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RESEARCH ARTICLE

Eugenol efficacy in preventing nicotine-induced seizures in mice

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ABSTRACT

Background: Seizure is the most common neurological disorder after stroke. In fact, the seizure is a chronic neurological disorder affected 1-2% of the global population. Given the side effects and toxicity of synthetic drugs, some herbal medicines are currently used in the treatment of seizure. **Aims and Objectives:** The aim of this study was to evaluate the effect of Eugenol, a compound extracted from clove plant, which is effective in preventing nicotine-derived seizures in mice. **Materials and Methods:** In this study, 64 mice were randomly selected. In the dose-response study, different doses of Eugenol (400, 600, and 800 mg/kg) were intraperitoneally injected to the experimental groups. The negative and positive control groups, respectively, received saline (10 ml/kg) and diazepam (0.15 mg/kg). After 30 min, the animals in all groups received intraperitoneal injections of nicotine (5 mg/kg) followed by the measurements of onset time, duration, and severity of seizures. In time-response examination, the most effective dose of Eugenol (600 mg/kg) was injected into the animals at 15, 30, 45, and 60 min before nicotine injection. Afterward, the onset, severity, and duration of seizure were determined. **Results:** The results of the dose-response test showed that Eugenol doses of 400, 600, and 800 mg/kg resulted in increased time of onset, and reduced severity and duration of seizure compared to the saline group. The results of time-response revealed that the best dose was 600 mg/kg and that the best time for Eugenol injection was in 30 min before injection of nicotine. **Conclusion:** The results of this study suggest that Eugenol can be effective in controlling nicotine-induces seizures.

KEY WORDS: Eugenol; Nicotine; Seizures; Mice

INTRODUCTION

Seizure is a sign of a synchronous, abnormal and excessive neuronal activity in the brain that can occur as a change in mental status, and tonic and colonic movements.[1] Factors such as infection, tumor, brain trauma, and inflammation may be involved in the incidence of seizures. Almost 30% of seizures are caused by central nervous system (CNS) disorders.[2] In general, convulsions are created due to an imbalance between the excitatory and inhibitory neurons with the most important roles of γ-aminobutyric acid (GABA) and Glutamate neurotransmitters.[3,4]

GABA is the most important inhibitory neurotransmitter of the brain. GABA receptor leads to chlorine influx into the cells causing membrane hyperpolarization, hence, inhibition of the cell. There are lots of GABA-producing neurons in the cortex with important roles in the control of the epileptic activity of neurons.[5] Glutamate level increases immediately before the onset of seizures while levels of GABA are low at the time of seizure.[6,7] Meanwhile, glutamate as the most important excitatory neurotransmitter in CNS activates N-methyl-D-aspartate (NMDA) receptor (affecting calcium influx). The NMD receptor antagonist has an antiepileptic activity as

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well. Some panic attacks happen due to electrical discharge or influx of calcium ions into the neurons.[8] Prostaglandins are made by cyclooxygenase-2 (COX2) enzymes resulting in increased glutamate release.[9] In addition, activation of the COX2 enzyme leads to apoptosis of GABA neurons by increasing free radicals and oxidative stress, which in turn increases glutamate release and elevates the risk of seizures.[10,11]

Seizures can be caused by several mechanisms, for example, three main mechanisms involved in the development of epilepsy include decreased GABA-induced synaptic inhibition, increased synaptic excitation especially NMDA-induced stimulation, and elevated endogenous discharges of neurons resulting from an increase in the voltage-dependent calcium influx.

The findings of this study showed that nicotine directly or indirectly increases the release of glutamate. The release of glutamate, in turn, stimulates NMDA receptors resulting in the production of nitric oxide and possible incidence of seizures.[12] The necessity of continuous long-term treatment with antiepileptic drugs provides the groundwork for side effects. Accordingly, the need to herbs becomes apparent to achieve low-risk drugs with minimal side effects.

