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The Effect of *Withania somnifera* Alcoholic Extract on Learning and Memory Disturbance in a Model of Temporal Lobe Epilepsy in the Rat

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ABSTRACT

Background and Objective: Temporal lobe epilepsy (TLE) usually leads to memory deficit. In this study, we tried to assess the effect of *Withania somnifera* extract on the impaired learning and memory in the intrahippocampal kainate model of TLE in the rat.

Materials & Methods: Male rats (n=32) were divided into sham, extract+sham, kainite, and kainite+extract. For induction of epilepsy, unilateral hippocampal injection of 1 microgram of kainate was made. Rats received the extract at a dose of 100 mg/kg daily for three days prior to surgery. At the end of 4 weeks, to assess learning and memory, initial latency and step through delay using passive avoidance test and the percentage of alternation behavior in the Y maze test were determined.

Results: Extract pretreatment of kainate group did not significantly attenuate severity and incidence rate of seizures, did not significantly improve retrieval and recall in passive avoidance and significantly ameliorated spatial memory deficit in Y maze ($p<0.05$).

Conclusion: These data suggest that *Withania somnifera* alcoholic extract could attenuate spatial memory disturbance in TLE model in rat.

1. Introduction

Epilepsy is a chronic neurologic disorder with recurrent seizures and with relatively high prevalence in the society (1). Temporal lobe epilepsy (TLE) is considered the most frequent type of epilepsy (1,2). Current therapies could only symptomatically mitigate seizures, therefore, new drugs with antiepileptic property are needed to correct the underlying abnormal processes involved in epilepsy (3). Even, new treatments are strictly needed to prevent or even reverse the cellular mechanisms of epileptogenic foci (4). A reliable animal model of TLE is induced via intrahippocampal injection of the excitotoxic glutamate analog kainate (KA) in rodents, with ensuing impaired learning and memory (5). Cognitive deficits and learning and memory impairments are relatively frequent in TLE patients (6,7).

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Withania somnifera L. (WS), also known as ashwagandha and as Indian ginseng, is a medicinal herb possessing different therapeutic properties such as memory enhancer and antioxidant properties (8). Root extracts from this plant could significantly lower hippocampal neurodegeneration due to its neuroprotective effect (9). WS could affect AMPA receptor function in the cerebellum of rats with TLE, ameliorate spatial memory deficits by enhancing antioxidant system and restoring altered NMDA receptor density in epilepsy with anticonvulsant effect (10-12). Therefore, we tried to assess whether WS alcoholic extract could mitigate learning and memory deficits in TLE model.

2. Materials and Methods

2.1. WS extract preparation

The plant was obtained from local grocery (Tehran) in July 2013 and was systemically identified at the Department of Botany of Shaheed Beheshti University. Then, it was finely powdered with a grinder. Thereafter, 50 g of powder was suspended in 500 ml of methanol for 48 h in dark (percolation method). The extract was then filtered and concentrated to obtain the solid residue which was and refrigerated until further use. The yield of this process was 23.2% (w/w). WS extract of lower concentrations were prepared by dilution of the stock with cold normal saline.

2.2. Experimental procedure

All experiments were conducted on adult male Wistar rats (210-260 g; n = 32) (Pasteur's Institute, Tehran, Iran). They were housed in a temperature-controlled colony room under light/dark cycle with food and water available ad libitum. Procedures involving animals were conducted in compliance with NIH guidelines for the care and use of laboratory animals.

Rats were randomly divided into equal-sized sham, WS-pretreated sham-operated (Sham+*Withania somnifera*), kainate, and *Withania somnifera*-pretreated kainate (kainate+*Withania somnifera*) groups. For intrahippocampal injections, rats were anaesthetized with chloral hydrate (300-350 mg/kg), placed into the stereotaxic frame (Stoelting Co., USA) with the incisor bar set at 3.3 mm below the interaural line. The dorsal surface of the skull was exposed and a burr hole was drilled in the skull using the following stereotaxic coordinates according to the atlas of Paxinos and Watson (13): anteroposterior (AP), 4.1 mm caudal to bregma; 4.2 mm lateral to the midline (right side), and 4.2 mm ventral to the dura. A 5 μl microsyringe filled with normal saline containing 0.2 μg/1 μl of kainate was placed over the burr hole and kainate solution was injected at a rate of 1 μl/min in order to induce experimental model of TLE. Kainic acid (Sigma-Aldrich, USA) was dissolved in cold normal saline just prior to surgery. The sham group received an equivalent volume of normal saline at the same stereotaxic coordinates. The microsyringe was slowly withdrawn after 5 min and the rat scalp was sutured. The sham+*Withania somnifera* group received WS alcoholic extract i.p. at a dose of 100 mg/kg/day starting 3 days before the surgery and the last treatment was 1 h before surgery.

