Study of Antiseizure Effects of *Matricaria recutita* Extract in Mice

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***Matricaria recutita* L. is a well-known medicinal plant that is suggested as being carminative, analgesic, and anticonvulsant in traditional medicine. In the present investigation the effect of hydro-methanolic percolated extract of this plant on seizure induced by picrotoxin was studied in male mice. This study was performed on animals pretreated with doses of 100, 200, and 300 mg/kg of extract or 40 mg/kg phenobarbital as the reference drug via intraperitoneal injection. After 20 min each animal received 12 mg/kg picrotoxin for induction of seizure. Latency of onset time of seizure, duration of seizure, death latency, and death rate were determined in experimental and control groups. The results showed that latency of the beginning time of seizure was increased in groups that were pretreated with different doses of extract. The most effective dose was 200 mg/kg ($P < 0.05$). In addition, this dose delayed the time of death in mice ($P < 0.01$). The extract had no effect on the death rate. The results indicate that the extract of *M. recutita* possesses suitable effects on seizure induced by picrotoxin, and more experiments are needed in this field.**

***Key words:* *Matricaria recutita*; seizure; picrotoxin**

Introduction

Epilepsy is one of the most common neurological disorders with no age, racial, social, sexual, or geographical boundaries and affects about 50 million people worldwide. In developed countries where drugs are easily available, epilepsy responds to treatment in up to 70% of patients.[1–3]

Antiepileptic drugs are one of the products used for control of seizures. Duration of treatment of seizure is long, and therefore most antiepileptic drugs induce a wide range of problems, such as the undesired effects of dependency during long-term therapy, that remain to be solved. This situation indicates the need for safe, novel, and effective antiepileptic drugs.[4]

There is renewed worldwide interest in the use of plants to relieve or cure different diseases, among which are neurological disorders, such as epilepsy, which has a high incidence in the world population.[5–8]

The empirical knowledge about medicinal properties of plants is the basis for their use

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as home remedies in Iran.[6–8] It is generally accepted by many Iranians and people elsewhere in the world that beneficial medicinal effects can be obtained by ingesting plant products.[1,6–8] *Matricaria recutita* L. is one of the most important medicinal plants in Iranian traditional medicine.[9–11] The dried flowers of aerial parts of *M. recutita* are largely used to provide sedative as well as spasmolytic effects in traditional medicine. The dried flower heads of *M. recutita* have been reported to exhibit spasmolytic and sedative properties, although the active components responsible for the sedative activity have not yet been fully characterized.[12,13] Furthermore, a depressive activity of a lyophilized infusion of flowers of *M. recutita* on the central nervous system was demonstrated in mice by Della Loggia *et al*.[14] The flowers of this plant have been used as demulcent, anti-inflammatory, and analgesic, anxiolytic, and sedative in Iranian folk medicine.[15,16] The pharmacological profile of apigenin, a flavonoid isolated from this plant, was studied and the results have shown that this compound may act on benzodiazepine receptors in the central nervous system.[12] *M. recutita* was selected for the present study because of the antiepileptic effect assigned to it by traditional medicine.[9–11,13,15,17–19] We focus on the potential antiseizure effects of *M. recutita*, which have not been assessed.

The present work was carried out to investigative the effect of hydro-methanolic extract of *M. recutita* on generalized seizures induced by picrotoxin, a widely used model for chemically induced convulsion in mice.[5,12,20] This study is an attempt to establish a scientific basis for the use of this plant as an antiepileptic drug in Iranian traditional medicine.

Materials and Methods

Animals

Male albino mice weighing 22–27 g were used. The animals were obtained from the Neuroscience Research Center of Kerman University of Medical Sciences. They were housed at room temperature (22 ± 2 °C) on a 12:12 h light:dark cycle. They had free access to food and water except during the time of the experiments. Animals were acclimatized to the laboratory for at least 1 h before testing and were used for one experiment only. The experiments were carried out between 8:00 AM and 3:00 PM. The animals were distributed into groups of seven as controls and test groups. According to international rules for animal experiments, all efforts were made to minimize animal suffering and to reduce the number of animals used.[21,22]

Plant Material

Matricaria recutita was collected from a farm in Kerman, Iran. It was authenticated by M. Mehrabani, and voucher specimens (No. 1003) were deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.

