Original Article

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Study of Anti-Epileptic Effect of Cannabis Sativa Extract on Pentylenetetrazol-Induced Kindling in Male Rats

S. Namvar Aghdash (PhD)*[1], R. Mirzae (MSc)[1], F. Pourabdolhossein (PhD)[2]

1.Department of Biology, Faculty of Basic Sciences, Azarbaijan Shahid Madani University, Tabriz, I.R.Iran
2.Neuroscience Research Center, Faculty of Medicine, Babol University of Medical Scineces, Babol, I.R.Iran

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ABSTRACT

BACKGROUND AND OBJECTIVE: Epilepsy is one of the most common neurologic disorders that presents in a large number of drug-resistant patients. Given the presence of the anticonvulsant compounds in *Cannabis* and its use in traditional medicine, this study aims to evaluate the effect of *Cannabis sativa* extract on the treatment of seizures.

METHODS: In this experimental study, 40 Syrian male rats were selected and randomly divided into five groups, including one control, one sham, and three experimental groups. The control group only received pentylentetrazol (PTZ), the sham group was administered distilled water, and the experimental groups were gavaged with 400, 600, and 800 mg/kg of body weight of the aqueous extract of Cannabis sativa for four weeks. Moreover, 45 mg/kg of PTZ was intraperitoneally administered 30 minutes later in the experimental and sham groups. Finally, the convulsive behaviors and the relevant parameters were recorded using a camera inside a specific cage for 25 minutes.

FINDINGS: Aqueous extract of Cannabis sativa increased the delay of seizure onset at the dose of 800 mg/kg compared to the control group (232.87±33.76 sec vs. 103.84±7.50 sec; $p<0.05$) and inhibited the progression of epilepsy phases equally at each three doses compared to the control group (1.91±0.5 vs. 5±0; $p<0.00$).

CONCLUSION: The results showed that cannabis extract had the maximum anticonvulsant effect on PTZ-induced seizures at the dose of 800 mg.kg of body weight. Therefore, the anticonvulsant properties of aqueous extract of *cannabis* are dose-dependent.

KEY WORDS: *Cannabis sativa*, *Epilepsy*, *Pentylenetetrazol*, *Seizure*.

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***Corresponding author: S. Namvar Aghdash(PhD)**
Address: Department of Biology, Faculty of Basic Sciences, Tabriz, I.R.Iran
Tel: +98 41 34327500
E-mail: siminnamvar2@gmail.com

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Introduction

Epilepsy is one of the most common mental disorders, and approximately 50 million people suffer from it worldwide. Epilepsy is a chronic, progressive disorder characterized by unpredictable and cyclical seizures caused by abnormal discharges of cerebral neurons (1). This abnormal discharge is induced by the inhibition-excitation imbalance in the brain (2). Various methods were proposed for the study of epilepsy, and kindling is one of the most common of them. Kindling is a functional model of epilepsy that facilitates behavioral, neuropsychological and neurochemical study of the brain without causing any severe morphological damage. Kindling is induced by subliminal electrical stimulation or subliminal levels of chemical convulsants, which are gradually intensified and eventually develop into generalized seizures (3). Kindling is caused by two electrical and chemical methods; pentylenetetrazol (PTZ) is the most commonly used material for inducing kindling (3, 4). PTZ crosses the blood–brain barrier, turns into a $GABA_A$ receptor, and stimulates the brain by inhibiting the chlorine channels (5).

Since the common convulsants have numerous undesirable side effects and their treatment duration is long, studying new drugs with fewer side effects seems to be mandatory (6). In addition, despite the use of different medications, a large number of patients suffer from intractable epilepsy. Furthermore, due to continuous and prolonged use of anti-epileptic drugs, patients develop unwanted side effects (7, 8). As a result, the use of medicinal plants has attracted particular attention in recent years.

Nature is a rich source of herbs used in traditional medicine for the treatment of various diseases. Researchers demonstrated beneficial properties of numerous herbs in the treatment of epilepsy (9-11). Studies showed the role of asafoetida plant on intensity, level, and duration of seizures (12). Additionally, Citrus aurantium extract and Rosmarinus officinalis were reported to play a role in proliferation of neural stem cells and neuronal protection (13, 14). Former studies exhibited that extract of Salvia officinalis could inhibit PTZ-induced seizures (15) and discussed the anticonvulsant effects of various plants including Tanacetum, Humulus iupulus, Melissa officinalis, and Datura (16-19).

