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Study of the anti-epileptic effects of *Foeniculum vulgare* essence and extract on male mice, using the PTZ and MES methods

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ABSTRACT

Epilepsy is the oldest nervous system disorder known to mankind and one of the most common diseases in human population. Despite extensive studies, multiple aspects of the disease and the nature of the disorder are still unclear. Currently 30-40% of the epileptic patients show resistance to all of the conventional therapeutic agents. In Iranian traditional medicine, various types of plants have been used to treat this disorder, one of them being *Foeniculum vulgare* (FV). This study aimed at evaluating the effects of FV extract and essential oil on seizures induced by Maximal ElectroShock (MES) and Pentylentetrazol (PTZ) in male mice. The first method involved inducing seizures in male mice using Pentylentrazol (1 mg/kg), administered intraperitionally (i.p.), and the second method involved inducing seizures by Electroshock (60 mA, 60 Hz, 0.10 sec.). Then, the seizures were tried to be stopped or brought under control using FV extract and essential oil. Our results indicated that although the fatal doses of PTZ leads to 100% fatality rate in the animals, the essential oil protects the mice against tonic convulsion induced by MES (TD50=0.3 ml/kg), and PTZ (LD_{50}=0.28 ml/kg). At anti-seizure doses, the FV essential oil causes sedation and impairment of motor functions. Also the result of the study shows that the oil has greater anti-seizure effect compared to the extract.

Key words: epilepsy, seizure, convulsion, mice, essential oil, extract, *Foeniculum vulgare*, pentylentetrazol, maximal electroshock.

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1. Introduction

Approximately, 1% of the world population suffer from epilepsy which is the most common neuralgical disorder after brain stroke. Epilepsy is a common neurological disorder characterized by periodic loss of consciousness (Guberman, 1999). It is a disorder of the central nervous system appearing in the form of abnormal automatic motor and sensory movements (Katzung, 1991; MacNamara, 1996; MacNamara, 1994; Dichter, 2001). Although advances in medical sciences has offered the epileptic patients very effective cures, still a large percentage of the patients suffer form incomplete drug treatments and the side effects of the current drugs (Guberman, 1999). About one third of the epileptic patients are resistant to all available treatment methods (Bazil, 2003). Since introduction of synthetic drugs, several agents of the kind have been taken out of the market due to their harmful side effects. This has motivated extensive research toward finding the therapeutic agents with natural origin, in particular herbal drugs and remedies. As herbal drugs are known to have little side effects, a growing trend has been emerged for substitution of synthetic therapeutic agents with them. Nowadays it is approximated that over 30% of the drugs are of herbal origin (Katzung, 1991; MacNamara, 1996). In this study we investigated the therapeutic effects of *Foeniculum vulgare* (FV) a conventional herbal drug to treat epilepsy in Iranian traditional medicine.

2. Materials and Methods

2.1. Sample collection

Male mice belonging NMRI race with a weigh range of 17-25 g were obtained from the Pasture Institute of Iran. The animals were transferred to the animal holding facility of the Department of Pharmaceutical Sciences of Shahid Beheshti University, and were kept under the photo cycle of 12h day/12h night at 25 ^{o}C. The animals were provided with water and moist enough living environment. Thirty minutes before starting the experiment, all the test subjects were weighed using a digital scale, and then transferred to the lab environment.

2.2. Materials

Electroshock apparatus MES-805 (Rayan Teb, Iran) with high degree of sensitivity was used. A digital scale with a sensitivity 0.0001 (Metter, Swiss), was used to weight the FV extract and the essential oil. Another type of digital scale with a sensitivity of 0.1 (Exell, Iran) was used to weigh the animals. A transparent container with the volume of 30×30×30 cm^3 was used to hold the animals for observation purpose after being injected with PTZ. The material used included PTZ (Sigma, USA), Flomazenil (Hoffman La Rosch, Swiss), normal saline solution (Daru Pakhsh, Iran), DMSO (Merck, Germany), sesame oil, distilled water, FV essence and extract.

2.3. Animal Treatment

In both PTZ and MES models, the animals were divided into 8 member groups. For each case group receiving FV essence or extract, a control and a positive control group is specified. The animals in the control group were given distilled water or sesame oil. Each animal was tested only once. The injection of PTZ was done using i.p. method. The essence and the extract of FV were dissolved in sesame oil and distilled water respectively. DMSO was used as the solvent for Flomazenil. A concentration of 5ml/kg pure DMSO was used to prevent its side effects on the central nervous system (CNS).