Extract Preparation

Seventy grams of powder of dried aerial parts of *M. recutita* was extracted with 80% aqueous methanol by percolation (72 h).[23] The extract was filtered, and the residue was concentrated by a rotary evaporator apparatus (45 °C) and then dried at room temperature.[23] The weight of the dried extract was 6 g, corresponding to an 8.6% yield. The residue was dissolved in normal saline for final suitable concentrations to give the desired concentration (100, 200, and 300 mg/10 mL).[7,8]

Convulsion Test and Data Recording

In the seizure test, *M. recutita* extract with doses of 100, 200, and 300 mg/kg (treatment groups), or 10 mL/kg normal saline (negative control group), or 40 mg/kg phenobarbital as the reference drug (positive control

group), was injected i.p. to groups of seven animals.[7,8] Picrotoxin with a dose of 12 mg/kg was injected i.p. 20 min after injection of different doses of extract, normal saline, or phenobarbital to induce generalized seizure.[5,7,8] The onset time of seizure, duration of seizure, latency (i.e., time between the injection and the onset of the first jerk or clonus), death latency, and death rate were measured in the test and control groups.[5,12] Mice were observed for 90 min after picrotoxin injection. The groups that were pretreated with normal saline and phenobarbital were considered as control and positive control, respectively.[5–8]

Statistic Analysis

Results are presented as mean ± SEM in each group of seven animals, and statistical differences between groups were analyzed by ANOVA followed by the Newman–Keuls test. $P < 0.05$ was considered significant.[7,8,24]

Results

Effect of *M. recutita* Extract on the Onset Time of Seizure Induced by Picrotoxin

As shown in Figure 1A , pretreatment of animals with different doses of extract increased the time of latency in the onset of convulsions. This effect was significant with doses of 100 and 200 mg/kg ($P < 0.01$).

Effect of *M. recutita* Extract on Duration of Seizure

The doses of 100 and 300 mg/kg of extract decreased the duration of seizure induced by picrotoxin, but the dose of 200 mg/kg increased it significantly ($P < 0.01$) (Fig. 1B). However, the severity of seizure in all treatment groups was milder than in the control group.

