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Anti-epileptic Properties of Terpineol Extracted from *Myristica fragrans* Houtt. Essential Oil in the Epileptic Rat Model

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Abstract

Epilepsy is the manifestation of the disease due to overstimulation of the brain. The common signs of this disease are chronic, episodic and recurrent seizures. This problem usually occurs in dogs and cats with the prevalence of the problem estimated about 5.0-5.7 % in dog population and about 0.5 % in the cat population. Today, there is a lot of studies that had been done to search for the new alternative anticonvulsant and antiepileptic using allopathy or traditional medicine, one of them that had been done was *Myristica fragrans* Houtt. The volatile oil from the *Myristica fragrans* Houtt. had been studied and proven to have anticonvulsant effect and one of the constituents suspected to contribute to the anticonvulsant activity was terpineol. This study was done to screen the anti-epileptic effect of terpineol that had been extracted from *Myristica fragrans* Houtt. essential oil to the kainic acid induced epileptic rat model by determining the effect of terpineol to the behavioural seizure activity and electroencephalogram (EEG) of this model. In this study, 3 adult male SpraqueDawley rats were established with a radiotelemetry system by implanting with electroencephalogram telemetry device and were induced for epilepsy using kainic acid and then treated with the terpineol with doses of 20 mg/kg and 50 mg/kg. The rats were observed for behavioral seizure activity and electroencephalograms were done qualitatively and quantitatively based on the Racine Scale and EEG semiology. The result of this experiment indicated that terpineol had the antiepileptic properties by inhibiting the behavioral seizure activity and seizure electroencephalogram. The effect of antiepileptic of terpineol also correlated with the doses that were used in this experiment. The possible mechanism of antiepileptic effect of terpineol is by enhancing the gamma-aminobutyric acid (GABA) inhibition mechanism.

Keywords: Terpineol, *Myristica fragrans* Houtt., epilepsy, antiepileptic, behavioural seizure activity, electroencephalogram

Introduction

Epilepsy is not a single disease but it is a manifestation of the disease that results from over-stimulation of the brain. The signs include chronic, episodic and recurrent seizures, with or without any detectable underlying brain lesion (Blood *et al*.,2007). Epilepsy is a common term used interchangeably with seizure and convulsion. The problem can occur in cats and dogs with the prevalence of 5-5.7 % in the general population of dogs and 0.5 % in cats (Berendt, 2008). Epilepsy can progress to a severe condition which is status epilepticus leading to euthanasia of the animals. Today, there are a lot of studies that had been done using the human traditional or allopathy medicine as anticonvulsant or alternative antiepileptic agent. One of them is *Myristica fragrans* Houtt. or nutmeg or mace. Nutmeg or mace also known as "Buah Pala" in Malay is the seed of an apricot-like fruit of the nutmeg tree which is native to Moluccas or Spice island of Indonesia (Bewley *et al*., 2006). Previous report had indicated that pharmacological activity of *Myristica fragrans* Houtt.exists in its volatile oil fraction extracted from the kernel. This oil was proven to have anti convulsant activity. Terpineol is a naturally occurring monoterpene alcohol that has been isolated from the nutmeg. terpineol is one of the compounds that issuspected to give anti-epileptic effect (Wahab *et al*., 2009). An experiment was conducted to screen the antiepileptic properties in terpineol extracted from nutmeg or *Myristica fragrans* Houtt. essential oil in the epileptic rat model by observing the antiepileptic effect on behavioral seizure activity in kainic acid induced epileptic rat model and to determine the antiepileptic effect of terpineol via electroencephalogram recording using a radiotelemetry system.

Materials and Methods

The experiment was performed on 3 male Sprague-Dawley rats, weighing 400-420 g, at Animal Laboratory House, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia. Experimental procedures were done with the permission from the Animal Research Committee of Universiti Sains Malaysia. The rats were divided into three groups: untreated group, terpineol 20 mg/kg treated group, and terpineol 50 mg/kg treated group.

In this study, EEG was obtained from the normal rat, during epilepsy and after treatment via a radiotelemetry system developed by the Division of Transomal Medical (DSI), United States. All rats used in this experiment were implanted with transmitters by surgical implantation. The injection of kainic acid was performed according to the protocol described previously by Dudek *et al*. (2006). The injection of 5 mg/kg kainic acid was performed intraperitoneally at lateral to linea alba. Treatment of epilepsy was performed by an intraperitoneal injection of terpineol 1 h after of the kianic acid induced epileptic rat model was established. The doses were terpineol at 50 and 20 mg/kg, respectively, for each group. The treatment was performed after the rats were confirmed in the status epilepticus.

Seizure behavior activity was determined using a scoring system as described by Racine (1972). Monitoring of seizure was conducted by visual observation and video recording. The behavior seizure activity was monitored qualitatively for 12 hours and quantitatively for 3 h. Methods of observation and interpretation of EEG were conducted based on the EEG semiology as described by Dr. Tahamina Begum of Universiti Sains Malaysia. The pattern was categorized into the four distinct EEG morphological patterns. Based on these data, EEG was monitored qualitatively for 12 h and quantitatively for 2 h. The EEG patterns were calculated based on the semiology.