Clove plant, *Dianthus caryophyllus*, belongs to the family Caryophyllaceae, which is one of the plants used in traditional medicine. Clove tree is native to the islands of Indonesia and the Pacific. It is now grown worldwide as an ornamental tree because of the special beauty.[13]

Eugenol is a transparent and almost colorless or yellow liquid categorized among phenolic drugs, which is one of the main constituents of the whole plant extract.[14] Some of the chemical compounds found in cloves include Eugenol, Eugenol acetyl, β-caryophyllene, vanillin, tannins, methyl salicylate, alpha and beta sesquiterpenes, caryophyllene, glutamic acid, and calcium oxalate.

Clove oil contains 83-87% of Eugenol. Essential oils have various biological activities such as antioxidant, antitumor, antimicrobial and analgesic in dentistry, useful for neurasthenia and neurological diseases, prevention of teratogenicity, and oxidative, and protective effects against neurotoxicity.[15,16] Since oxidants and free radicals are among the causes of seizures, this study aims to achieve the scientific basis for the traditional use of Eugenol as a compound of clove plant in the treatment of seizures.

MATERIALS AND METHODS

A total of 64 adult male mice weighing approx. 25-30 g were obtained from the animal reproduction and maintenance center, Jundishapur University of Medical Sciences, Ahvaz, and kept in the animal room located at the School of Pharmacy. The room temperature and humidity were set as 23 ± 2°C and 40-50%, respectively, with a photoperiod of 12 h light: 12 h dark condition for the animals. Sufficient food and water were readily available to the experimental mice. The experimental protocol was approved by the Institutional Ethics Committee of School of Medicine, Ahvaz Jundishapur University of Medical Sciences guidelines for animal care and use, and all the experimental procedures were conducted in accordance with international guidelines for care and use of laboratory animals (Approval No. IR.AJUMS.REC.1395.123).

Dose-response Study

To perform the test, the animals were divided into octamerous groups, weighed, numbered, and administered as follows. The experimental groups were intraperitoneally injected 400, 600, and 800 mg/kg of Eugenol. The negative and positive controls, respectively, received saline (10 ml/kg) and diazepam (0.15 mg/kg) through intraperitoneal injections. After 30 min, dissolved nicotine (5 mg/kg) was injected intraperitoneally to all groups followed by measurements of seizure onset, severity, and duration. The intervals between nicotine injections and the first observation of convulsion symptoms were measured to determine the time of onset. To measure the intensity of seizures, the mice were placed on a table. If the animal showed normal movements, it was given a score of zero. It scored one if the head and jaws moved slowly. When the animal's jaw trembled violently, it was assigned a score of two. In case the animal's body trembled strictly, it was given a score of three. Finally, it scored four if the body was shaking strongly. The duration of seizure was measured as the interval between the start and complete disappearance of convulsions.

Time-response Study

After determining the effective dose (600 mg/kg) of Eugenol, it was injected to the mice. After 15, 30, 45, and 60 min, nicotine (5 mg/kg) was injected intraperitoneally followed by determination of seizure onset, severity, and duration.

Statistical Analyses

The experimental groups were compared with SPSS software using one-way ANOVA and least significant difference (LSD) auxiliary test. Significant differences were considered when $P < 0.05$.

RESULTS

The average delay in the onset of seizures significantly increased with Eugenol doses of 400, 600, and 800 mg/kg compared to the negative control (saline) group. In addition, the average delay in the onset of seizures at doses of 600, and 800 mg/kg showed a significant increase as opposed to the positive control (diazepam) group (Figure 1).

The mean severity of seizure in groups injected Eugenol doses of 600 and 800 mg/kg significantly decreased compared to the negative control (saline). The average severity of seizures in all groups receiving Eugenol was significantly higher than those received diazepam (Figure 2).

