J Health Scope.2012;1(1):44-48. DOI: 10.5812/jhs.4816

Health Scope
International Quarterly Journal
www.jhealthscope.com

Comparing Anticonvulsive Effect of Melissa Officinalis` Hydro-Alcoholic Extract and Phenytoin in Rat

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ARTICLE INFO

Article type:
Original Article

Article history:
Received: 15 Feb 2012
Revised: 11 Mar 2012
Accepted: 28 Mar 2012

Keywords:
Melissa officinalis
Seizures
Pentylenetetrazole
Phenytoin
Rats

ABSTRACT

Background: Epilepsy originates from brain function disorders that might appear in the forms of overt disorders or fainting (losing consciousness), abnormal motional activities, behavioral abnormalities and sensational impairment and/or disorders in autonomic performance; all these symptoms are observable in early sleeping stages.

Objectives: Epilepsy is one of the most common neural disorders in human beings and with regard to the conducted studies , the *Melissa officinalis* plant has been used to treat epilepsy disease. Therefore, in this empirical work the effect of pretreatment with hydro-alcoholic extract of this plant compared to Phenytoin in the prevention of the epileptic convulsions caused by Pentylenetetrazole was studied.

Material and Methods: In this research the following groups receive the following drug doses intraperitoneal : four groups received different concentrations of extract (25, 50 , 100 , 200 mg/kg body weight), the positive control group was tested by Phenytoin (5 mg/kg) and the negative control group was tested by normal saline. Data were analyzed by kruskal – Wallis test and Tukey test.

Results: Injection of 50 and 100mg of the extract per kilogram of the body weight during the30 minutes interval before the systemic injection of Pentylenetetrazole, resulted in delay in the average onset time of the clonic convulsion Seizures with respect to the control group ($P = 0.001$) and also delay in the average onset time of the tonic – clonic Seizures with respect to the control group ($P = 0.02$) and besides the rates of mortality in that group of animals which were pretreated with 50 and 100 mg concentrations of the extract per kg of body weight indicated a significant difference with respect to the control group ($P = 0.004$) . Mortality rate was 100 % in the negative control group,37.5 % in the50 mg/kg weight group and 12.5 % in the 100 mg/kg weight group and 12.5 % in the group treated by Phenytoin.

Conclusions: This study indicated that the hydro-alcoholic extract of the *Melissa officinalis* plant can cause helpful effects on Seizures induced by Pentylenetetrazole in rats. Therefore, further studies are needed to understand the extent and mechanism of these effects.

▸ *Implication for health policy/practice/research/medical education:*

The results of this article may be used by other investigators for future study about precise mechanism of anticonvulsive effects of this plant. If these results approved by other research, can be used with clinicians for prevention of seizure in human trails. These results can be used with other investigators for analysis of this extract for effective components.

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▸ *Please cite this paper as:*
Gorgich E, Komeili Gh, Zakeri Z, Ebrahimi S. Comparing Anticonvulsive Effect of Melissa officinalis` Hydro-Alcoholic Extract and Phenytoin in Rat. *Health Scope*. 2012; **1**(1):44-8.

1. Background

Epilepsy is the second most prevailing neurological disorder, just coming after brain stroke (1-3).Epilepsy prevalence is reported to be between 1-2 percent of the population in different societies; the most common emergence of this syndrome in children is tonic-clonic seizure referred as "grand mal epilepsy" (4-6). Epilepsy in fact originates from disorder in brain function that might appear in the forms of overt disorder or fainting (losing consciousness), abnormal motional activities, behavioral abnormalities and sensational impairment and/or disorder in autonomic performance; all these symptoms are observable in early sleeping stages (7, 8). Epilepsy can be divided into two main types: generalized seizure and partial or focal seizure. Generalized seizure is recognized with extremely severe neuron discharges all over the brain (in cortex, in deeper parts of cerebral lobes and even in brainstem and thalamus). Also, some discharges all along the routes are transferred to spinal cord and lead to generalized tonic convulsions in entire body, which is called "tonic-clonic seizure" (9). Focal epilepsy can nearly damage any part of the brain including limited regions of the cortex up to deep formations of cerebral lobes and brainstem (9). In this paper, grand mal seizure will be studied, which is also referred to "large epilepsy" and "generalized tonic-clonic seizure". Administration of herbal medicines has been widely taken under consideration due to chronic nature of epileptic diseases and abundant complications and side effects arising from taking anti-epileptic drugs (10, 11). In Iranian traditional medicine, the herb " *Melissa officinalis*" has been used for curing diseases such as epileptic seizures. *Melissa officinalis* is a grassy, scented, and durable plant, having quadrilateral stem and quite glandular or simple lint (10). This herb is indigenous to Mediterranean regions but is dispersed in Europe and Asia and grows in Iran as well. Leaves of this plant have 0.1 - 0.25% of oily essence. This oily essence is known as "Melissa essence" and contains 0.5% of aldehyde and terpene alcohol. The other compositions include phenol acids, tri-terpenes, tannins, and flavonoids. Leaves of this herb are used in traditional medicine as perspiring agent, stomach stimulator, anti-spasm, anti-bloat, anti-convulsion, anti-hysteric seizures, anti-nausea, gum strengthening, and also, for treatment of many other illnesses (3, 12-14). Thus, this research is devoted to investigation of pre-treatment effect of hydro-alcoholic extract of *Melissa officinalis* herb on preventing epileptic seizures progress caused by 90 mg/kg overdose of Pentylenetetrazole (PTZ) in rats.

