**) Toluene treated rats with TQ: Mild degenerative changes on CA1 hippocampal neurons are seen (H&E, scale bar, 50 μm)

CA3 and CA4 region of the hippocampus. However, the neuronal damage in the CA1 (Fig. 2a–d) region of the hippocampus was not widespread as compared to the CA2, CA3 and CA4 region (Fig. 1b). In the TQ (Fig. 1d) and especially NS-treated (Fig. 1c) rat hippocampus, the intensity of neuronal changes was less than in the only toluene treated group. The dark stained nucleus and the distorted nerve cells were mainly absent in the NS and TQ-treated rats. In these groups, the severity of degenerative changes in the cytoplasma and especially in the nuclei of cells was less than that in the only toluene treated group. On the other hand, the number of neurons was decreased significantly in toluene treated rats compared to control ($P < 0.001$) and toluene treated with NS ($P < 0.01$) or TQ ($P < 0.05$) rats' hippocampal areas (Table 1).

Immunohistochemical findings

Light micrographs showed apoptotic neurons by caspase-3 immunohistochemistry after chronic toluene exposure in rats (Fig. 3a–d). Moderate caspase-3 immunoreactivity was observed in the cytoplasm of neurons in control rats (Fig. 3a). While the caspase 3 immunopositivity was increased in neurons of CA2, CA3 and CA4 region of the hippocampus following toluene exposure (Fig. 3b), the immunoreactivity was decorated the cytoplasm of neurons moderately in the CA1 region of the hippocampus. Treatment of TQ (Fig. 3d) and especially NS (Fig. 3c) markedly reduced the immunoreactivity of degenerating neurons after chronic toluene exposure.

Electron microscopic findings

In the control group, the ultrastructure of neurons in the hippocampus was normal (Fig. 4a). In the toluene treated group, the most consistent findings occurring in the ultra-thin sections stained with Mg-uranyl acetate and lead citrate were those indicating severe degenerative changes, shrunken cytoplasma, slightly dilated cisternae of endoplasmic reticulum and markedly swollen mitochondria

Table 1 The numbers (number/mm^2) of neurons in the hippocampal tissues of A (control), B (toluene treated), C (toluene treated with NS) and D (toluene treated with TQ) groups

Groups	CA1	CA2	CA3	CA4
A	124 ± 13.5	113 ± 12.3	114 ± 11.6	86 ± 7.7
B	73 ± 6.4[a]	64 ± 5.5[a]	62 ± 5.2[a]	51 ± 4.1[a]
C	108 ± 10.6[b]	96 ± 9.8[b]	95 ± 9.6[b]	67 ± 5.9[b]
D	87 ± 8.8[c]	78 ± 7.1[c]	77 ± 6.9[c]	59 ± 4.7[c]

Kruskal-Wallis test was used for statistical analysis. Values are expressed as means ± SD, $n = 10$ for each group; [a]$P < 0.001$ compared to A group, [b]$P < 0.01$ compared to B group, [c]$P < 0.05$ compared to B group, [c]$P < 0.05$ compared to C group