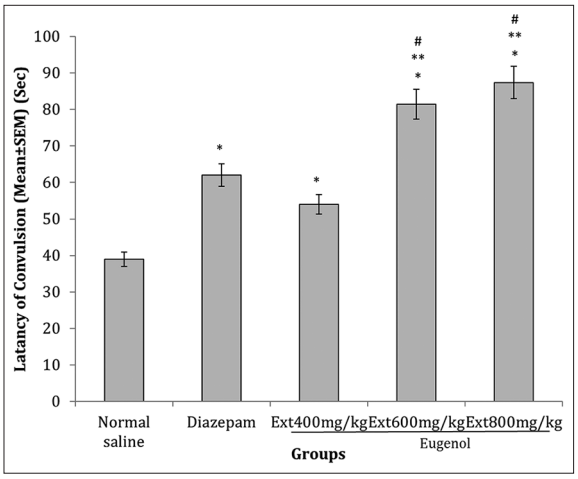

Figure 1: Comparison of average delay in the onset of seizures in the groups tested with positive (diazepam) and negative (saline) control groups. Different doses of Eugenol (400, 600, and 800 mg/kg) were intraperitoneally injected into the mice in 30 min before injecting a dose of nicotine (5 mg/kg). *There is a significant difference ($P < 0.05$) with the group received saline (10 ml/kg). **There is a significant difference ($P < 0.05$) with the group received diazepam (0.15 mg/kg). #There is a significant difference ($P < 0.05$) with the group received a Eugenol dose of 400 mg/kg. There were eight animals in each group

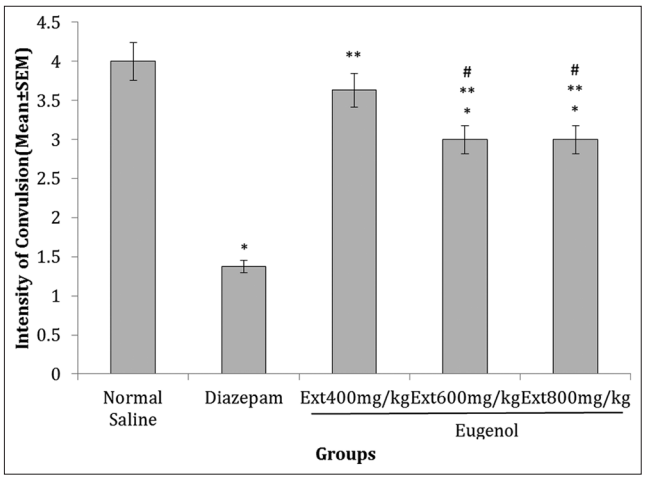

Figure 2: Comparison of mean severity of seizure in the experimental groups with positive (diazepam) and negative (saline) control groups. Different doses of Eugenol (400, 600, and 800 mg/kg) were intraperitoneally injected into the mice in 30 min before injecting a dose of nicotine (5 mg/kg). *There is a significant difference ($P < 0.05$) with the group received saline (10 ml/kg). **There is a significant difference ($P < 0.05$) with the group received diazepam (0.15 mg/kg). #There is a significant difference ($P < 0.05$) with the group received a Eugenol dose of 400 mg/kg. There were eight animals in each group

The average duration of seizure in all groups received different doses of Eugenol significantly decreased compared to the negative control (saline) group ($P < 0.05$). The average duration of seizure in the groups administered different doses showed a significant increase compared to the positive control (diazepam) group ($P < 0.05$) (Figure 3).

Time-response Study

The onset of seizure significantly delayed in the groups received Eugenol in 30 min before nicotine injection in comparison with the group injected Eugenol in 15, 45, and 60 min before nicotine administration ($P < 0.05$) (Figure 4).

Seizure severity was not significant among all the groups received a Eugenol dose of 600 mg/kg at different times before nicotine injection (Figure 5).

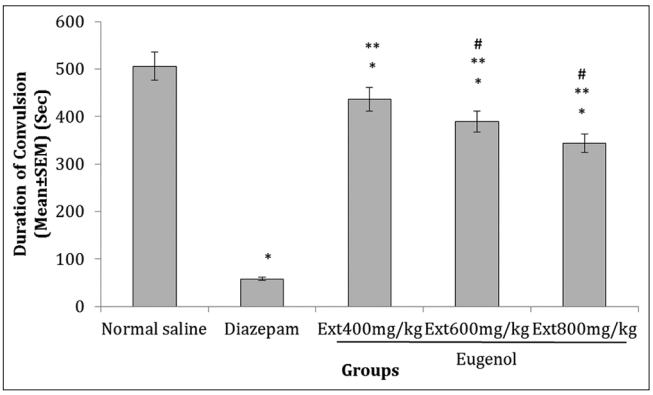

Figure 3: Comparison of mean duration of seizure in the experimental groups with positive (diazepam) and negative (saline) control groups. Different doses of Eugenol (400, 600, and 800 mg/kg) were intraperitoneally injected into the mice in 30 min before injecting a dose of nicotine (5 mg/kg). *There is a significant difference ($P < 0.05$) with the group received saline (10 ml/kg). **There is a significant difference ($P < 0.05$) with the group received diazepam (0.15 mg/kg). #There is a significant difference ($P < 0.05$) with the group received a Eugenol dose of 400 mg/kg. There were eight animals in each group

