Effects of Methanolic Extracts of Papaver Somniferum on Picrotoxin Induced Seizure in Mice

1Reihaneh Sabbaghzadeh and 2Mohsen Asadbegi

1Department of Biology, Faculty of Science, Hakim Sabzevari University, Iran.
2Pharmacist Employed in Lorestan University of Medical Sciences, Lorestan, Iran.

ABSTRACT

The current study investigates the anticonvulsant effect of methanol extract of the seeds of this plant on picrotoxin-induced seizures in male mice. In this study, the animals were pretreated by intraperitoneal injection of different doses of methanolic extract of the seeds of this plant (12.5, 25, 50, 100 and 200 mg/kg), and after 20 minutes each animal was injected with 12 mg/kg picrotoxin to induce seizures. The change in seizure onset, duration of seizures and seizure-induced death in experimental and control groups were measured and compared, as well. The results showed that pretreatment of animals with different doses, especially 200 mg/kg doses causes delayed the onset of seizures (p<0.01). On the other hand, the duration of seizures increased in all groups compared to the control group, but the severity of seizures was milder. Different doses, especially doses 50 mg/kg caused delayed of death in mice (p<0.05). So poppy seed extract caused a delay in the onset of seizures, as well as reduce the severity of seizures induced by picrotoxin and delay in death, therefore can be a good candidate for further research as an antiepileptic drug.

INTRODUCTION

Epilepsy has been one of the world's common neurological disorders in all ages [1]. The term “Epilepsy” refers to a condition in which a person is faced with disability and dependency with abilities and performance affected. Epilepsy is a common disease in all countries, so that its incidence is approximately 7.9 per thousand, and occurs in all ages, all races and both sexes [17]. Before the discovery of antiepileptic drugs, man attempted to treat epilepsy by acts like boring through the skull and applying phlebotomy as well as using plants [1], until could finally treat this disease through the use of drugs. In 1912, the first drug, Phenobarbital, was used as a new and useful anticonvulsant drug, and then phenytoin and other drugs entered the field of medication [1]. Epilepsy treatment requires prolonged, continuous and concurrent use of medicine, so this leads to further side effects of the various drugs in patients [13].

There are no credible evidence of anticonvulsant effect of this plant in bibliographic resources and databases, so methanolic extracts of papaver somniferum on generalized tonic seizures and clonic seizures induced by picrotoxin in mice has been studied.

MATERIALS AND METHODS

Poppy plant is the species of Papaveraceae plant with the scientific name of Papaver somniferum L. Plant seeds used in this study was obtained from Kerman valid herb stores. The species are Iran native plants and has been approved by botany department of the Faculty of Agriculture, Kerman experts.

Animals: albino male mice weighing 22-28 g prepared from Science and Neuroscience Research Center, Kerman University of Medical Sciences.

Extraction method: Percolation method was used for extraction. Under this method, the plant grains were crushed first, (dried weight = 100 g), then entered percolator so that plants will take less than 23 volume percolator. A filter paper was placed on the chopped plant, and solvent was added slowly to keep smooth. It should note that the solvent must always cover the plant. Percolator discharge rate of 5 ml per minute extract...
was considered [5]. Then extracts were concentrated at a temperature of 40 °C by vacuum distillation method using rotary operator and finally completely dried in an oven at 30 °C. Weight of dried extract was 8 g.

**Study groups:**

- The negative control group: mice received the amount of 10 ml/kg normal saline 20 min before picrotoxin injection.
- The positive control group: mice received the amount of 40 ml/kg phenobarbital 20 min before picrotoxin injection.
- Experimental groups: mice treated with different doses of zhish extract 20 min before picrotoxin injection.

**Test Procedure:** 24 hours before the experiment, the animals were transferred to whereabouts of animals kept in special cages. The 12 hours light and 12 hours dark cycle was maintained, and the animals had access to urban water and food. One hour before the experiment, the mice were transferred to individual cages [15].

Different concentrations of extracts prepared with normal saline and different doses of 12.5, 25, 50, 100 and 200 mg/kg or 40 mg/kg dose of phenobarbital or 10 ml/kg normal saline were injected to different groups of mice and 20 min after the injection, 12 mg/kg dose of picrotoxin were administered to in addition to the anticonvulsant effect of FU, the most effective dose is determined.

**Injection volume per mouse was 10 ml/kg (0.1 ml 10 g):**

Mouse body weight and all injections were administered intraperitoneally. The onset of seizures, duration of seizures and deaths were recorded.

**Statistical analysis:**

In each series of experiments the effect of different doses of the plant extract and phenobarbital on seizure onset, duration of seizures and death induced by picrotoxin were recorded as mean and SEM Mean for 5 mice. Calculations to determine significant differences between experimental groups were performed using ANOVA followed by Newman-keuls and difference of p< 0.05 was considered significant [15].

**Results:**

**Injection of 12 mg kg picrotoxin-induced seizure in mice:**

12 mg/kg dose of picrotoxin Intraperitoneal injection first caused mild to moderate and often scattered contractions in mice. The contractions as minor ticks (especially the ears and neck), lying ears, straight tail, and hands gathered and rarely feet contractions occurred. Feet were apart and the ability of motion was limited and at first times, the animal’s body was dragged on the ground. However, waist and height of animals were normal. This phase lasted for about 1-2 minutes, was regarded as a tonic seizures. Then, suddenly mouse suffered from successive involuntary high frequency vibration that was clearly visible at this stage, especially when compared with mice that had received the carrier. At this stage the animal suffered from severely jerky movements and agitation, often involving the entire body and was almost generalized. Unlike the tonic phase, in this stage animal back often abnormally bent back and animals would have coil mode. Wild running was also observed occasionally. While not exactly well-documented pattern of behavior by animals at seizure can be determined, we take this step with this particular observation as the seizure in mice.

