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Original article

The effects of *Nigella sativa* on neural damage after pentylenetetrazole induced seizures in rats

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ABSTRACT

Nigella sativa (NS) has been suggested to have neuroprotective and anti-seizures properties. The aim of current study was to investigate the effects of NS hydro-alcoholic extract on neural damage after pentylenetetrazole (PTZ) – induced repeated seizures. The rats were divided into five groups: (1) control (saline), (2) PTZ (50 mg/kg, i.p.), (3–5) PTZ-NS 100, PTZ-NS 200 and PTZ-NS 400 (100, 200 and 400 mg/kg of NS extract respectively, 30 min prior to each PTZ injection on 5 consecutive days). The passive avoidance (PA) test was done and the brains were then removed for histological measurements. The PTZ-NS 100, PTZ-NS 200 and PTZ-NS 400 groups had lower seizure scores than PTZ group ($P < 0.01$ and $P < 0.001$). The latency to enter the dark compartment by the animals of PTZ group was lower than control in PA test ($P < 0.01$). Pre-treatment by 400 mg/kg of the extract increased the latency to enter the dark compartment ($P < 0.05$). Meanwhile, different doses of the extract inhibited production of dark neurons in different regions of hippocampus ($P < 0.001$). The present study allows us to suggest that the NS possesses a potential ability to prevent hippocampal neural damage which is accompanied with improving effects on memory.

1. Introduction

Epilepsy is a common and heterogeneous neurological disorder arising from biochemical and molecular events that are incompletely understood. The results of human studies suggest that epilepsy affects cognition, learning and memory.[1] Animal studies have also confirmed that prolonged or recurrent seizures cause memory and emotional deficits.[2] The rats which had experienced recurrent seizures also showed the damages to the parts of the brain such as the hippocampus and other parts of the limbic system.[3] Even brief induced seizures lasting 60–70 s by administering a single convulsive dose of pentylenetetrazole (PTZ, 50 mg/kg, i.p.) has been shown to impair learning and memory.[4]

Dark neurons are considered as a group of cells undergoing early damage in their cytoskeleton such as microtubules or microfilaments, representing small dense nuclei inside of them.[5] Dark neurons appear under specific conditions such as mechanical forces (head injuries or an electric shock), pathological metabolic conditions (hypoglycemia or ischemia) and epilepsy. It has been reported that a percentage of the dark neurons produced under such conditions recover while, the others can no more be alive.[6] No specific method is considered for detecting the dark neurons, but they are almost diagnosed by their hyperbasophilia, hyperargyrophilia, and

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high-electron density properties in histological sections.[6] Production of dark neurons has also been reported during epilepsy.[7] Dark neurons during seizures are produced due to a cellular stress caused by markedly increased intracellular Ca^{2+} concentration, which in turn, results in ultrastructure compaction in the neurons.[8,9] Baracskay *et al.,* suggested that generalized seizure produces widespread dark cells throughout the brain especially in the hippocampus and the pontine reticular formation.[8,9] On the other hand, experimental evidence indicates that antioxidant compounds protect against the neuronal damage observed during epilepsy and seizures.[10] The protective effects of antioxidant compounds against learning and memory impairments due to epilepsy and seizures has also been well documented.[11,12]

Nigella sativa L. (NS) is an annual flowering plant native to different regions of southern Europe and some parts of Asia. The flowers are delicate and are usually colored pale blue and white with small black seeds.[13] NS seeds are the source of active components such as 30–40% fixed oil, 0.5–1.5% essential oil, various sugars and proteins and pharmacologically active components containing thymoquinone (TQ), ditimoquinone (DTQ) and nigellin.[13–15] In traditional medicine, this herb was identified to have healing power so that it has been used in the Middle East and Far East for treating diseases such as asthma, headache, dysentery, infections, obesity, back pain, hypertension and gastrointestinal problems. Finally, there is a common Islamic opinion that the NS is useful for all diseases except death.[16] The results of previous experimental studies have confirmed the extract of NS seeds and TQ have inhibitory effects on inducible nitric oxide synthase and production of nitric oxide[13,17,18] as well as anti-inflammatory and anticancer activities.[19,20] Both NS and TQ also showed the beneficial effects in lipopolysaccharide – induced depression like behavior in rats.[21] The anti-oxidant effects of NS and TQ in carbon tetrachloride (CCl4)-induced oxidative injury in rat liver,[22] isolated rat hepatocytes,[23] hypercholesterolemic rats[24] and gentamicin and cyclosporine induced kidney injury have been reported.[25] The antioxidant effects of NS oil and TQ in hippocampal tissues of the rats subjected to cerebral ischemia-reperfusion has also been reported.[26] Neuroprotective effects for NS and TQ has also been suggested.[27–30]

Based on the properties of NS which has been reported in traditional medicine and in experimental studies, the present study was designed in order to evaluate possible effects of the plant hydro-alcoholic extract on neural damage after PTZ-induced seizures in rats.

