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# EVALUATION OF ANTICONVULSANT ACTIVITY OF *MENTHA PIPERITA* LINN AQUEOUS AND ETHANOL EXTRACT IN WISTAR ALBINO RATS.

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## ABSTRACT

Our main objective of this study is to find the anti-convulsive activity of *Mentha piperita Linn* aqueous and ethanol extract in waster albino rats. The study was started after obtaining the institute animal ethics committee approval. The Wister albino rats of either sex with 200 - 250gm was obtained from the central animal house of the institution. For MES induced seizure- Rats were divided into 4 groups. Each group was having 6 rats. MES seizure was electrically induced by means of an Electro convulsiometer. 150mA current was delivered transauricularly for 0.2sec, to produce tonic hind limb extension in animals. Suppression of tonic hind limb extension was taken as a measure of efficacy of the drugs in this test. Onset and duration of tonic hind limb extension was noted and compared in all groups. The aqueous and ethanol extract (400mg/kg & 400 gm/kg), normal saline and standard drug (phenytoin) was administered to test, control and standard groups by orally respectively, 60 minutes before application of MES. For PTZ induced seizure- PTZ (60mg/kg, ip.) was used to induce generalized clonic or tonic convulsion. After the injection of PTZ, all the rats were observed for 30 min to detect the onset of generalized clonic or tonic convulsion. The onset of convulsion and the number of animals convulsing within the observation period were noted. The ability of the plant extract to prevent or delay the onset of the convulsion in the animals was taken as an indication of anticonvulsant activity. Diazepam (4mg/kg) was used as the standard drug. Percentage of seizure protection and mortality was measured. All the drugs were given orally. Values were expressed as mean ± SEM from 6 animals. Statistical difference in mean was analyzed by one way ANOVA. It was found to be significant in both MES & PTZ induced animal models. *Mentha piperita linn* aqueous and ethanol extracts were found to be effective in decreasing MES & PTZ induced seizures.

**KEY WORDS:** **Mentha piperita linn, MES seizure, PTZ induced seizure, hind limb extension.**

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# INTRODUCTION

Epilepsy is the term used to describe a group of disorders characterized by recurrent spontaneous seizures that apparently result from complex processes involving several neurotransmitter systems such as the glutaminergic, cholinergic, and gabaergic systems [1]Actual estimations of the prevalence rate for epilepsy are 1-2% of the world population[2], and despite the fact that there are a considerable number of classic and more modern anticonvulsant drugs available for the pharmacological treatment of epilepsy patients worldwide, seizures remain refractory in more than 20% of the cases [1]Moreover, all current drugs have a synthetic origin that causes severe side effects and leads to dependency development [3] So many herbal products have been tried in the treatment of epileptic models to prove their action against epilepsy. One among them is *Mentha piperita linn*. *Mentha piperita* belongs to the family *Labiatae*. It is an herbaceous rhizomatous perennial plant growing to 30–90 cm (12–35 in) tall, with smooth stems, square in cross section. The rhizomes are wide-spreading, fleshy, and bare fibrous roots [4]The leaves are 4–9 cm (1.6–3.5 in) long and 1.5–4 cm (0.59–1.6 in) cm broad, dark green with reddish veins, and with an acute apex and coarsely toothed margins [5]The leaves and stems are slightly hairy. The flowers are purple, 6–8 mm (0.24–0.31 in) long, with a four-lobed corolla about 5 mm (0.20 in) diameter; they are produced in whorls around the stem, forming thick, blunt spikes. It has the chemical constituents like menthol, menthone, 1,8-cineole, methyl acetate [6]It is used as stimulant [7]stomachic, carminative and anti anemic [8,9]The anticonvulsive effect of *Mentha piperita* mixed with other herbs was found in one study [10] In the wider frame of the ongoing research on the treatment of epilepsy, the present study attempts to evaluate the possible anticonvulsive property of *Mentha piperita linn* aqueous and ethanol extract isolated from the leaves in MES & PTZ induced convulsive models. We would like to analyze the anticonvulsive activity of *Mentha piperita* alone in this study.

# MATERIALS AND METHODS

### *Plant Material*

The fresh leaves of *Mentha piperita linn* was collected from the local gardens of Coimbatore district and it was authenticated by a botanist for its botanical idendity (Mr. P Parameswaran, Voucher No 5046).. Aqueous extract and ethanol extract of the leaves of *Mentha piperita linn* was used in this study. The extract was prepared by maceration procedure after identification.

