The Therapeutic Effect of the Aqueous Extract of *Boswellia Serrata* on the Learning Deficit in Kindled Rats

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Original Article

ABSTRACT

Background: It has been reported that epilepsy is a disorder of the central nervous system that causes memory impairment. This study examines the role of the aqueous extract of *Boswellia* on the learning disability of the pentylenetetrazol (PTZ)-induced kindled rats.

Methods: In this experimental study, 64 male rats were used. Kindling seizures were induced by three injections of 25 mg/kg of PTZ every 15 min. Control animals received normal saline instead. To evaluate the therapeutic effect of *Boswellia* extract on the PTZ-induced cognitive deficits, the aqueous extract (0, 0.1, 0.5 or 1 g/kg, i.p.) were administrated to all animals for three consecutive days. At 24 h later, passive avoidance learning of animals was examined using shuttle box apparatus, respectively. The time required for the animal stepping through the dark chamber was determined as step-through lateney (STL). Data were subjected to the *t*-test and analysis of variance and followed by Tukey's test for multiple comparisons.

Results: The STL of the kindled rats was significantly reduced compared with control ones (22/375 ± 4/19 for kindled and 295 ± 15/71 for control groups, respectively). Aqueous extract of *Boswellia* improved passive-avoidance learning ability in both control and PTZ-kindled animals ($P < 0.05$).

Conclusions: The results can be stated that the *Boswellia* extract is offset by harmful effects of seizures on cognitive function and consumption of *Boswellia* extract increases the learning ability in epileptic animals.

Keywords: *Boswellia*, passive-avoidance learning, PTZ-kindling, rat

INTRODUCTION

Epilepsy is a chronic neurological disorder with the prevalence of 1% which is characterized by recurrent spontaneous seizures due to neuronal hyperactivity in the brain.[1] A lot of data support the idea that prolonged frequent seizures in animals and patients lead to later cognitive deficits These recurrent seizures, by affecting hippocampus, can produce cognitive, memory and emotional impairments and psychological problems.[2] Experimental animal studies can be useful in investigating the effects of seizures on neurodevelopment

and in identifying the potential mechanisms involved. A periodic systemic injection of convulsive drugs, such as pentylenetetrazol (PTZ) has been shown to induce seizures in animals.[3] PTZ-induced kindling is an accepted animal model for the study of epilepsy and its consequences on memory has a direct effect on the knowledge of individuals and knowledge is a crucial factor in enhancing the individuals' quality-of-life. Therefore, according to the studies previously done, finding appropriate ways to increase satisfaction in patients has always been considered important.[4] Now-a-days pharmacotherapy with psychoactive drugs are available, however they are not effective in all cases and exert numerous side-effects, especially upon long-term administration.[5] Herbal medicine is commonly used for treating the diseases such as amnesia as well as reinforcing memory. One of the most common herbs used for improving memory performance is frankincense. The resin of this plant is known by various names like olibanum and frankincense obtained from Burseracea tree of genus *Boswellia* which is native to regions such as Ethiopia, India and Saudi Arabia.[6] This gum has been used for more than thousands of years and provides an integrated approach treating illness through life-style intervention and natural therapies. Frankincense resin is edible and often used in various traditional medicines in Asia. In Ayurvedic medicine Indian frankincense (*Boswellia serrata*) commonly called "dhoop" has been used for hundreds of years for treating arthritis, healing wounds, strengthening female hormone system and purifying atmosphere from undesirable germs.[7] Moreover, many other properties such as induction of cancer cell's death, inflammatory bowel disease and liver damage treatment, decreasing the symptoms of asthma attack.[8] Relieving pain[9] and glioma treatment.[10] Can be accounted for this resin. Frankincense has been mentioned as an effective resin in Iran's traditional medicine and by Islamic physicians like Ave Sina, al-Razi and others.[10] Therefore, the present study was aimed to investigate the effect of *Boswellia* extract on learning disorders resulting from PTZ-kindling for the first time.

METHODS

Preparation of extract

A total of 100 g of the dry powder of frankincense resin was soaked in 1000 ml boiled water and this Solution was gently heated for 1 h and centrifuged to obtain a clear aromatic and then the solution was dried in a rotary device. Twenty-four g of the dry extract was obtained from every 100 g of powder. This extract was dissolved in normal saline and injected intraperitonealy (i.p., injections) to the animals.