Considering the role of various plants on laboratory models of seizure and their neuroprotective roles, to achieve a more effective and safer treatment broader studies should be performed. Cannabis, known by the scientific name of Cannabis sativa and with English names of Marijuana or Indian hemp, is an annual dioecious plant reaching heights of up to 4 meters, which is used in traditional medicine.

Cannabis sativa leaves form as a pair on opposite sides of the stem, but as we get closer to the end of the stem, the leaves form a singular pattern. Typically, this plant is dioecious, and female flowers and the leaves surrounding them are used for resin production and its seeds have medicinal uses. Chemical composition of Cannabis includes choline, trigonelline, acids, and cannabinoids such as cannabinol and cannabidiol; the hallucinogenic effect of this plant is only attributed to its tetrahydrocannabinol (THC) agent. Marijuana and hashish contain about 1% and 5% THC, respectively. Additionally, flavonoids, alkaloids, and terpenoids are found in this plant.

Cannabis is used in traditional medicine for the treatment of rheumatoid arthritis, asthma, and inflammatory conditions such as skin diseases and cardiovascular diseases. Given the sedative, analgesic, and hypnotics properties of Cannabis sativa extract, this study was conducted to evaluate the anticonvulsant effect of this extract using the chemical kindling model and to examine the anticonvulsant effects of choline-containing compounds such as alkaloids, and flavonoids (20)

Methods

Laboratory animals: In this experimental study, we used 40 adult male rats, weighing about 25-31 g. They were purchased from Veterinary Medicine Faculty of Tabriz University, Tabriz, Iran. To adapt the mice to the new circumstances, no interventions were applied over the first five days. They were kept in a room at 22°C, 40% humidity, and 12 hours of light and 12

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hours of dark cycle. Except for the test period, enough food and water were provided for the animals. The International Association for the Study of Pain ethical principles were maintained throughout the study (21). The animals were randomly divided into one control, one sham, and three treatment groups, each containing eight animals.

Extraction: After supplying *Cannabis* from local stores and having it confirmed by Phytochemistry Professors of Shahid Madani University of Azarbaijan, Azarbaijan, Iran, its impurities were e3liminated, and to improve the efficiency of seed extraction, the dried seeds were powdered by an electric mill device. Approximately 500 g of the powder was soaked in 5 liters of distilled water for 24 hours.

Afterwards, it was heated and stirred by hot plate magnetic stirrer (Heather Styrene, Sahand Azar, Iran) for four hours. The resulting solution was passed through a paper filter, and then was condensed by Rotary Evaporator device (Rotary, Heidolph, Germany) and distilled inside a spinning vacuum at 50°C. Finally, to eliminate water, it was placed inside a freeze dryer device (drying at low temperature: Christ, Germany) for three days. The dried extract of the plant weighted about 20 g.

In order to produce 200 ml of stock solution, 6,000 mg of *Cannabis* extract powder was dissolved in 200 ml of distilled water. Each ml of stock solution contained 30 mg of the extract powder (22).

The effect of the extract on PTZ-induced seizures: To induce seizures, 30 minutes after gavage we injected the 400, 600, and 800 mg/kg of body weight (at volume of 0.4, 0.6, and 0.8 ml) of aqueous extract of *Cannabis* and distilled water to the treatment groups and 45 mg/kg of PTZ to the sham group. The control group only received PTZ. The solution was administrated to the rats through intraperitoneal injection of insulin syringes, which is the most common and important type of injection in rodents. After dissolving 45 mg/kg of PTZ in a normal isotonic saline solution, a maximum of 0.1 cc of this solution was prepared for each 10 kg of body mass and was injected to each rat 12 times on a 48-hour basis. After PTZ administration, animal behaviors and seizure responses (attack phase) were monitored for 25 minutes and classified into (23) phase zero: there was no sign of behavior, phase one: clonic contraction of ear and face muscles and tail erection, phase two: epileptic up-and-down motion of the head and rhythmic movement of the hands, phase three: myoclonic contraction of the body, phase four: generalized clonic seizures and turning on the ipsilateral side, phase five: generalized tonic seizure, and phase six: death.

Statistical analysis: To analyze the data, One-way ANOVA and Tukey's post-hoc tests were performed. $p<0.05$ was considered statistically significant.