### *Animals*

48 Wistar Albino rats of the either sex weighing 200-250 gm were used in the study. They were fed with standard animal pellets, tap water and ad libitum. The temperature of animal house was maintained at 22 ± $2^0$C in the standard cages with 12hrs light/dark cycle. Animal ethics committee approval was obtained before the start of the study ((KFMSR/IAEC/2013/008).All efforts were made to minimize animal suffering and to reduce the number of animals used [11].

### *Acute toxicity study*

Acute Oral toxicity study was carried out according to the OECD 425 guidelines. Five Swiss Albino mice of either sex were fasted prior to the dosing for 3–4 h. Extract of 2,000 mg/Kg was administered orally to one animal and observed at least once during the first 30 minutes after dosing, periodically during the first 24 hours with special attention given during the first 4 hours, and daily thereafter, for a total period of 14 days. The importance was given for its changes in skin, fur, eyes and mucous membranes, and also for its respiratory, circulatory, autonomic functions, somatomotor activity and behavioural pattern. After 24hrs, the remaining four animals were given the test compound in the same dose and observed for14 days [12].

### *Methods*

The aqueous extract and ethanol extract of *Mentha* piperita were studied for its anticonvulsant effect on maximal electroshock-induced seizures (MES) and pentylenetetrazole (PTZ) induced seizures in Wister albino rats.

### *MES induced seizures*

The electrical stimulus (150mA; 50 Hz; 0.2sec duration) was applied through ear clip electrode using electro convulsiometer. The onset and duration of hind limb extension was noted. A complete abolition of hind limb tonic extension was considered as 100% seizure protection[13]. Phenytoin 25mg/kg b.w was used as the standard drug [14]All the rats were divided into 4 groups (n=6). Group 1 received 0.5ml of normal saline, Group 2 received standard drug, Group 3 received aqueous extract (400mg/kg), and Group 4 received ethanol extract (400mg/kg). All the drugs were given orally.

### *PTZ induced seizures*

PTZ (60mg/kg, i.p.) was used to induce absence seizures. The standard and test drugs were administered orally 60 min before the intraperitoneal injection of PTZ (60 mg/kg b.w). After the injection of PTZ, all the rats were observed for 30 min to detect thelatency to the first forelimb clonus, and number of animals convulsed. . The onset of convulsion and the number of animals convulsing within the observation period were noted. The ability of the plant extract to prevent or delay the onset of the convulsion in the animals was taken as an indication of anticonvulsant activity [15]Diazepam (4mg/kg,.b.w) was used as the standard drug [16]All the animals were divided into 4 groups (n=6). Group 1 received 0.5ml of normal saline, group 2 received standard drug, group 3 received aqueous extract (400mg/kg), and group 4 received ethanol extract (400mg/kg). All the drugs were given orally.(Since it is a simple observational study, and not a comparative study single dose was used.. In the next phase we will be using multiple doses to have a elaborate study )

### *STATISTICAL ANALYSIS*

Tukey test was used. Results were expressed as Mean ± SEM, and the data obtained was analyzed using one-way analysis of variance (ANOVA). The level of significance was set at $P<0.05$.

## RESULTS

### *Acute toxicity study*

No adverse effect and mortality was detected with the dose of 2000mg/kg of b.w. of aqueous and ethanolic leaf extract of *Mentha piperita linn* in all the five mice. The animals were alive, healthy and active for all the observational period. There was no significant weight change during the period of all the 14 days (for both aqueous and ethanol extract separately). So the LD 50 was considered as > 2000mg/kg. Table 1 shows the anticonvulsant activity of aqueous and ethanolic leaf extract of *Mentha piperita* in Maximal Electro Shock (MES) induced convulsion in Wistar albino rats. Phenytoin blocked the MES induced convulsion in the dose of 25 mg/Kg b.w. in all the Wistar albino rats. There was significant increase in the time taken for the onset of Tonic Hind Limb Extension (THLE) in the aqueous and ethanolic extract of *Mentha piperita* leaf extract and there was significant reduction in the duration of Tonic Hind Limb Extension in aqueous and ethanolic extract of 400 mg/Kg. The mean time taken for the onset of THLE in Group I is 2.9 ± 0.18 seconds, Group III (400mg/Kg of aq.extract) is 7.8 ± 0.15 seconds ($p < 0.001$), and Group IV (400mg/Kg of ethanolic extract) is 8.2 ± 0.32 seconds ($p < 0.001$). The mean duration of THLE of Group I is 8.7 ± 0.83 seconds, Group III (400mg/Kg of aq.extract) is 5.7± 0.2Group IV((400mg/Kg of ethanolic extract) is 4.7±0.33 ($p < 0.001$). In the dose of 400 mg/Kg of aqueous and ethanolic extracts blocked the MES induced convulsion .The duration of post ictal depression also was significantly reduced in both aqueous and ethanolic extract groups.