2.3. Behavioral assessment of seizure

During the first 24-h post-surgery, all animals were assessed for behavioral progression of kainate-induced seizures 4 h/day and scored according to Racine's standard classification: 0, no reaction; 1, stereotypic mounting, eye blinking, and/or mild facial clonus; 2, head nodding and/or multiple facial clonus; 3, myoclonic jerks in the forelimbs; 4, clonic convulsions in the forelimbs with rearing; and 5, generalized clonic convulsions associated with loss of balance (14).

2.4. Y-maze task

Short-term spatial recognition memory performance was assessed by recording spontaneous alternation behavior in a single-session Y-maze as described before (15, 16). Each rat was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded visually. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The number of maximum spontaneous alternation was then the total number of arms entered-2 and the percentage is calculated as the ratio of actual to possible alternations (defined as the total number of arm entries - 2).

2.5. Single-trial passive avoidance test

This test was conducted 2-3 days after Y-maze task and was conducted as described before (15, 17). On the first and second days of testing, each rat was placed on the apparatus and left for 5 min to habituate to the apparatus. On the third day, an acquisition trial was performed. Rats were individually placed in the illuminated chamber. After a habituation period (5 min), the guillotine door was opened and after the rat entering the dark chamber, the door was closed and an inescapable scrambled electric shock (1 mA, 1 s once) was delivered. In this trial, the initial latency (IL) of entrance into the dark chamber was recorded and rats with ILs greater than 60 s were excluded from the study. Twenty-four hours later, each rat was placed in the illuminated chamber for retention trial. The interval between the placement in the illuminated chamber and the entry into the dark chamber was measured as step-through latency (STL up to a maximum of 180 s as cut-off).

2.6. Statistical analysis

Values were expressed as means ± SEM. To compare the groups, data were analyzed using Kruskall-Wallis and Mann-Whithney U tests and the null hypothesis was rejected at a level of 0.05.

3. Results

3.1. Seizure activity and behavior

Sham and sham+WS groups showed no signs of seizure activity during the first 24 h post-surgery and/or after 4 weeks. In contrast, all rats (100%) in kainate group exhibited high scores of seizures. Meanwhile, rats injected with KA and pretreated with WS also exhibited rather severe behavioral signs as compared to kainate group and this difference was statistically non-significant versus kainate group (Fig. 1).