Data analysis was performed on EEG and behavioral seizure activity for qualitative and quantitative data. Qualitative data were behaviour video recordings and EEG data were analyzed by visual observation and quantitative data were analyzed by manual calculation.

Results

Qualitative Behavioural Seizure Activity

Behaviour Characteristics

After kainic acid was injected to the rat, the behavioural seizure activities such as head nodding or wet dog shakes, forelimb clonus, lordosis posture, rearing behaviour and loss of righting reflex were scored using Racine Scale (Racine, 1972) and were present in all rat groups in this study. But without treatment the condition continued and became severe and with treatment with terpineol the condition was absent.

Survivability

The rats with the treatment had a much better survivability compared to untreated rats. Treated rats for both 20 and 50 mg groups survived the whole 12 h observation period but untreated rats died within 4 hours after kainic acid was injected.

Severity of Seizure

The severities of the seizure in treated groups compared to before and after treatment were significantly reduced. For terpineol 20 mg there were only 2 seizures observed which was of class 2 in the early minutes after treatment and the seizure did not occur after that. For terpineol 50 mg/kg there was no seizure activity observed immediately after the treatment was given. However, for untreated rats all seizures were still present and most of the seizure were of class 5 seizure and similar to the severity before treatment.

Quantitative Behavioural Seizure Activity

There were reductions in total number of behavior seizure activities in treated groups compared to untreated group. Figure 1 presents the total behavior seizure activity for these experimental groups.

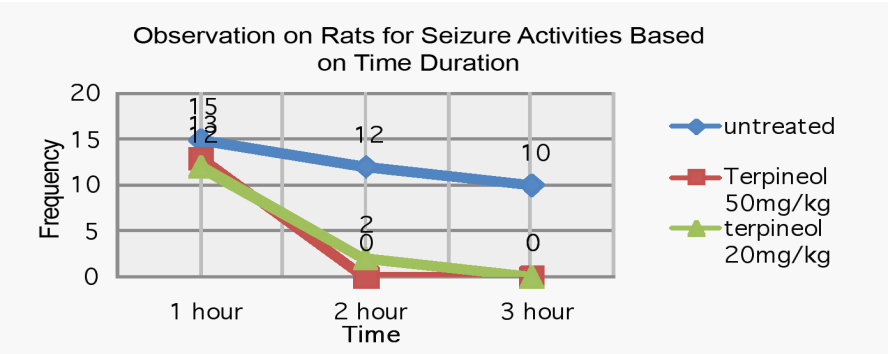

Figure 1. Total Behavioural Seizure Activity for Experimental Groups

Qualitative Electroencephalogram

Figure 2 presents the portion of the EEG wavelength taken from each group during normal, before and after treatments. In normal rats, EEG was stable with straight wave observed. The amplitudes were less than 0.2 mv and no spike was present. After kainic acid induced seizure, all 4 distinct patterns of epileptiform discharges were present. The amplitudes of the wave were variable in the range of 0.5 -0.8 mv and spike frequency was in the range of 3 – 5 hertz. This pattern of amplitudes and spike frequency occurred continuously when no treatment was given. However, for the treated group, there was a decrease in the pattern from severity to lesser severity.

Quantitative Electrocephalogram

There was a reduction of the EEG seizure patterns after the treatment was given in the terpineol groups compared to untreated group as showed in the Figure 3. Based on this result we concluded that terpineol had decreased the severity of seizure EEG pattern and total number of seizure pattern in EEG.

Discussion

Based on the results, we concluded that terpineol has a potential in minimizing the behavioral seizure activity and seizure electroencephalogram. This finding actually is novel because there are no studies prior to this to test terpineol as an antiepileptic and to examine the antiepileptic effects of terpineol extracted from the nutmeg.

The effects of terpineol as found in this study were consistent with other similar studies using a similar model to examine antiepileptic or anticonvulsant (Cepeda *et al*., 1984; Hashizume *et al*., 2000; Grabenstatter *et al*., 2007). All of these studies reported inhibition of seizure activity and electroencephalogram. Based on these studies, we can suggest that terpineol also has an anti-epileptic effect to behavioral seizure activity and seizure electroencephalogram.