Animals

In this experimental study, 64 male wistar rats (220-250 g) obtained from Pasteur institute (Tehran-Iran) were placed in the same experimental conditions: 12 h light/dark cycle and temperature of 24 ± 2°C with free access to sufficient food and water. All experiments were carried out according to the guidelines of German protection of animal act (Deutsches tiers chutzges etz., BGBI 1998 pant I no 30, S.1109 ff) approved by local comity of Kermanshah University of Medical Sciences.[11]

Kindling

Kindling seizures were induced by repetitive i.p., injections of PTZ (25 mg/kg, 1 ml/kg) every 15 min, but the total dose did not exceed 75 mg/kg (these injections).[12] Control animals received normal saline instead. Immediately after injection, the incidence of seizures activity of rats was observed in an isolated Plexiglas box for 45 min. The severity of seizures was evaluated using five-score scale as follows: 0: No change in behavior, 0.5: Atypical behavior (e.g., intensive grooming, sniffing and moving arrests), (1) isolated myoclonic Jerks, ear and facial twitching, (2) atypical minimal seizure, convulsive wave through the body, (3) fully developed minimal seizure, clonus of head muscles and forelimbs and righting reflex, (4) major seizure (generalized without the tonic phase), (5) generalized tonic clonic seizures begun with running followed by the loss of righting ability, then short tonic phase (flexion or extension of fore-and hind limbs) progressed to the clonus of all four limbs.[13] In the kindled group, only the animals were selected for the experiments that had reached stages 4/5. LD_{50} (2 g/kg) has been reported for *Boswellia* extract in mice and rats.[14] The first injection of the extract was done in the 1st day 45 min after the PTZ or normal saline administration. The extract with specified doses (0, 0.1, 0.5 or 1 g/kg, 1 ml/kg) was administered to each group (n = 8) in the 2nd and 3rd days as well.

Shuttle box

At 24 h later, the passive-avoidance learning ability of animals were evaluated using shuttle-box apparatus, respectively.[15] The apparatus consisted of two separate chambers (20 cm × 30 cm × 20 cm) that were separated by a guillotine door from which the animal can pass through when it was open. The walls and floor of one of the chambers were white (light chamber) and for another one were black (dark chamber). The floor of both chambers had parallel metal bars through which electric stimulation with desired voltage and time could be delivered to animals' feet using the stimulator attached to them. Passive-avoidance learning was evaluated in three stages: Adaptation: The rats were brought to the laboratory environment 1 h before each of the training or testing sessions. Each animal was placed in the light compartment for 20 s, after which the door was opened and the animal was moved around in dark/light chambers for 180 s to become familiar with the environment. Training: 24 h later, the animal was placed in the light compartment for 20 s, the door was raised and the time the animal waited before crossing to the dark (shock) compartment was recorded as the latency. Once the animal completely entered dark compartment, the door was closed and a foot shock (1, 5 mA, 50 Hz) was delivered for 2 s. After 20 s the animal was removed from the apparatus and 2 min later, the procedure was repeated. Training was terminated when the animal did not enter the dark compartment for 120 consecutive seconds. All the animals were trained with a maximum of 3 trials. Retention test: 24 h after training, while the guillotine door was closed, the rat was placed in the light compartment for 20 s, then the door was opened and the delay time for the animal stepping through the shock compartment was determined as memory criterion and registered as step-through latency (STL). During these sessions, no electric shock was applied. The criterion for retention was 600 s. All experiments were carried out between 8:00 a.m. and 12:00 a.m.[16]

Statistical analysis

All data are presented as mean ± standard error of the mean. Significance of the mean of the STL in the shuttle box was determined by the student *t*-test and one-way analysis of variance followed by Tukey's test for multiple comparisons. $P < 0.05$ was considered to be significant.

RESULTS

The rats that received PTZ developed the characteristic features of seizures, whose severity reached stage 4/5 of kindling. Control animal that were subjected to normal saline instead of PTZ never experienced seizures activity.