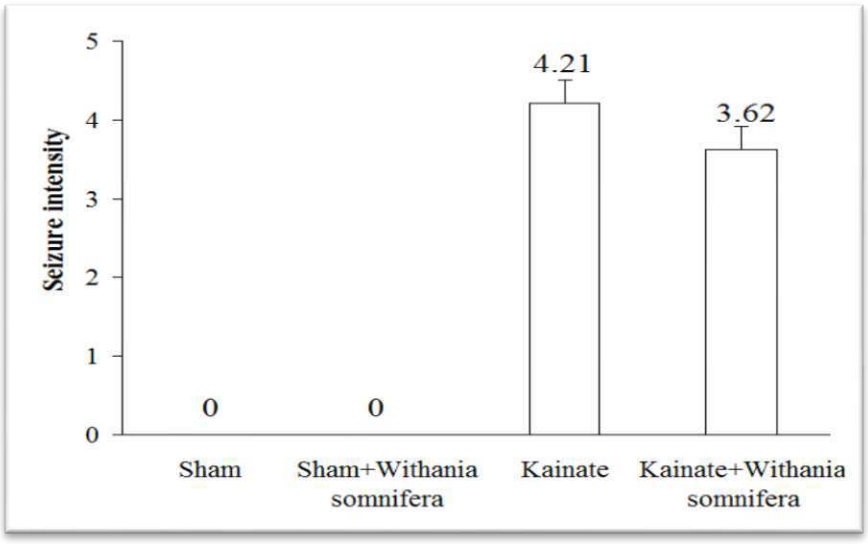

Fig.1: Averaged seizure intensity in experimental groups

3.2. Spatial recognition memory in Y maze

Figure 2 shows the results for the performance of rats in Y-maze task, in which short-term spatial recognition memory as an alternation behavior can be examined. In this respect, the alternation score of the WS-pretreated sham group was not statistically different from that of the vehicle-treated sham group. In contrast, kainate group had a significantly lower alternation score as compared to sham-operated rats ($p<0.05$) and WS treatment of kainate group did improve it ($p<0.05$). In our study, locomotor activity of the animals was also assessed by counting the total number of arms visited by each rat to avoid the compounding effect of locomotor activity on memory processes in experimental groups (Fig. 2). In this respect, there were not any significant differences among the groups.

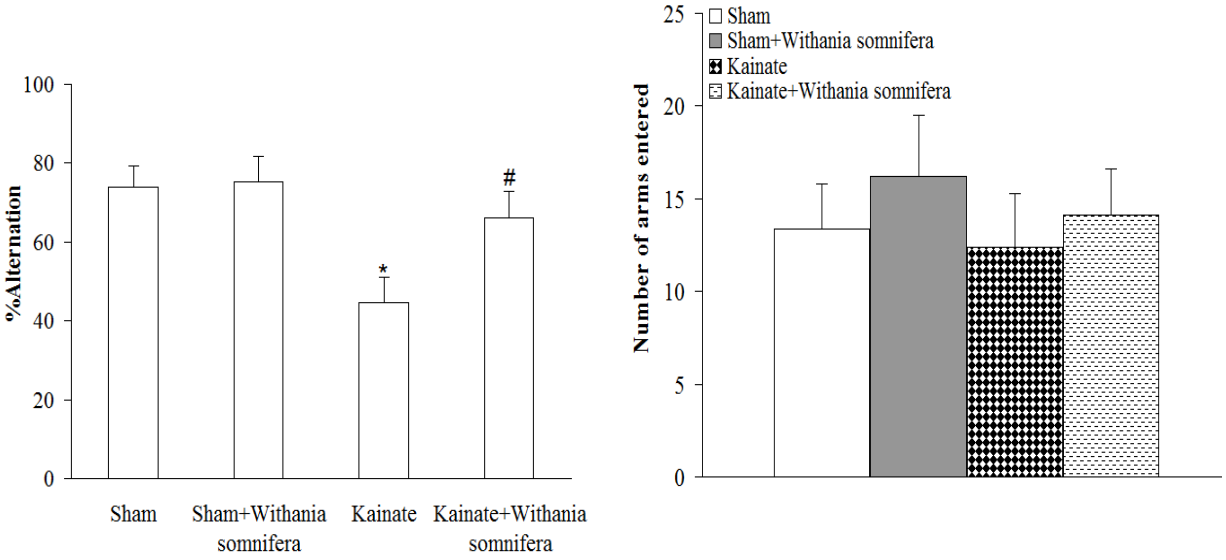

Fig. 2: Spontaneous alternation behavior (left panel) and locomotor activity of animals as shown by the total number of arms entered (right panel) in Y-maze task. * $p<0.05$ (as compared to sham)

3.3. Learning and memory in passive avoidance test

Figure 3 shows the performance of rats in passive avoidance paradigm as indicated by initial (IL) and step-through latencies (STL). Regarding IL, there was no significant difference among the groups. With respect to STL, WS-pretreated sham group did not show a significant change as compared to sham group. In contrast, kainate and WS-pretreated groups showed a significant impairment in retention and recall in passive avoidance test ($p<0.01$) in comparison with sham group, as it was evident by a lower STL and WS pretreatment of kainate group did not significantly improve it in comparison with kainate group.