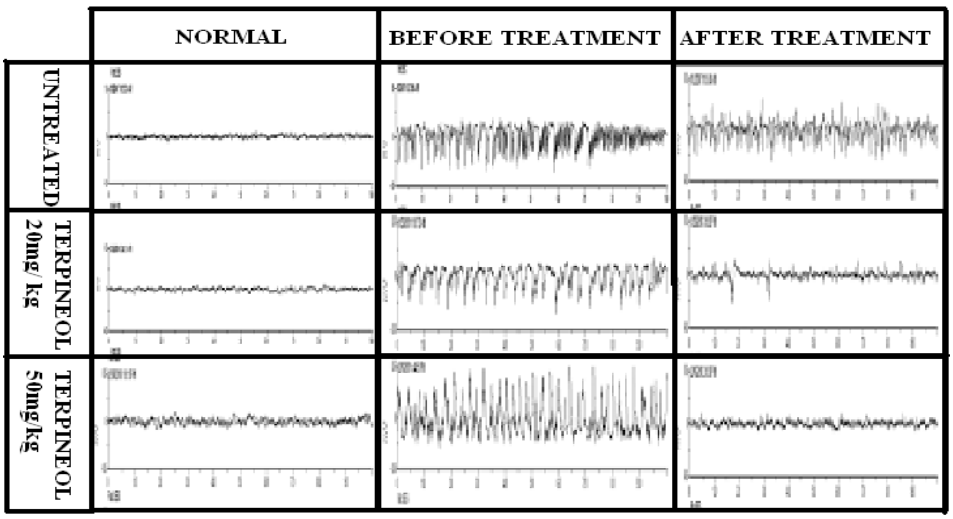

Figure 2. Portion of EEG wavelength taken via radio telemetry system from each group during normal, before treatment and after treatment

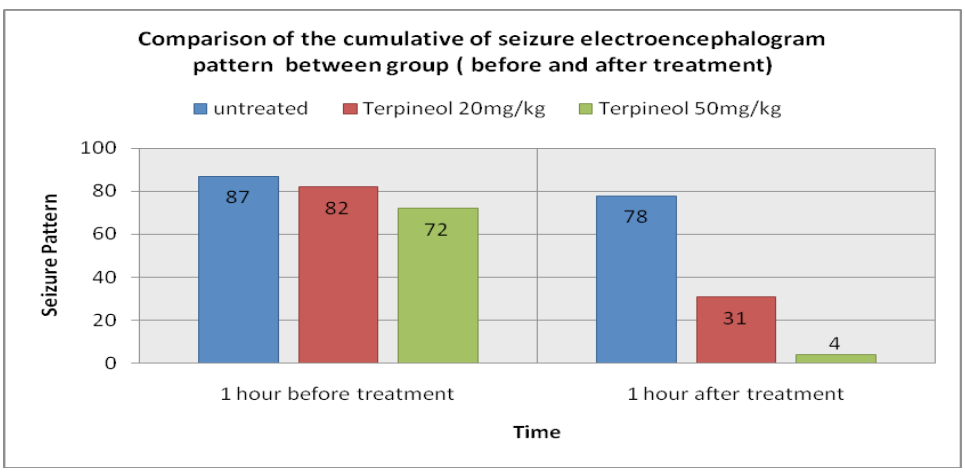

Figure 3. Total seizure electroencephalogram (EEG) pattern for experimental groups

Qualitative observation in this study had also indicated that terpineol may be beneficial to reduce motor activity in the rat and has some effect of anesthesia such as sleep when injected systemically. This may correlate with the effect of terpineol having one of these anesthesia activities (Moreira *et al*., 2001). Qualitatively, we have also found that terpineol may be possible to enhance the survivability in the rat with seizure but due to a limited number of animals used in this experimental study, the result obtained was cogent.

The rats in untreated group died at the end of experiment and were a common occurrence in the kainic acid induced epilepsy rat. This is consistent with the previous study of kainic acid induced epileptic model. Rats usually will have continuous episodes of seizure and some studies indicated that rats died within 24 hours after kainic acid induced seizure. This may bedue to neuronal damage that occurs after induction by kainic acid (Berg *et al*., 1993).

The seizure activity was different between the groups and could be due to various individual factors of the rat. As described in the literature, the aim of the kainic acid induced epileptic model is to have repetitive convulsive motor seizure activities longer than 3 hours. Generally, an animal should have a minimum of 1 obvious seizure per hour and typically an animal will have 7 to 22 episodes of seizure every hour. Seizure activity may last for 72 hours after kainic acid injection (Dudek *et al*., 2006).

The mechanism of the terpineol to reduce seizure in this study is most likely the reversing of the effect of kainic acid through enhancement of GABA inhibition mechanism of the neuron. As mentioned earlier, kainic acid is an excitatory neurotoxin that was proven to reduce GABA inhibitory mechanism by disinhibiting the GABAB receptor (Haas *et al*., 1996). GABAB is presynaptic receptor for GABA, which is important in regulating the secondary messenger system. Activation of this receptor will decrease the neurotransmitter release. Kainic acid will inhibit the function of GABAB, resulting in constant activation of the receptor which leads to a decrease of GABA release, impaired GABA inhibition mechanism, excitation of the brain and finally, epilepsy. However, when rat was treated with terpineol, this effect was reversible, as evident of inhibition of seizure activity and seizure electroencephalogram. This could be due to the effect of terpineol by enhancing the GABA release and lead to the increase of the GABA inhibition effect. Further studies need to be conducted to confirm the enhancement of GABA inhibition effect by terpineol.

Conclusion

terpineol extracted from the *Myristica fragrans* Houtt. essential oil may be beneficial for the inhibition of behavioral seizure activity and seizure electroencephalogram. The antiepileptic effect of terpineol was correlated to the dose of terpineol as

used in this experimental study. Possible mechanism of antiepileptic properties of terpineol is by enhancing GABA inhibition mechanism.

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