Learning performance in the shuttle-box apparatus

The shuttle-box apparatus was used to assess the step-through passive-avoidance learning and as shown in Figure 1, PTZ-induced kindling affected memory retention of passive avoidance learning. The data indicated that retention latencies of kindled animals were significantly reduced compared with the control ones ($P < 0.05$). When we tested the effect of *Boswellia* extract (0, 0.1, 0.5 or 1 g/kg) on the learning ability of the saline received (control) animals, we found a trend toward increased STL which was significant in the group received 0.5 g/kg of the extract ($P < 0.05$), respectively [Figure 2]. In our experiments, the therapeutic effects of the extract on the passive-avoidance learning deficits of the PTZ-kindled rats were also investigated. As shown in Figure 3, there are differences among performance of studied groups. *Post-hoc* analysis revealed that the retention latencies of the kindled animals received *Boswellia* extract (0.1, 0.5 or 1 g/kg) were significantly increased compared with control ones ($P < 0.05$).

DISCUSSION

Neurological testing in epileptic patients demonstrated moderate to severe impaired

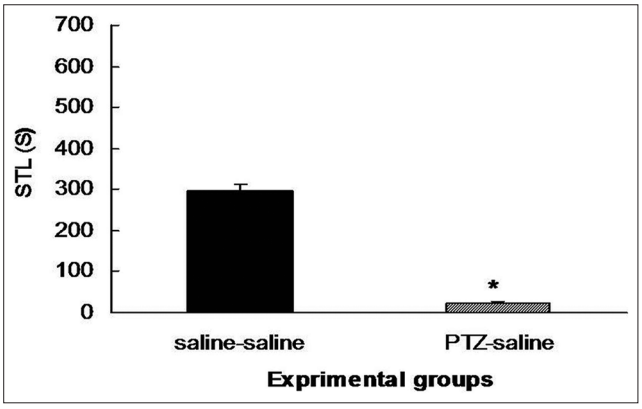

Figure 1: The effect of PTZ-induced kindling on the step-through latency (*$P < 0.05$)

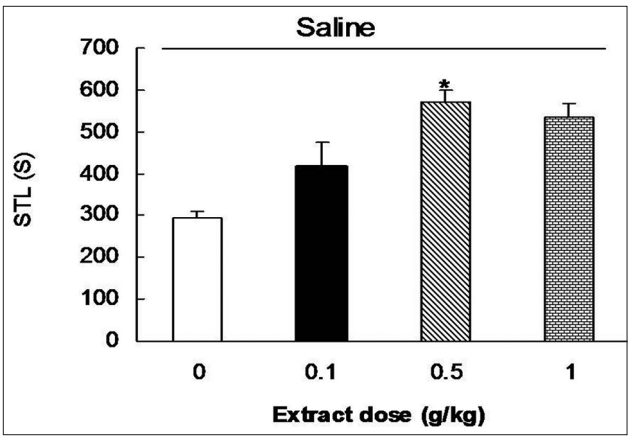

Figure 2: The effects of the Boswellia extract (0, 0.1, 0.5 or 1 g/kg) on the learning ability of the saline received (control) animals. Administration of the extract increased step-through latency in a dose dependent manner which was significant in the group received 0.5 g/kg of the extract compared to control ones (**P* < 0.05)

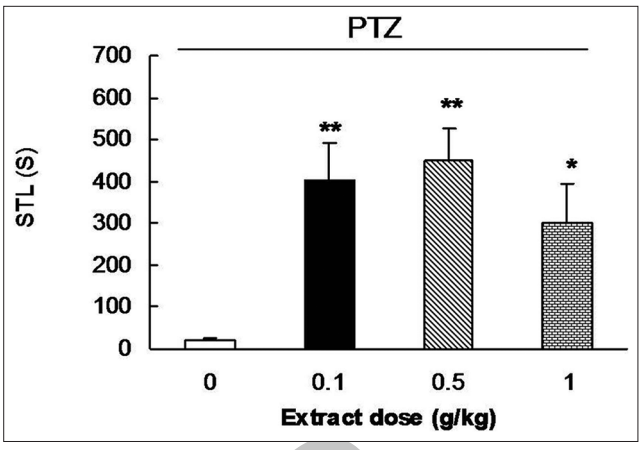

Figure 3: The effects of the Boswellia extract on the passive-avoidance learning deficits of the PTZ-kindled rats. Administration of the extract significantly increased retention latencies of the kindled animals (**P* < 0.05, ***P* < 0.01)

verbal and visual memory Moreover, weak communication skills and deficient social behaviors have been reported in epileptic patients.[17] Thus, memory defect in neurodegenerative diseases like epilepsy have always been considered a challenge, because amnesia and learning difficulties are the most common symptoms of cognitive disorders that causes depression in the epileptic patient.[18] At the cellular level, PTZ interact with the N-methyl-D-aspartate receptor and/or gamma-aminobutyric acid (GABA) GABAergic system activity.[19] GABA is intimately involved in the regulation of synaptic inhibition in the adult brain since reduction in the efficacy of synaptic inhibition mediated by GABA receptors can lead to seizure, GABA receptor antagonist has been used to induce epileptic seizures in experimental animals.[20] PTZ, as a GABA receptor antagonist has been shown to reduce the GABA-mediated inward chloride current in the adult brain.[21]