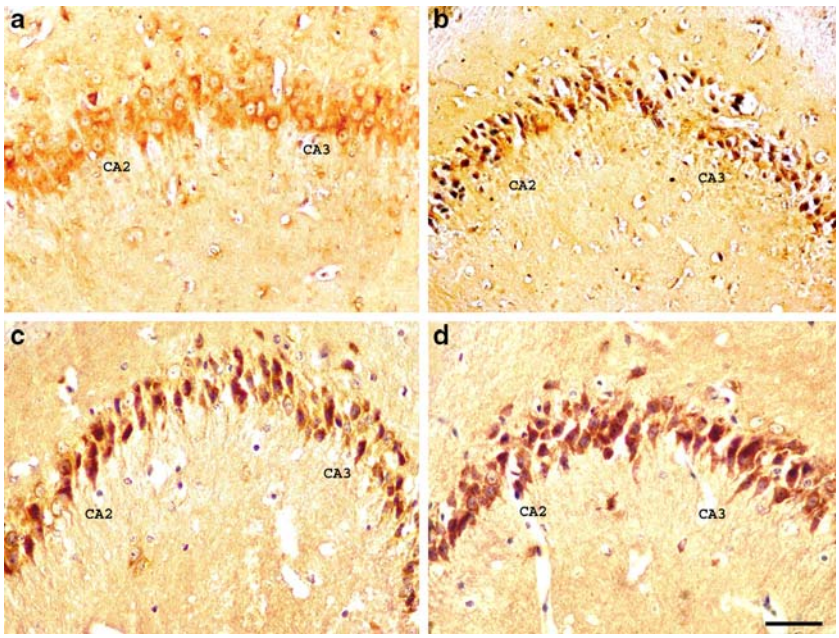

Fig. 3 Representative light microphotographs showing apoptotic cortical neurons of CA2 and CA3 region of the hippocampus after chronic toluene exposure by caspase-3 immunohistochemistry (**a**–**d**). (**a**) Control rats: Neuronal cells are moderately stain with the anti-caspase-3 antibody. (**b**) Toluene treated rats: The caspase-3 immunopositivity was strongly increased in neurons of CA2 and CA3 region of the hippocampus after chronic toluene exposure. (**c**) Toluene treated rats with NS: Treatment of NS markedly reduced the immunoreactivity of degenerating neurons after toluene exposure. (**d**) Toluene treated rats with TQ: Treatment of TQ also reduced the immunoreactivity of degenerating neurons, but less extent than NS treatment (Immunoperoxidase, haematoxylin counterstain, scale bar, 50 μm)

with degenerated cristae were observed in neurons of the hippocampus. The nucleus of these cells seem to have increased evidence of nuclear membrane breakdown with chromatin disorganization and severely shrunken appearance, all signs of advanced cell death (Fig. 4b). Although NS treatment was effective preventing the dilatation of endoplasmic reticulum, mitochondrial degeneration and irregularly shaped nuclei, the irregularly shaped chromatin clumps and moderately mitochondrial swollen was still observed in neurons (Fig. 4c). In the TQ treated rat hippocampus, the severity of degenerative changes in the cytoplasma and especially in the nuclei of cells was less than that in the only toluene treated group (Fig. 4d).

Discussion

Neurological symptoms have been noted in chronic solvent abusers as well as in those exposed to volatile solvents through environmental or industrial contact. Toluene also causes memory impairment in humans, an increased tendency to sleep, frequent headaches, and eye irritation [44]. Paint workers, who are chronically exposed to solvents such as toluene, often complain of dizziness, depression, and fatigue [45, 46]. Brain magnetic resonance imaging shows cerebral and hippocampal atrophy as well as a loss in brain volume in toluene/solvent abusers [47–49]. Despite substantial epidemiological data regarding toluene's neurobehavioral and neurotoxic effects, the sites and mechanisms of action for toluene in the brain are not fully known.

Several studies [50] have developed a chronic toluene inhalation model in the cat to address the neuronal changes at prefrontal cortex, cerebellum and hippocampus. All this structures showed varying degrees of neuronal degeneration to necrosis. Even if injury signs were widespread, the neuronal layers were not equally affected and there were clear differences in injury severity. In the prefrontal cortex, injured neurons were observed in layers II, III and V/VI extending over several gyri. Lesions were time related, as was more clearly observed in Purkinje cells. In dorsal hippocampus alterations were particularly severe in CA1 and CA3. In ventral hippocampus damaged neurons were scarce and located mainly in CA2. The possible relation of this findings with behavioral changes observed during chronic toluene inhalation are noted.

Although several studies have examined the effects of chronic toluene exposure on gross brain morphology, the effects of prolonged toluene treatment on ligand-gated ion channels have not been previously characterized. Neuronal toxicity has been observed in the CA2 and the CA3 regions of the rat hippocampus following withdrawal from chronic