2. Materials and Methods

Animals and test conditions: Mature male rats of Wister race weighing approximately 200 ± 30 gr were used in the current research. The rats were kept in Animals Room of Zahedan University of Medical Sciences. According to former studies, 8 animals were selected for each group and preserved under 12 hours of illumination and 12 hours of darkness at 21-25 °C with access to water and standard prepared food (concentrated). Every rat was used for testing only once. All tests were performed between 8 to 14 hours. Extraction preparation: The plant was identified and confirmed by Herbarium Committee of Biology Group of Sistan and Baluchistan University. 10 mg of milled powder of the herb was then put inside a small thread bag using Soxlet apparatus; the bag was fastened and another similar bag was prepared in the same way. The sample bags were placed in Soxlet apparatus and extraction started; the resulting extract was filtered after 24 hours, then poured onto plate and placed in oven. The dried extract was cut from the plate after 24 hours and dissolved in a certain amount of normal saline to be injected into rats' bodies.

2.1. Convulsion Induction

pentylenetetrazol (PTZ) is used for inducing convulsions. PTZ was systematically injected with 90 mg/kg weight dose. The respective concentration was suitable for creating epileptic seizures and caused animal's death in 90% of cases. The time interval between extract and PTZ injections was considered to be 30 minutes. The rats were under observation at least for 30 minutes following PTZ injection. The epileptic responses of animals were categorized as follows:

Zero stage (no response), stage 1 (starting time of clonic seizures), stage 2 (number of clonic seizures) (*Table 1*), stage 3 (starting time of tonic seizures), stage 4 (duration of generalized tonic-clonic seizures) (*Table 2*), stage 5 (death time). Impact degree of the extract on prevention from epileptic symptoms depended on the following variables: lengthening of starting time of clonic seizures, number of myoclonic seizures, starting time of tonic-clonic seizures, durability interval of tonic-clonic seizures and mortality of animals.

2.2. Test Groups

In this step, the rats were divided randomly in six groups (8 rats per each group) and studied as described below: positive control group (phenytoin 5 mg/kg weight), negative control group (normal saline), treatment groups

with different doses of extract (25, 50, 100 and 200 mg/kg weight). Volume of the injected liquid was nearly the same for all groups, equal to 0.5 ml for each animal.

2.3. Statistical Analyses

Data were analyzed by KRUSKAL_WALIS test to compare averages of occurrence time of different stages; TUKEY test was also utilized for binary comparison of occurrence time averages. The study was conducted in the Physiology laboratory of School of Medicine, and was approved by Medical Ethics Committee of Zahedan University of Medical Sciences.

3. Results

This research was intended to investigate the effect of *Melissa officinalis* extracton epileptic seizures in laboratory animal pattern. Adjusting or lowering the epileptic effects of PTZ on lab rats by a medicine is the best way to assess its anti-epileptic performance. In this study, injection of 50 and 100 mg/kg weight doses of extract 30 minutes before systematic injection of PTZ caused a delay in average starting time of clonic seizures ($P = 0.001$) and tonic-clonic seizures ($P = 0.02$) compared to control group (*Table 3* and *Table 4*). On the other hand, mortality rate in animal groups pretreated with 50 and 100 mg/kg weight doses of extract indicated a statistically significant decrease compared to that of control group ($P = 0.004$). Death percentage was 100% in negative control group, 37.5% in the group with injection of 50 mg/kg weight hydro-alcoholic extract, 12.5% for the group with 100 mg/kg weight injection of hydro-alcoholic extract, and 12.5% for the positive control group (*Table 5*).

Table 1. Comparing the Number of Clonic Seizures in the Groups Under Study

	Mean, No	S.D [b]
positive control	24.63	15.43
negativecontrol	55.12	28.06
Extract25 mg/kg	45.50	24.59
Extract50 mg/kg	27.75 [a]	17.46
Extract100 mg/kg	32.13 [a]	18.44
Extract200 mg/kg	32.63 [a]	23.80

[a] $P < 0.05$ compared to negative control group
[b] Abbreviation: SD, Standard deviation

Table 2. Comparing Tonic-Clonic Seizures Duration in the Groups Under Study

	Mean, No	S.D [b]
positive control	167.12	27.7
negative control	790	95
Extract25 mg/kg	588.75	134.4
Extract50 mg/kg	460.75	141.6
Extract100 mg/kg	185 [a]	65
Extract200 mg/kg	241.75 [a]	122.4

[a] $P < 0.01$ compared to negative control group
[b] Abbreviation: SD, Standard deviation