2. Materials and methods

2.1. Preparing the plant extract

Powdered seeds (100 g) of NS were extracted in a Soxhlet extractor with ethanol (70%). The resulting extract (yielded 32%) was concentrated under reduced pressure and kept at −20 °C until being used. The extract was dissolved in saline.[30]

2.2. Animals and the experimental protocol

Thirty male Wistar rats (8 weeks old and weighted 230 ± 20 g) were kept at 22 ± 2 °C and 12 h light/dark cycle at 7:00 am. They were randomly divided to five groups and treated according to the experimental protocol. Group 1 (control group) received saline instead of NS extract or PTZ. The animals in group 2 (PTZ group) were treated by saline instead of NS extract and were injected PTZ (50 mg/kg, i.p.). Groups 3 (PTZ-NS 100), 4 (PTZ-NS 200) and 5 (PTZ-NS 400) were treated by 100, 200 and 400 mg/kg of NS (i.p.) respectively, before each PTZ injection.

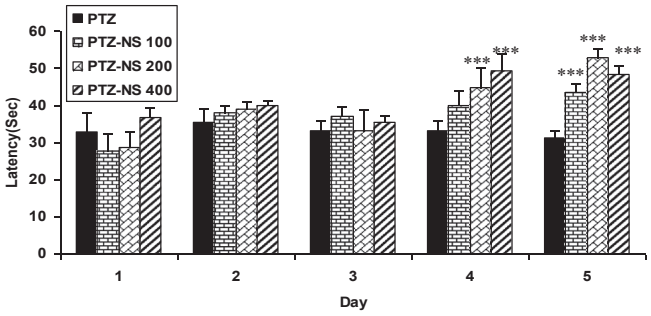

Fig. 1. Comparison of latency to the onset of seizures between groups. Data are presented as mean ± SEM (n = 6 in each group). $^{***}P < 0.001$ in comparison with PTZ group. The animals were injected by PTZ and observed for 60 min. The animals of PTZ-NS 100, PTZ- NS 200 and PTZ-NS 400 groups were treated by 100, 200 and 400 mg/kg of *Nigella sativa* (NS) extract before PTZ injection. The animals of PTZ group received saline instead of NS extract.

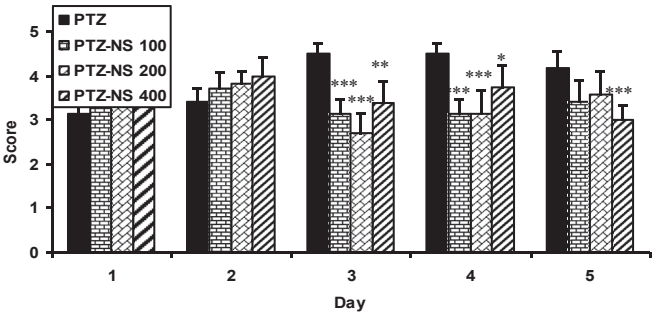

Fig. 2. Comparison of seizure score between groups. Data are presented as mean ± SEM (n = 6 in each group). $^{*}P < 0.05$ and $^{***}P < 0.001$ in comparison with PTZ group. The animals were injected by PTZ and observed for 60 min. The animals of PTZ-NS 100, PTZ-NS 200 and PTZ-NS 400 groups were treated by 100, 200 and 400 mg/kg of *Nigella sativa* (NS) extract before PTZ injection. The animals of PTZ group received saline instead of NS extract.

2.3. Behavioral procedures

2.3.1. PTZ-induced repeated seizures

The animals were injected by 50 mg/kg PTZ. Following each injection the rats were placed a Plexiglas cage separately, and observed for 60 min. The resultant seizures were classified according to a modified Racine scale as follows: 1- Mouth and facial movements; 2- Head nodding; 3- Forelimb clonus; 4- Rearing; 5- Rearing and falling. The latencies to the first sign of seizure were also recorded.[31,32]

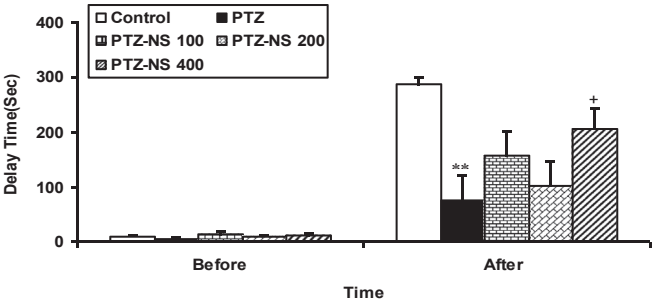

Fig. 3. Comparison of time latency for entering the dark compartment. Data are presented as mean ± SEM (n = 6 in each group). $^{**}P < 0.01$ in comparison with control group, $^{+}P < 0.05$ in comparison with PTZ group. The animals were injected by PTZ and observed for 60 min. The animals of PTZ-NS 100, PTZ-NS 200 and PTZ-NS 400 groups were treated by 100, 200 and 400 mg/kg of *Nigella sativa* extract before PTZ injection. The animals of PTZ group received saline instead of *Nigella sativa* extract. The animals of control group received saline instead of both the extract and PTZ.