**Effect of methanolic extract on picrotoxin-induced seizure onset:**

As seen in Figure 1, 12 mg/kg dose of picrotoxin alone in mice triggered seizure at duration of 35330 seconds and pretreatment of animals with different doses of extract of opium caused delayed seizures onset. At this stage, the most effective dose was 200 mg/kg, which delays the onset of seizures up to 20478 seconds (p<0.01).

**Papaver somniferum effect on seizures induced by picrotoxin duration:**

As seen in Figure 2, duration of seizures induced by dose of 12 mg/kg picrotoxin alone in mice is 98 739 seconds. The animals pretreated with different doses, duration time was prolonged, at the dose of 50 mg/kg duration of seizures increased up to 198 1275 (p<0.05).

**Methanolic Papaver somniferum effect on death induced by picrotoxin:**

According to Figure 3, the time of death due to picrotoxin by dose of 12 mg/kg alone was 113 1069 seconds in mice. The animals pretreated with different doses death time was delayed, among which the most effective dose was 50 mg/kg with the time of death at 180 1650 seconds.

Different doses of Phenobarbital or extract with dose of 40 mg/kg administered to the animals by intraperitoneal 20 min before injection of picrotoxin (12mg/kg) n = 5.
Discussion:

Poppy plant with the scientific name of Papaver somniferum has been used for long in traditional medicine and modern medicine as a sedative, hypnotic and analgesic [3, 4]. The results obtained in this study show that methanol extract possesses anticonvulsant effects in mice. Pretreatment of animals with different doses of extract significantly delayed onset of seizures, and death compared to the control group. The results of this study were comparable to findings on the effects of rosemary extract on picrotoxin-induced seizures in mice [2].

The most effective dose of opium extract on the seizures onset was the dose of 200 mg/kg that delayed seizure onset up to 20 478 seconds and comparable with the dose of 50 mg/kg rosemary extract [2]. It seems that dose of 200 mg/kg could produce enough blood concentrations and delays seizure onset more. Lower doses cannot cause blood concentrations levels sufficient to create maximum effect. However, increasing the dose reduces extract effect that is probably due to the high concentration of the extract and non-pharmacological effects and toxicity of the extract [14].

Poppy plant extract doses causes longer seizure duration that the most effective dose is 50 mg/kg mice, which have increased seizure duration up to 198 1275 seconds, comparable to dose of 500 mg/kg of rosemary extract [2]. But the severity of seizures in the groups that received different doses is much lower than the control group. To explain the observed fact, it seems that different extract doses, especially dose of 50 mg/kg through reduction of seizure, increased adjusted seizure bearing and therefore the delayed animal death with more animal survival. Pretreatment of animals with different doses of poppy plant delayed the time of death that at this stage doses of 50 mg/kg and above (100 and 200 mg kg) caused more delay in death among which the most effective dose was 50 mg/kg with up to 180 1650 second delay comparable to dose of 500 mg/kg of rosemary extract [2].

Because the plant extract could affect on generalized tonic and clonic seizures induced by picrotoxin (because significant effects and the possibility of involvement of GABA-A receptors in the anticonvulsant effects of poppy seed extract exists [2, 10, 19].

It is recommended to consider medicinal plants used in traditional medicine to achieve a safe drug with minimal side effects.

The evaluation of information sources, including Medline, the articles found investigated the effect of different crops on epilepsy. These plants are:
- Toothbrush tree extract (alvadora persica) [20]
- Leonotis leonurus [10]
- Portulaca extract (sativa var.Portulaca oleacea) [22]
- Methanol extract of Vitex negundo [16]
- Methanol extract of Asparagus (Asparagus pubescens) [21]
- Estonia seed extract galbanum (Sesbania grand flora) [18]
- Snow White Plant (Goodyera schlechtendaliana) [12]

And a number of other plant species that often delay the onset of seizures.
The methanolic extract of toothbrush tree [20], five fingers (16) and Persimmon [9] with the scientific name “Diospyros mespiliformis” reduces mortality rate of seizures in mice. Each part of the poppy plant with different chemical composition and active ingredients are used differently. The main component of this plant is alkaloids compounds such as morphine, codeine, Narcheine and Papaverine that are very important in terms of medical treatment [4]. Poppy seeds consists a little papaverine and morphine (7). Poppy peel and seeds are traditionally used as analgesic, sedative and hypnotic [3]. Probably the anticonvulsant effect of this plant is due to its alkaloids. To prove requires removing the plant components and conducting specific researches on each. Also, it is not clear that poppy extract the effective compound act via GABA receptors or not. In the evaluation of information sources such as Medline, IPA, Pubmed and Google motor search no scientific and classical studies investigated the anticonvulsant effects of opium were found. Since these seeds as traditional herbal medicines, are effective and acceptable in Iranian traditional medicine and modern medicine as a sedative, hypnotic and tranquilizer; therefore it is an important to pursue this study in more accurate and extensive analysis of Phytochemical and Pharmacological Studies.

REFERENCES