We also investigated the effect of seizures on cognitive function of rats as previous data reported that occurrence of seizures might result in cognitive impairment in animals.[22] Our results also clearly demonstrate that the PTZ-induced seizure has deleterious consequences on the learning ability as indicated by passive-avoidance test. A decrease in the learning ability has been reported in clinical studies on memory disorders in epileptic patients.[23] According to the sources of traditional medicine in East Asia, frankincense is able to obviate problems such as amnesia.[24] Our data indicated that administration of *Boswellia* extract in non-kindled groups increased learning ability in rats compared to control ones. In the review of literature, various studies confirm this issue, too. Oral administration of *Boswellia* extract during pregnancy and lactation strengthens short-and long-term memory in infants in field models.[25] Further, administration of the extract during pregnancy causes an increase in the size of the neurons in pyramidal cells of the hippocampus CA3 area as well as increase in the number of dendritic process in these cells.[26] Extract administration during lactation promotes memory function in infants through increasing cell volume, neurotransmitters release and number of synaptic contacts.[27] Another study indicated that frankincense can prevent Alzheimer and be effective in treating it.[28] So far, the components of *Boswellia* resin have been identified. The most important of which are *Boswellia* acids (BAs). According to pharmacokinetic studies, 11-keto-β-boswellic acid (KBA) and 3-acetyl-11-keto-β-boswellic acid (AKBA) are the most potent BAs. Due to being lipophilic, AKBA is able to pass blood brain barrier.[29] These substances, via activating protein kinase pathways including protein kinase-C (PKC) and protein kinase-A (PKA) signaling pathways, lead to synaptic plasticity in the hippocampus There is strong evidence that PKC signaling pathways are causally involved in associated with memory storage.[30] PKA has been strongly implicated in the expression of specific forms of long-term potentiation (LTP), long-term depression and

hippocampal long-term memory.[31] According to the current our data, administration of *Boswellia* extract significantly improved learning deficits in kindled animals.

Frankincense is known as a potent anti-inflammation agent. In various studies, epilepsy has been introduced as a neurodegenerative disease, which can be created following the events that induce inflammatory responses in the central nervous system.[32] It has been also reported that BAs possess potent anti-inflammatory properties by inhibiting 5-lipoxygenase, human leukocyte elastase and the nuclear factor-K-B pathway, without exerting the adverse effect known for steroids.[33] *B. serrata* extract as well as KBA and AKBA are identified as potent inhibitors of p-glycoproteins in brain capillary endothelial cells and consequently, it prevents leucocytes from sticking to the veins' epithelium in the inflammatory processes.[34] These protective effects can be attributed to the presence of incensole acetate (IA). IA as a major active constituent of *Boswellia* resin; showed an anti-inflammatory activity it seems that IA and its derivatives play an important role in the effects of *Boswellia* extract on biologic processes. It is considered a potent anti-anxiety and a hippocampal LTP through activating $TRPV_1$ channel (transient receptor potential cation channel subfamily V member). Further, the activation of $TRPV_3$ canal causes an increase in calcium influx.[35] This, in turn, plays a role in the synaptic enhancement in hippocampus. Thus, Ca is a major stimulus for releasing neurotransmitters, so it plays an essential role in the synaptic facilitation and molecule mechanisms of memory.[36] In addition, the anti-inflammatory quality has been accounted for IA.[37] This suggests that IA may inhibit nuclear factor κappaB activation in neurological disorders, hence attenuating inflammation, postponing deterioration and perhaps ameliorating degenerative conditions.[38] Considering that the purpose of this study has not been evaluate the anti-inflammatory properties. But maybe we can attribute this results to antiinflammatory agent and effects of *Boswellia* gum resin.

CONCLUSIONS

Based on the obtained findings from this study and other relevant studies, it can be argued that the consumption of *Boswellia* extract increases the learning ability in epileptic animals. However, further studies are needed to identify the advantages and possible complications of this substance.

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