2.4. Study of the effect of essence in the PTZ method

First, the solutions of the FV essence and PTZ with the required concentrations were prepared in dry condition and away from direct exposure to the light. Thirty minutes after that the animals were transferred to the lab, they recieved injection of the essence solution (0.1 ml/0.01kg) with i.p. method. Thirty minutes later animal was injected with PTZ solution (100 mg/kg). After administration of the injections, the animals were kept under observation for 30 minutes and the rate of fatality was recorded. Each animal was also injected with a constant dose (10 mg/kg) of Flomazenil, 15 minutes prior to induction of seizure so that the effect of FV essence could be studied through the Benzodiazpini receptors. The animals in the control group were injected with a solution of distilled water.

2.5. Study of the effect of FV essence in the MES method

First, the solutions of the FV essence with the required concentrations were prepared in dry condition and away from direct light source. Thirty minutes after that the animals were transferred to the lab, the injection of essence solution was administered using i.p. method. 30 minutes later electrical shock (60 Hz, 60 mA, duration= 0.1 second) was administered. The state of HLTE (plus/minus) was recorded for a period of 30 seconds after the administration of electric shock. The animals in the control group were injected with a solution of distilled water. Each animal was also injected with a constant dose (10 mg/kg) of Flomazenil, 15 minutes prior to induction of seizure so that the effect of FV essence could be studied through the Benzodiazpini receptors. The animals in the control group were injected with a solution of distilled water.

2.6. Study of the effect of methanol containing FV essence in the PTZ method

First, the solutions of the methanol containing FV essence and PTZ with the required concentrations were prepared in dry condition and away from direct light source. Thirty minutes after that the animals were transferred to the lab, the injections were administered using i.p. method. The test group was injected with varying concentrations of methanol containing FV essence. Thirty minutes later they were further injected with PTZ (100 mg/kg). The animals were then kept under observation and the fatality rate after HLTE was recorded. Each animal was also injected with a constant dose (10 mg/kg) of Flomazenil, 15 minutes prior to induction of seizure so that the effect of methanol containing FV essence can be studied by the Benzodiazpini receptors. The control group was injected with a solution of distilled water.

2.7. Study of the effect of methanol containing FV essence in the MES method

First, the solutions of the methanol containing FV essence and PTZ with the required concentrations were prepared in dry condition and away from direct light source. Thirty minutes after that the animals were transferred to the lab, the injection methanol containing essential oil solution were administered using i.p. method. 30 minutes later electrical shock (60 Hz, 60 mA, duration= 0.1 s) was administered then the state of HLTE (plus/minus) was recorded for a period of 30 seconds. The animals in the control group were injected with distilled water.

2.8. Ethical Considerations

All animals in the experiment were treated according to the Animal Rights Doctrine.

2.9. Statistical analysis

The statistical analysis of the data was performed using SAS 90.0 software (Institute Inc., Cary, NC, USA, 2002). Also, SPSS program and Microsoft Excel were used to analyze the data in certain parts of this study.

3. Results

ED_{50} value of diazepam was used as a basis for measuring the value of the ED_{50} for the FV essential oil and extract. Administration of the fatal dose of PTZ led to a 100% fatality rate in the animals.

3.1. Anti-seizure effect of FV essential oil in PTZ method

Essential oil of FV effectively controlled the seizure induced by PTZ, but the effect was dose-dependant. As shown in Table 1, the maximum effect was observed when the dose was 400 mg/kg. The LD_{50} value for the FV essential oil was 274.1 mg/kg. In this group Flomazenil could block the anti-seizure effect of the FV essence only by 50%. The result of the statistical analysis is described in Fig. 1.

Table 1. The anti-seizure effect of FV essence and the fatality rate in PTZ method

Treatment agent	Dosage	Living animal	Dead animal	% of fatality
Sesame seed oil+FV essence	400mg/kg+5ml/kg	8	0	0
Sesame seed oil+FV essence	300mg/kg+5ml/kg	3	5	63
Sesame seed oil+FV essence	200mg/kg+5ml/kg	2	6	75
Sesame seed oil+FV essence	100mg/kg+5ml/kg	0	8	100