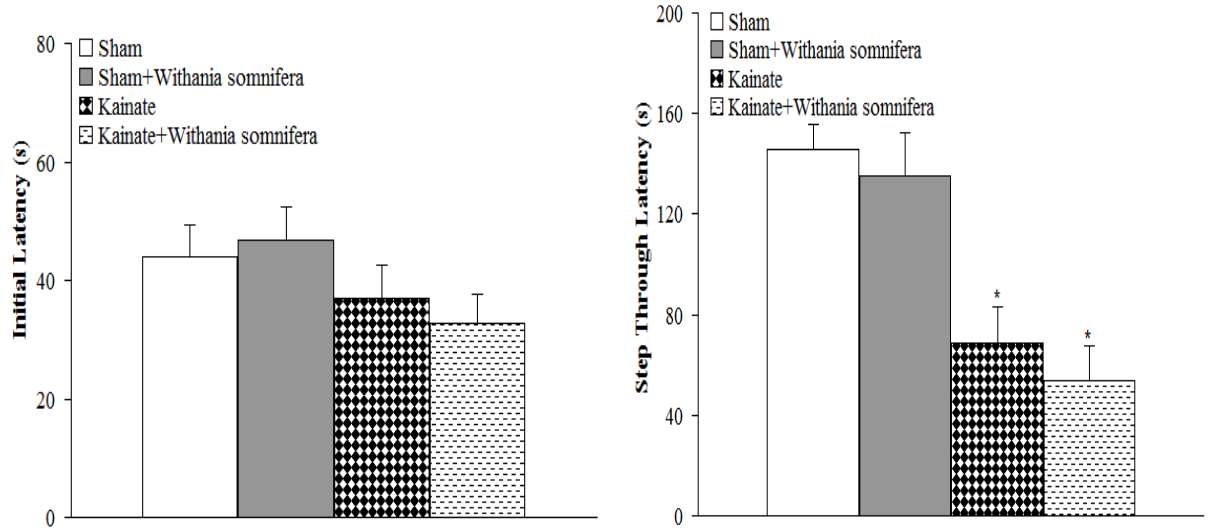

Fig. 3: Initial (IL) (left panel) and step-through latencies (STL) (right panel) in single-trial passive avoidance test. * $p<0.01$ (as compared to sham)

4. Discussion

Temporal lobe epilepsy is regarded as a recurrent debilitating neurological disorder as a result of excitatory or inhibitory circuits (18). Intracerebral injection of kainate into the CA3 area causes development of epileptic seizures, followed by a pattern of cell loss that is similar to that seen in patients suffering from TLE (19). For this reason, kainate-induced brain damage has been routinely used for modeling TLE (5, 20). Spatial hippocampus-dependent memory impairment in TLE has been previously reported (21, 22). Mice with intrahippocampal injection of KA show deficits of spatial learning and short- and long-term memories in a hippocampus-dependent large diameter pool Morris water maze task (MWM) (23). Learning and memory were also impaired in pilocarpine epileptic mice (24). Adult mice that received unilateral injection of kainate into the dorsal hippocampus develop epilepsy and ipsilateral and to some extent contralateral neuronal loss in the hippocampus, which is associated with impairment of acquisition and retention of memory (5, 23). Cognitive deficits in TLE is due to the neurodegenerative processes in the brain structures including hippocampal areas (23), astrocytic hypertrophy (25), and sprouting of new connections (26). In our study, although acquisition process in passive avoidance test as determined by IL did not change, but retention and recall of stored information (as shown by STL) was impaired in kainate injected rats, that was consistent with previous studies using other memory assessment tests (5, 23).

Following kainate injection, there is an increased oxidative stress (27) and development of seizures is also associated with such stressful condition (28). An increased production of reactive oxygen species could reduce cognitive function (28). On the other hand, deficiency of antioxidant redox systems could exacerbate the etiology of epilepsy (29). WS could protect tissue cells against free radicals (9). Although the role of inflammation in this model of TLE was not evaluated in the present study, but WS may also exert neuroprotective effect against seizures and cognitive deficit in kainate-injected rats through its anti- inflammatory activity (9).

In conclusion, these data suggest that *Withania somnifera* alcoholic extract could attenuate spatial memory disturbance in TLE model in rat.

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