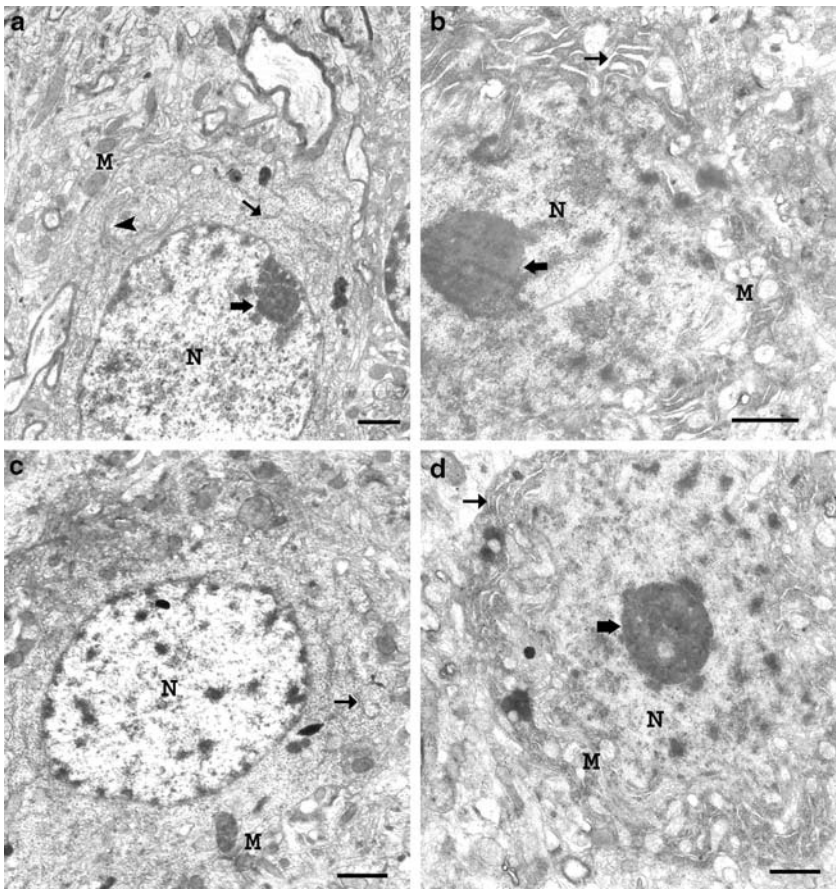

Fig. 4 Representative transmission electron microphotographs showing the ultrastructure of neurons in the hippocampus after chronic toluene exposure (**a**–**d**). (**a**) Control rats: Normal neurons are shown in *a* for reference. Uniformly condensed, regularly shaped, round chromatin clumps were present in cells. The ultrastructure of cytoplasma and cytoplasmic organelles showed a normal appearance. (**b**) Toluene treated rats: Disintegrating neuron containing shrunken cytoplasma, slightly dilated cisternae of endoplasmic reticulum and severely damaged mitochondria in varying stages of destruction were observed. Typical necrotic neurons had smaller clumps of irregularly shaped, condensed chromatin with nuclear membrane breakdown. (**c**) Toluene treated rats with NS: Although NS treatment was effective preventing the above mentioned findings, moderately damaged mitochondria was still observed. (**d**) Toluene treated rats with TQ: Disintegrating neurons, which had a relatively homogeneous nucleus with a few irregular chromatin clumps with nuclear membrane breakdown and, condensed granular cytoplasm with slightly dilated cisternae of endoplasmic reticulum and markedly swollen mitochondria were typically found in this model. (thick arrow: nucleolus, N: nucleus, arrowhead: Golgi apparatus, thin arrow: cisternae of endoplasmic reticulum, M: mitochondria, Scale bars, 1 μm)

toluene exposure [51]. This toxicity may result from increased neuronal excitability that may predispose neurons to glutamate-mediated excitotoxicity [52]. However, the results from Bale et al. [53] suggest that toluene induces compensatory responses in the functional expression of ion channels that regulate neuronal excitability.

Pathological studies of the effects of toluene inhalation based on biopsies of the sural nerve have shown swelling of the axons and an extremely thin lamella of the myelin sheath [54]. Coskun et al. [55] suggested that increased lipid peroxidation, reduced antioxidant enzyme activities, and ultrastructural changes found in their study indicates that chronic toluene inhalation might be involved free radical processes.