Table 3. Comparing the Clonic Seizures Onset in the Groups Under Study

	Mean, No	S.D [b]
positivecontrol	163	17.14
negativecontrol	71.62	4.53
Extract25 mg/kg	93.75 [a]	20.84
Extract50 mg/kg	108.62 [a]	36.17
Extract100 mg/kg	143 [a]	40.45
Extract200 mg/kg	116.87 [a]	22.96

[a] $P < 0.001$ compared to negative control group
[b] Abbreviation: SD, Standard deviation

Table 4. Comaring the Tonic-Clonic Seizures Onset in the Groups Under Study

	Mean, No	S.D [b]
positivecontrol	157.37	44.86
negativecontrol	107.12	23.55
Extract25 mg/kg	168.12 [a]	65.86
Extract50 mg/kg	191.75 [a]	66.62
Extract100 mg/kg	286.40 [a]	57.07
Extract200 mg/kg	244.71 [a]	73.95

[a] $P < 0.05$ compared to negative control group
[b] Abbreviation: SD, Standard deviation

Table 5. Mortality Rate in the Groups Under Study

	Animal Number	Mortality Rate, NO. (%)
Negative control	8	8 (100)
Positive control	8	1 (12.5)
Extract 25 mg/kg	8	4 (50) [a]
Extract 50 mg/kg	8	3 (37.5) [b]
Extract 100 mg/kg	8	19 (12.5) [b]
Extract 200 mg/kg	8	4 (50) [a]

[a] $P < 0.05$
[b] $P < 0.01$ compared to negative control group

4. Discussion

Injection of the extract within 30 minutes before injection of Pentylenetetrazole, resulted in delay in the average onset time of the clonic and tonic – clonic convulsion Seizures with respect to the control group. Rates of mortality in that group of animals which were pretreated with 50 and100 mg/kg weight concentrations of the extract indicated a significant decrease compared to control group. Mortality rate was 100% in the negative control group, 37.5% in the 50 mg/kg weight group, 12.5% in the 100 mg/kg weight group and 12.5% in the Phenytoin group. Also, administration of this extract caused decrease in number and duration of convulsion seizures.In the current study, the herbal extract was injected into the rats' peritoneum as pretreatment and before injection of PTZ. The results indicated that the extract affects the seizure trend caused by PTZ injection and balanced its different stages. Protective impact of extract on some stages of epileptic process such as reduction in number of animal deaths

was analogous to effect of technical twin treatment. However, it did not considerably affect some other stages of epileptic trend including number of clonic seizures and duration of tonic-clonic attacks. As mentioned in the results, generally in studies and tests, injection of 50 and 100 mg/kg weight doses of extract within 30 minutes interval prior to systematic PTZ injection would lead to a delay in average starting time of epileptic clonic seizures compared to control groups (P=0.02) and also a delay in average starting time of tonic-clonic seizures in comparison with control group (P = 0.2). On the other hand, death percentage was 100% in physiology serum group, 37.5% in the group with injection of 50 mg/kg weight hydro-alcoholic extract, 12.5% for the group with 100 mg/kg weight injection of hydro-alcoholic extract, and 12.5% for technical twin group. The above results are suggesting the fact that hydro-alcoholic extract of *Melissa officinalis* lead to a delay in starting time of clonic seizures and starting duration of tonic-clonic attacks, and, protective impact of the extract in some stages of convulsion process including reduction in death number was similar to effect of technical twin medicine. Regarding occurrence of epilepsy, it is believed that any agent causing change of potential difference between interior and exterior of neuron membrane can induce epilepsy (15).Under this assumption, it can be asserted that the extract is able to somewhat stabilize the cellular membrane, and as a result, reduces the excitability effect of neurons, which leads to alleviation of epilepsy symptoms. From another point of view, taking into account the role of PTZ and its influence mechanism which causes an increase in transfer of neural intermediates in the receptors (16), the chemical molecules of the extract might have been involved in this transfer, leading to its reduction and consequently weakening of the epilepsy induced by PTZ injection; however, proof of this hypothesis requires further studies. The available information concerning chemical composition does not guide us on what substance is responsible for the anti-epileptic behavior of this herbal extract.

5. Conclusion

In the subsequent steps of this research, pharmacological examinations are required for separating the useful compositions of the extract or avoiding any kind of its poisonous and adverse effects. According to results of the current study, it can be stated that hydro-alcoholic extract of *Melissa officinalis* positively affects prevention of the occurrence of seizure symptoms caused by PTZ injection in rats, and finding the precise mechanism needs to be investigated more deeply(17). Yet, the studies conducted on chemical compositions of other medical herbs are indicating the presence of caffeic acid, which is presumed to have anti-epileptic properties and can be also found in the extract of the herb.

Acknowledgements

We are grateful to Vice Chancellor for Research of Zahedan University of Medical Science for his/her financial support and Laboratory of physiology staff for their assistance.

Authors' Contribution

Elaheh Gorgich (40%), Gholamreza Komeili (40%), Zahra Zakeri (10%), and Soheila Ebrahimi (10%).

Financial Disclosure

None declared.

Funding/Support

None declared.

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