Results

The effect of different doses of *Cannabis* on progression of seizure phases: The inhibitory effects of *Cannabis* were higher on the last treatment days, that is, the mechanisms involved in the inhibitory effect were intensified over time. On the last day of excitation, the seizure phases showed greater inhibition compared to the control group (5±0) at the doses of 400 mg/kg (2±0), 600 mg/kg (1.5±0.5), and 800 mg/kg (2.25±0.5; fig 1). At the final phase of excitation, different doses of *Cannabis* equally inhibited the development of convulsive stages in the treatment groups compared to the control group (1.91±0.5 vs. 5±0; $p<0.05$).

There were significant differences between the treatment groups on the third, sixth, eighth, and ninth days of treatment. These variations were eliminated by kindling and stabilization of seizure phases; therefore, on the last three days of treatment, 400, 600, and 800 mg/kg doses produced the same inhibitory effect, and there was no significant difference between the three groups.

The effect of different doses of *Cannabis* on the required time for the onset: The onset time (in seconds) increased in the treatment groups with doses of 400 (188.56±30.43), 600 (180.70±27.56), and 800 (232.87±33.76) mg compared to the control (103.84±7.50) and sham (99.45±10.56) groups (fig 2). The results also indicated that the dose of 800 mg/kg

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had the most influential effect, and this effect might be enhanced by increasing the dose of the active ingredient. Statistical analysis demonstrated a significant difference between the sham and control groups receiving 800 mg/kg of the extract ($p<0.05$), but no significant difference was observed between the treatment groups.

The effect of different doses of Cannabis on the duration of seizure: Administration of *Cannabis* reduced seizure duration (in seconds) in the treatment groups receiving 400 mg/kg (420.78±35.98), 600 mg/kg (380.98±57.0956), and 800 mg/kg (390.87±60.46) compared to the control (510.87±50.78) and sham groups (490.76.47.98; fig 3). Although the effect of the extract was more prominent at the doses of 600 and 800 mg/kg, no significant difference was observed between all the studied groups. As can be noted in Figure 3, there was no significant difference between the sham, control, and treatment groups.

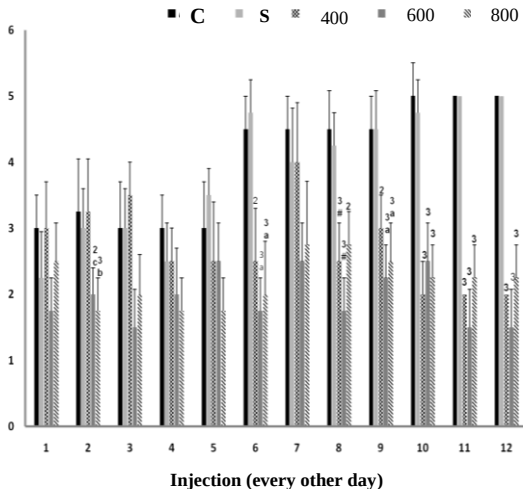

Figure 1. The effect of aqueous extract of *Cannabis* at doses of 400, 600, and 800 mg/kg of rat body weight on the seizure phases, $p<0.05_{(1)}$, $0.01_{(2)}$, and $0.0001_{(3)}$ represent a significant difference from the control group.

$p^a<0.05$, $p^b<0.01$, and $p^c<0.001$ represent a significant difference between the treatment groups at doses of 600 and 800 mg/kg and the treatment group receiving the 400 mg/kg dose. $p<0.05$ shows a significant difference between the treatment groups receiving 400 and 600 mg/kg of *Cannabis* and the group receiving the 800 mg/kg dose.

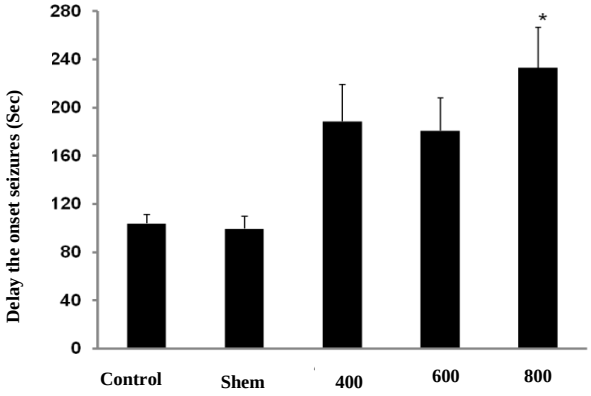

Figure 2. The effect of aqueous extract of *Cannabis* at doses of 400, 600, and 800 mg/kg of rat body weight on the onset of seizure.