In the present study, chronic toluene exposure caused severe degenerative changes, shrunken cytoplasma, slightly dilated cisternae of endoplasmic reticulum, markedly swollen mitochondria with degenerated cristae and nuclear membrane breakdown with chromatin disorganization in neurons of the hippocampus. These data are corroborated by previous studies reported by other investigators on toluene -induced neurotoxicity in different animals. [50, 51, 54, 55].

Coskun et al. [55] conclude that ebselen therapy causes biochemical and morphologic improvement on sciatic nerves which have been subjected to toluene exposure. Baydas et al. [56] investigated that the effects of melatonin on the glial reactivity induced by the exposure of rats to the fumes of thinner, which due to the presence of toluene (3,000 ppm, for 45 days) are known to be neurotoxic. But to date, no histopathological changes of toluene induced oxidative damage by NS or TQ treatment have been

reported. Recent studies [35] have attempted to identify the NS has some neuroprotective and restorative effects on secondary pathochemical events after spinal cord injury in rats. These restorative effects mainly observed on oxidative stress and neuronal numbers and neuronal morphology.

Most properties are mainly attributed to quinone constituents, of which TQ is the main active constituent of the volatile oil of the NS [57]. TQ has been demonstrated to possess strong antioxidant properties [37], and has been shown to protect non-tumor tissues from chemotherapy-induced damage [38, 58] and suppresses expression of inducible nitric oxide synthase in rat macrophages [59]. Al-Majed et al. [60] investigated that the oral administration of TQ protected rats from ischemia-induced brain injury. The protection may be due to the reduction of oxidative stress. These observations suggest that TQ may be a clinically viable protective agent against a variety of conditions where cellular damage is a consequence of oxidative stress. In addition, TQ may have the potential to be used in the prevention of neurodegenerative diseases such as forebrain ischemia. In our study, in the TQ and especially NS-treated rat hippocampus, the severity of degenerative changes in the cytoplasma and especially in the nuclei of cells was less than that in the only toluene treated group. The dark stained nucleus and the distorted nerve cells were mainly absent in the NS and TQ-treated rats.

For hippocampus on postnatal day 6, the caspase-3 activity was higher in the toluene group compared to the control group [61]. Wen et al. [62] demonstrated for the first time that erythropoietin protects hippocampus neurons in vivo from apoptosis after status epilepticus, and that anti-apoptotic signaling pathways initiated by erythropoietin included caspase-3 activation in hippocampal neurons. The reproductive and developmental toxicity of toluene in animals and man is well characterized [63, 64]. Studies have shown that in rats toluene just like ethanol, has toxic effects on the brain development as well as disturbances in the cognitive functions [65]. Previously it has been shown that toluene affects the apoptotic neurodegeneration in the cerebellar granular cell layer after prenatal exposure [22]. In this study, moderate caspase-3 immunoreactivity was observed in the cytoplasm of neurons in control rats, whereas the caspase 3 immunopositivity was increased especially in neurons of CA2 and CA3 region of the hippocampus following toluene exposure. Treatment of TQ and especially NS markedly reduced the immunoreactivity of degenerating neurons after chronic toluene exposure.

Some studies [51] have attempted to identify the structural basis of the toxic effects of toluene in the hippocampus, a region of the brain known to be involved in learning and memory processes and well suited for stereological analysis. Rats were exposed to 1,500 ppm of toluene, 6 h per day, 5 days per week for 6 months. This was followed by a 4-month-period without exposure prior to sacrifice. The total number of neurons in each of the five subdivisions of hippocampus of six exposed and six control rats was estimated with the optical fractionator. A statistically significant neuron loss of 16% was found in region inferior (CA3 and CA2) of the exposed rats. In a study the influences of toluene intoxication on quantity of the pyramidal and granular cells in hippocampus was analyzed in rats. The number of the pyramidal neurons decreases by 26% in the CA-3 field will induce deterioration of the hippocampal neural circuits and destroy of memory and learning processes in the animals [66]. In the present study, the number of neurons was decreased significantly in toluene treated rats compared to control and toluene treated with NS or TQ rats' hippocampal areas.

We conclude that TQ and especially NS therapy causes morphologic improvement on neurodegeneration in hippocampus after chronic toluene exposure in rats. 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.

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