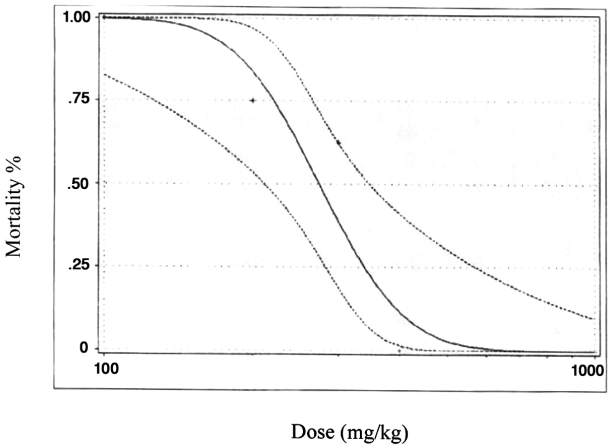

Figure 1. The plot of dose-response of FV essence in PTZ model

3.2. Anti-seizure effect of FV essential oil in MEZ method

Essential oil of FV was effective in controlling the seizure induced by MES, but the effect was dose-dependant. As shown in Table 2, the maximum effect was observed at the dose of 600 mg/kg. The TD_{50} value for the FV essential oil in this method was 278.3 mg/kg. Flomazenil, in this group, could block the anti-seizure effect of the FV essence only by 63%. The result of the statistical analysis is given in Fig. 2.

3.3. The anti-seizure effect of methanol containing FV extract in MES method

Methanol containing FV extract effectively controlled the seizure induced by MES, but the effect was dose-

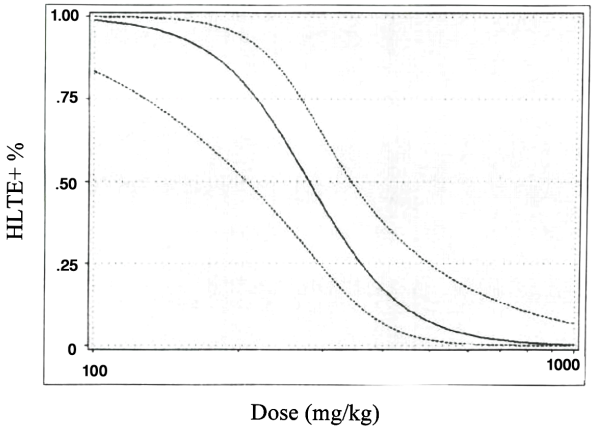

Figure 2. The plot of dose-response of FV essence in MES model

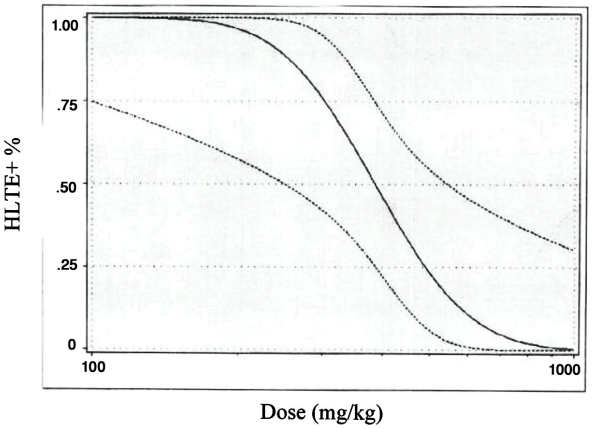

Figure 3. The plot of dose-response of FV methanolic essence in MES model

dependant. As shown in Table 3, the maximum effect is related the administered dose of 1000mg/kg. The TD_{50} value obtained was 385.8 mg/kg. Flomazenil could not significantly limit the anti-seizure effect of the FV extract. The result of probit analysis is shown in Fig. 3.

3.4. The anti-seizure effect of methanol containing FV extract in PTZ method

Methanol containing FV extract was effective in controlling the seizure in PTZ method, but the effect was dose-dependant. The maximum dosage required for the extract effectiveness was found to be 2000 mg/kg which does not fall within the acceptable range.

3.5. Anti-seizure effect of Diazepam in PTZ and MES methods

Diazepam effectively controlled the seizure in both the MES and PTZ methods, but the effect was dose-dependant. The obtained ED_{50} values were 1.57 and 1.87 mg/kg respectively. Flomazenil was able to significantly limit the anti-seizure effect of Diazepam in both methods (P-value < 0.001). Figures 4 and 5 illustrate the effect of Diazepam in both methods.