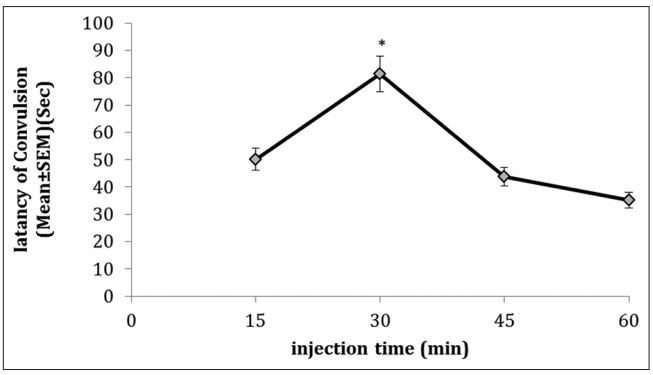

Figure 4: Comparison of average delay in the onset of Seizure between groups received a Eugenol dose of 600 mg/kg at different times before the injection of nicotine. *There is a significant difference ($P < 0.05$) with the group received a dose of 600 mg/kg in 60 min before the injection of nicotine. There were eight animals in each group

Seizure duration was significantly lower in the group injected Eugenol in 45 min before nicotine administration compared to the group received Eugenol in 60 min before injection of nicotine ($P < 0.05$) (Figure 6).

DISCUSSION

Seizure results from an imbalance of inhibitory and excitatory neuronal communication, which is in turn caused by sudden and uncontrolled discharges of neurons in the CNS. If seizures are frequent and unexplained, they are referred to as idiopathic epilepsy. Statistics suggest that around 1-2% of the world's population suffer from this neurological disorder.[17,18] The need for continuous long-term treatment with antiepileptic drugs paves the ground for the incidence of side effects.

According to the World Health Organization, about 80% of the world's population prefers to use plant extracts or their active ingredients for primary health care. The use of traditional medicine is on the rise in both developed and developing countries.[19,20] Medicinal plants have been medically used in the control and treatment of diseases for centuries, and their fewer fallouts and therapeutic effects have been proven over many years.

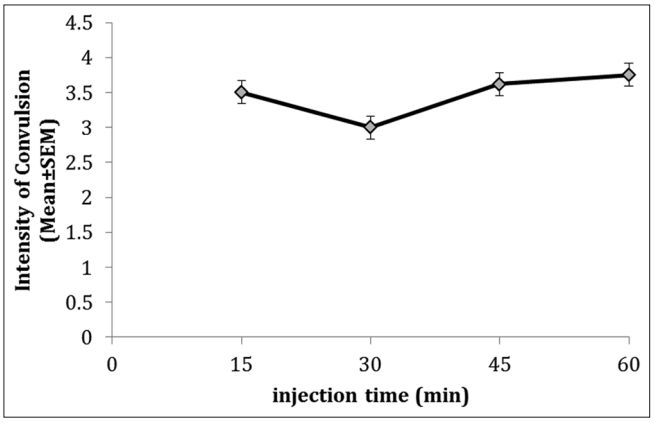

Figure 5: Comparison of seizure severity between the groups administered a Eugenol dose of 600 mg/kg at different times before nicotine injection. There were eight animals in each group