$p<0.05$ represents a significant difference from the control group. There was no significant difference between the treatment groups.

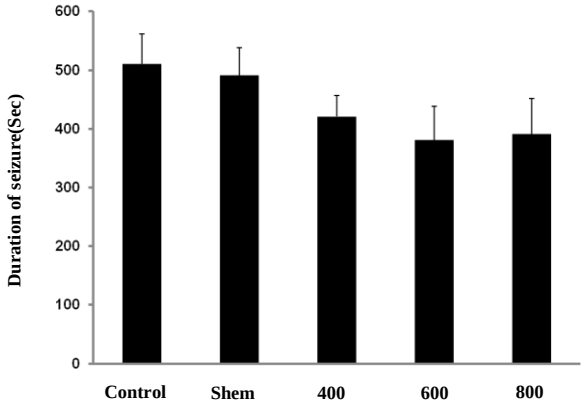

Figure 3. The effect of aqueous extract of Cannabis at doses of 400, 600, and 800 mg/kg of rat body weight on seizure duration

Discussion

Our findings revealed that *Cannabis* seeds produced anticonvulsant effects on PTZ-induced kindling chemical model. In this experiment, while nearly all the doses of *Cannabis* extract could prevent progression of epilepsy, at the dose of 800 mg/kg it had the greatest impact due to its influential properties on duration and onset of seizure, which suggests that higher doses of drug are more effective, but none of the doses could significantly shorten the duration of seizure. Cannabis contains cannabinoid compounds; in one study, it was indicated that cannabidol could inhibit caffeine-induced seizures; it applies its

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anticonvulsant effect through reduction of glutamate release (24). Since pretreatment with an antagonist of palmitoylethanolamide lowers the anticonvulsant effect, it regenerates its impact through cannabinoid receptors such as cannabinoid receptors type 1 and type 2 (25). The study of the effects of cannabinoid antagonist administration on healthy mice showed that the endocannabinoid system exerts protective effects against cryptogenic seizures (26), which is in line with our results. The endocannabinoid system is one of the various mechanisms justifying effects of *Cannabis*. Endocannabinoid system exists in almost all excitatory and inhibitory synapses containing various types of neurotransmitters in the central and peripheral nervous systems (27, 28).

Previous studies indicated that exogenous and endogenous cannabinoids cause nerve growth, brain maturation, and protection against toxins and trauma. The cannabinoid system plays a role in cognitive function, learning ability, sensory motor control pathway, and sleep-wake cycle (27). Given that under normal circumstances, inhibitory (GABAergic) or stimulatory (glutamatergic) adjustment is dominant in a pathway, exogenous and endogenous cannabinoids can have stimulatory or inhibitory effects in different directions. However, dose-dependent curves of cannabinoid system are diphasic in most cases. Intense and persistent activity of endocannabinoid system leads to desensitization and down regulation of receptors, and as a result, it diminishes and even reverses its effects (27). The endocannabinoid system exerts its impact through its own receptors known as cannabinoid receptors type 1 and type 2 (CB1 and CB2); these receptors belong to the G protein-coupled receptor family. CB1 receptor is frequently present in the central nervous system and exerts its impact through Gi, inhibition of adenylate cyclase, and reduction of cyclic adenosine monophosphate. CB1 and CB2 act through Gq, which inhibits voltage-gated calcium channels and activation of potassium channels (29). Consequently, this system increases the inhibitory function of the brain via the above-mentioned signaling pathways (27, 29).

Given that excitatory-inhibitory imbalance of the brain, that is, increased stimulation or low inhibition, leads to brain seizures (29), it can be inferred that due to having abundant cannabinoids and activating the endocannabinoid system, *Cannabis* boosts the inhibitory function of the brain and controls seizures. On the other hand, *Cannabis* contains flavonoid compounds that are proven to act as benzodiazepine-like molecules and serves as ligands for $GABA_A$ receptors.

Through activating GABA receptors, flavonoids strengthen the GABAergic system and exert anxiolytic, hypnotic, and anticonvulsant effects (30-34). These findings confirm the current results. According to the reports obtained from the current and other studies, *Cannabis* seeds have anticonvulsant effects by activating GABAergic system, boosting the inhibitory mode of the brain, inhibiting glutamatergic system, and reducing cerebral stimulation.

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