Table 2. The anti-seizure effect of FV essence, observation of HLTE and the fatality rate due to MES

Treatment agent	Dosage	HLTE-	HLTE+	% of seizure
Sesame seed oil+FV essence	600mg/kg+5ml/kg	8	0	0
Sesame seed oil+FV essence	500mg/kg+5ml/kg	7	1	13
Sesame seed oil+FV essence	400mg/kg+5ml/kg	7	1	13
Sesame seed oil+FV essence	300mg/kg+5ml/kg	4	4	50
Sesame seed oil+FV essence	200mg/kg+5ml/kg	2	6	75
Sesame seed oil+FV essence	100mg/kg+5ml/kg	0	8	100

Table 3. The anti-seizure effect of FV essence, observation of HLTE and the fatality rate due to MES

Treatment agent	Dosage	HLTE-	HLTE+	% of seizure
Distilled water+ FV extract	1000mg/kg+5ml/kg	8	0	0
Distilled water+ FV extract	500mg/kg+5ml/kg	6	2	25
Distilled water+ FV extract	300mg/kg+5ml/kg	2	6	75
Distilled water+ FV extract	100mg/kg+5ml/kg	0	8	100

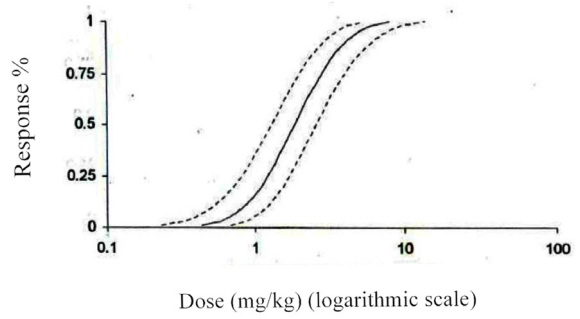

Figure 4. The plot of dose-response of Diazepam in PTZ model

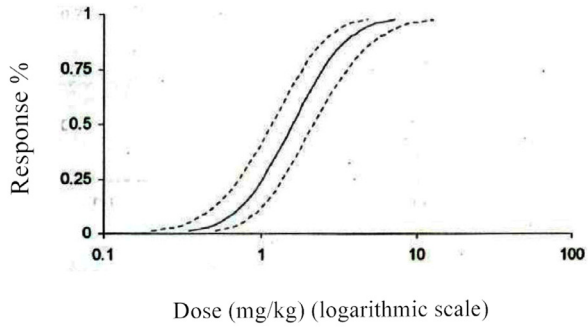

Figure 5. The plot of dose-response of Diazepam in MES model

4. Discussion

In the present study, the anti-seizure effect of FV essential oil and extract on male mice was investigated. The results of this study confirm the anti-seizure effects of the FV essence and extract already acknowledged in traditional medicine. The FV essence was able to control the seizures induced by MES method with TD_{50} of 385.8 but it was dose-dependant. Conversely the essence could not control the seizures induced by PTZ significantly. The seizures induced through PTZ method are similar to the seizures in Absenca Petitmal Epilepsy, and the seizures induced through MES method are resemble to those in Grandmal Epilepsy.

PTZ induced seizure occurs as a consequence of the PTZ antagonistic effect on the GABA receptors (GABAA) and its controlling effect on the flow of Chlor. It has also been found that the direct stimulation of cell membranes or controlling the effect of Benzodiazepine compounds can cause seizure (Litchfield and Wilcoxon, 1947; Royawski and Porter, 1990). On the other hands the drugs that control the MES induced tonic convulsions, exert their control by preventing the seizure spreading (Royawski *et al*., 1990). The mechanism used by effective drugs controlling the MES induced seizures including Phenitoien, Walproate and Felbamat, involve blocking the ionic channels particularly the sodium channel (Litchfield and Wilcoxon, 1947; Carter *et al*., 1997; Malacangio *et al*., 1991). The drugs known to be the antagonists of the NMAD receptors also control the MES induced seizures (Litchfield and Wilcoxon, 1947; White, 1997; Subramaniam, 1995).

According to our results, Flomazenil which is a specific antagonist of Benzodiazepam could significantly control the anti-seizure effects of FV essence or concentrate. This observation suggests that anti-seizure effects of FV essence and concentrate are at least partially related to the Benzodiazepine receptor. Flomazenil, one of the derivatives of 1, 4-Benzodiazepam, has a great affinity to the Benzodiazepine receptors which is currently the only Benzodiazepine receptor antagonist agent available for the clinical use (Katzung, 1991). Based on literature data, it can be postulated that the FV essence affects seizures through interfering GABA receptors, blocking the sodium channels sensitive to voltage, interfering NMAD receptors or a combination of these mechanisms.

The results of this study show that FV essence features anti-seizure properties in both PTZ and MES laboratory models. The essence possesses stronger controlling effect in PTZ model than in MES model, and in some doses, it can induce sedation and motor function disorders. The anti-seizure effect of the FV extract is significantly less than that of the essence. Further comprehensive study of FV fruit is needed for making a more certain judgment.

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