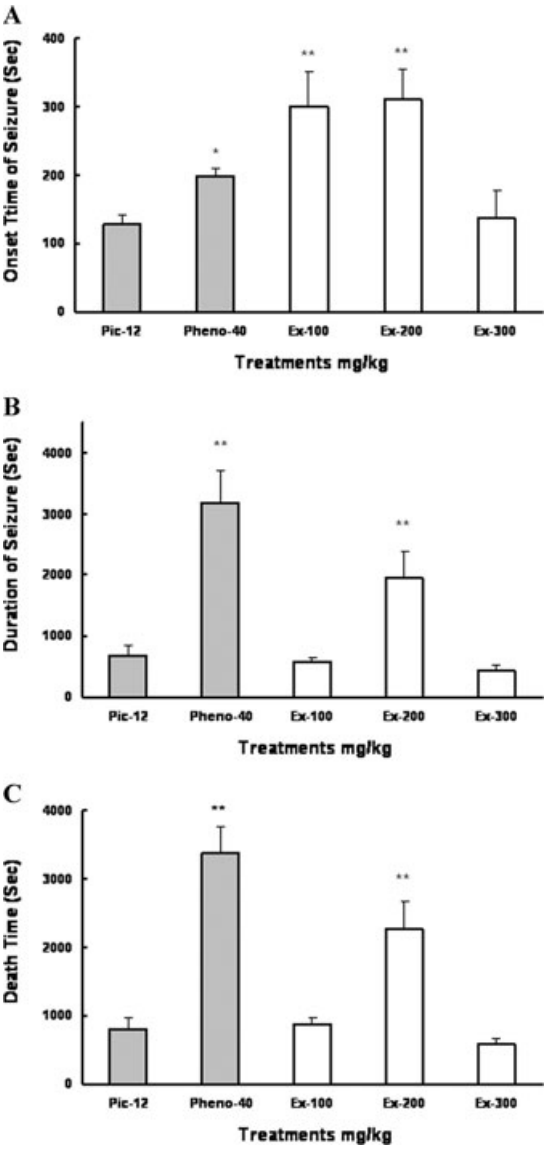

Figure 1. The effect of *Matricaria recutita* L. extract on the onset time of seizure (**A**), duration time of seizure (**B**), and death time (**C**) in mice. Normal saline (10 ml/kg), phenobarbital (40 mg/kg), or different doses of extract were injected intraperitoneally 20 min before picrotoxin (12 mg/kg). Each column indicates the mean ± SEM of time in seven animals. *, $P < 0.05$ and **, $P < 0.01$ show a significant difference from the control group.

Effect of *M. recutita* Extract on Death Time

Pretreatment of animals with doses of 100 and 200 mg/kg of extract delayed the death

time, which was signficant ($P < 0.01$) with a dose of 200 mg/kg (Fig. 1C). The dose of 300 mg/kg decreased the death time.

Effect of *M. recutita* Extract on Death Rate

None of the doses of extract were able to protect animals from death induced by seizure from picrotoxin, and therefore the death rate was 100%.

Discussion

Picrotoxin as a γ-aminobutyric acid-A ($GABA_A$) antagonist has been widely used as a model for chemically induced convulsion and produces a generalized clonic-tonic convulsion that leads to death in most cases.[7,21,24] The results of the present study showed the anticonvulsant effect of the hydro-methanolic extract of the dried aerial parts of *M. recutita* in mice. The most effective dose of the extract was 200 mg/kg. This dose significantly delayed the onset time of seizure and death time compared to the control group ($P < 0.01$). The higher dose of 300 mg/kg exerted a less anticonvulsant effect. Higher doses of the extract may produce concentrations higher than the therapeutic level[25] and produce nonpharmacologic or toxic effects. These nontherapeutic effects can be attributed to unknown ingredients in this plant. This inverted U-shaped dose-response relationship is relatively common with complex herbal products and also reported by others.[7,8,26] It suggests that other constituents in the herbal complex have opposite or toxic effects. Thus, the effect of a mixture is a sum of the relative doses and maximal effects of the individual components within the mixture.[26]

The duration of seizure was increased significantly ($P < 0.01$) with a dose of 200 mg/kg compared to the control group. However, the severity of seizure was milder than in the control group, and this resulted in the seizure being tolerable so that animals survived longer.

The ingredients in this plant include azolen, sesquiterpens, chamomilol, matricarine, chamazoline, bisabolol, matricarine, capric ethers, amino acids, fatty acids, phenolic acids, choline, cumarins, and flavonoids.[9,10,12,15,16,18,19] There is some evidence of an anticonvulsant effect of this fatty acid and some of the flavonoid compounds.[7,8,12]

During the last 10 years, several Bzs and Bz-like ligands have been found in natural sources, food, and medicinal plants. Authentic diazepam and nordiazepam have been identified in wheat, potato, lentils, and mushrooms, while flavonoids exerting Bz-like activity, such as chrysin and apigenin, have been found in *Passiflora coerulea* and *M. chamomilla*, respectively.[12]

Some reports have shown anxiolytic effects of some natural and synthetic flavonoids in rats and have found that these compounds exert their effects through the central benzodiazepine receptors.[27–29] Therefore, it seems that the antiseizure effect of *M. recutita* may be related, in part, to linoleic acid and/or flavonoid compounds present in the extract. However, determination of the role of each of these compounds in the anticonvulsant effect of the extract requires more investigations. In conclusion, the findings reported in this study indicate that *M. recutita* may contain novel bioactive principles with anticonvulsant properties. These results confirm the antiepileptic properties assigned to the plant in traditional medicine. However, more pharmacological and toxicological experiments are needed for use of this plant as an official herbal drug in clinical use. Biological testing of these plants, using different models of convulsions, is suggested.

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Conflicts of Interest

The authors declare no conflicts of interest.

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