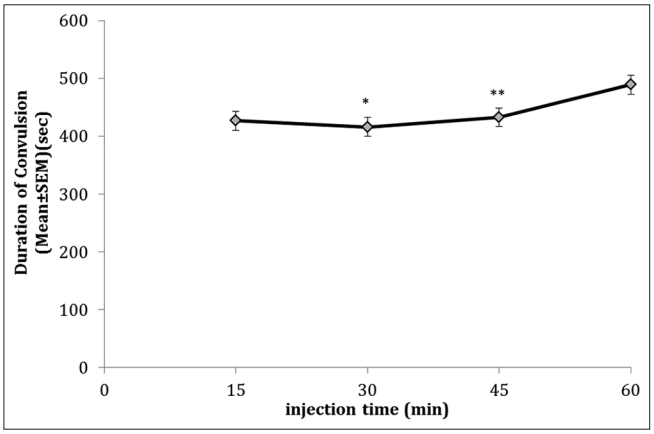

Figure 6: Comparison of average seizure duration between the groups administered Eugenol (600 mg/kg) at different times before nicotine injection. *There is a significant difference ($P < 0.05$) with the group received a Eugenol dose of 600 mg/kg in 15 and 60 min before the injection of nicotine. **There is a significant difference ($P < 0.05$) with the group received a Eugenol dose of 600 mg/kg in 60 min before the injection of nicotine. There were eight animals in each group

Therefore, medicinal plants are brought in the focus of disease treatment aiming at the achievement of a safe drug with minimal side effects and toxicity and Eugenol is one of them. Eugenol is one of the most effective compounds found in clove plant allocating 83-87% of the clove essential oil.[15]

On the subject of the limitation of studies on the effect of clove plant extract on seizure status in comparison with common anticonvulsants such as diazepam. This study attempt was to analyze the effect of Eugenol in different doses on seizure status in mice and compared its effect with those who treated with diazepam.

Therefore, in this study, two models of dose-response and time-response effects were analyzed. To assess the effect of dose-response in the prevention of nicotine-induced seizure (5 mg/kg) in mice, doses of 400, 600, and 800 mg/kg of Eugenol were used. 30 min before injection of nicotine, these doses were applied intraperitoneally to mice in different groups. The results of this study indicate that the dose of 600 mg/kg of Eugenol in comparison with other groups had the greatest effect on parameters such as the onset time of seizure, seizure severity, and seizure duration. Since the time of injection of different Eugenol doses, before injection of nicotine was the same in all mice (30 min). On the other hand, with an increase in the dose of Eugenol, the obtained results were much better. Therefore, it can be determined that the effect of Eugenol is probably in a dose-dependent manner. To study the time-response effect, the most appropriate dose of Eugenol (600 mg/kg) was chosen and intraperitoneally was given into groups and time for 15, 30, 45, and 60 min before nicotine injection (5 mg/kg). Concerning on the results of Eugenol injection (600 mg/kg) at 15, 30, 45, and 60 min before injection of nicotine (5 mg/kg), and on the parameters such as the onset of seizure, seizure severity, and seizure duration, the most effective was 30 min which may be due to high dose of Eugenol in this time period in animal's body. It worth to be noted that the effect of nicotinic seizure can be due to its effects of intermittent on glycine which is a chemical interference of the spinal cord. It can also be considered as the result of the nicotine stimulating effect on CNS motor controls such as basal ganglia.[21] Other studies have indicated that nicotine injections in the globus pallidus or the substantia nigra of the dogs and monkeys cause colonic seizures.[22]

Eugenol prevents oxidative damage and has protective effects against neurotoxicity induced by NMDA through the mechanism of inhibiting calcium absorption.[23] Arzi et al.

(2011) noted that lavender extract can apply its anticonvulsant effect through blocking calcium channels.[12] Sayyah et al. (2002) conducted a study on the anticonvulsant effect of a volatile oil in the leaves of *Laurus nobilis* on pentylenetetrazole-induced seizure in mice and observed that the plant essential oils effectively prevents the occurrence of seizures. The plant contains Eugenol, methyl Eugenol, pinen, and linalool, which can explain the anticonvulsant impact of the plant.[24]

CONCLUSION

Concerning on the results of this study and their association with many other studies, it can be determined that Eugenol can probably be used as an adjuvant or a most common medicine for the prevention or treatment of seizures. Furthermore, that this research was only a preliminary study, accordingly, to achieve study objectives, therefore another animal model was generated using different administration methods. Based on the prescription in case of the efficacy of Eugenol and the acceptability of its side effects, the work began on a human specimen.

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