**Table 1**
***Effect of Mentha piperita Linn in MES induced seizures***

| S.NO | Groups | Onset of hind limb extension(HLE) ± SEM (Seconds) | Duration of hind limb extension ± SEM (Seconds) | Duration of Post ictal depression (PID) ± SEM (Seconds) |
|---|---|---|---|---|
| 1 | I (Control) | 2.9±0.18 | 8.7±0.83 | 94.6±3.73 |
| 2 | II (Phenytoin) | -*** | -*** | 40.1±2.49*** |
| 3 | III (Aqueous extract) | 7.8±0.15*** | 5.7±0.2* | 61.2±2.21*** |
| 4 | IV (Alcohol extract) | 8.2±0.32*** | 4.7±0.33** | 42.03±1.9*** |

***n= 6, *p<0.05, ***p<0.005 compared to control group (One way ANOVA).***

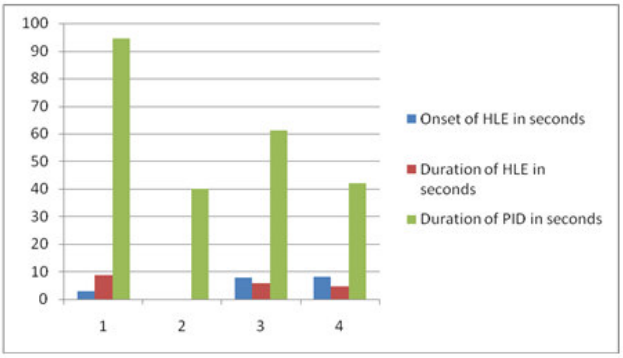
Table 2 shows the anticonvulsant activity of aqueous and ethanolic leaf extract of *Mentha piperita linn* in pentylenetetrazole (60mg/Kg ip, b.w.) induced convulsion in Wistar Albino rats. Diazepam (4mg/Kg b.w.) blocked the PTZ induced convulsion in all the six rats. Mean latency period of convulsed animals in control rats is 73.7±2.44 seconds, group III (aqueous extract) is 334.6 ±70.69 seconds ($p<0.01$) and group IV(ethanolic extract) is 401.905 ± 84.72 seconds ($p<0.001$). The percentage of protection of convulsion with the dose of 400mg/Kg of aqueous extract is 33% (number of animals convulsed is 4/6) and with 400mg/Kg of ethanolic extract is 66.7% (number of animals convulsed is 2/6).

**Table 2**
***Effect of Mentha piperita Linn in PTZ induced seizures***

| S.NO | Groups | Mean Latency period± SEM (seconds) | Number of animals convulsing | % of protection |
|---|---|---|---|---|
| 1 | I (Control) | 73.7±2.44 | 6/6 | 0% |
| 2 | II (Diazepam) | -*** | 0/6 | 100% |
| 3 | III (Aqueous) | 334.6±70.69*** | 4/6 | 33.3% |
| 4 | IV (Alcohol) | 401.905±84.72*** | 2/6 | 66.7% |

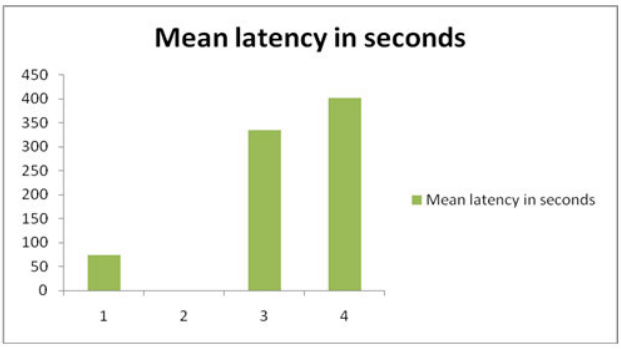
***n=6, *p< 0.05, ***p<0.005 compared to control.***

**Figure 1**
***Effect of Mentha piperita Linn in MES induced seizures***




***n= 6, *p<0.05 compared to control group (One way ANOVA).***

**Figure 2**
***Effect of Mentha piperita Linn in PTZ induced seizures***




***n=6, *p< 0.05 compared to control. (One-way ANOVA)***

## DISCUSSION

Epilepsy is a major neurological disorder and up to 5% of the world population develops epilepsy in their lifetime [17]The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy ()[18-20].*Mentha piperita* is an herbaceous rhizomatous perennial plant. Peppermint is commonly used to soothe throat, treat symptoms such as nausea, vomiting, abdominal pain, indigestion, irritable bowel, and bloating ()[14,21]. Previous studies have shown that menthol engages in synergistic excitation of $\gamma$-amino butyric acid (GABA) receptors and sodium ion channels resulting in analgesia so it may prove valuable as a leading structure for the synthesis of drugs that target multiple receptors involved with a number of pharmacological effects [22]In that experimental protocol, *M. piperita* essential oil (EO) was the most effective form of all other, as animals pretreated with it experienced no seizures at all after the administration of PTZ. Furthermore, the survival of animals after the treatment was 100%, showing that it is extremely tolerable by the animals and has the best anticonvulsant results[23,24]The administration of the tested EOs delivered different results to the PTZ-induced seizures. *M. piperita* EO gave very encouraging results as, despite that the PTZ dose was considered lethal, all animals of the specific group managed to survive for 24 hours after the injection without experiencing any seizures at all. It is possible that the cause of this different antiepileptic action is the dominant component of menthol found in the oil [22] Further researches including the administration of the pure leading compound of EO lowering the development of PTZ-induced seizures thus providing possible anticonvulsant agents of a more natural origin [10] *Mentha arvensis Linn (Labiatae),* a plant used in traditional medicine in India was explored for its pharmacological activities [25] It was observed that the methanolic extract of the leaves showed potentiation of pentobarbitone induced sleeping time. The sedative activity was based on the fact that the CNS depressant drugs potentiate a sub-hypnotic dose of pentobarbitone [26,27] All the above studies proved that, *Mentha piperita* has sedative as well as anticonvulsive property in the animal models. So we tried with both aqueous and ethanol extract of *Mentha piperita linn* in MES and PTZ animal models. In our study, in the MES induced seizure models, the duration of onset of Tonic hind limb extension (THLE) was increased in both aqueous and ethanol extracts administered rats. The mean duration of the Tonic hind limb extension was decreased in both aqueous and ethanol extracts treated animals. The mean duration of post-ictal depression (PID) was significantly decreased in rats of both the groups (Group III, Group IV). All these parameters were significantly ($p < 0.05$) changed both in aqueous and ethanol extracts compared to the control, that too in ethanol extract (Group IV) showed more significant ($p < 0.005$) changes in the parameters. In PTZ induced convulsive models, the mean latency period was significantly increased with aqueous and ethanol extracts. The number of animals convulsed also was less compared to the control. With aqueous extract the animals convulsed was 4/6, and with ethanol extract it was 2/6. The percentage of protection was significant ($p < 0.05$) with aqueous (33.3) and very significant with ethanol ($p < 0.005$) extract. In the MES & PTZ convulsive models, all the results of the parameters were significant with aqueous extract and ethanol extract of *Mentha piperita linn* but more significant with ethanol extract. This may be because of the sedative property of alcohol [28] which would have potentiated the action of *Mentha piperita linn*. Further studies are needed to provide more details on this.

## CONCLUSION

From the above study, it is clear that *Mentha piperita linn* increased the duration of onset of THLE and decreased duration of hind limb extension in both aqueous and ethanol extract which is statistically significant ($p<0.05$). It also decreased the duration of post-ictal depression which also statistically highly significant. The latency period in PTZ induced seizures also increased significantly with both aqueous and ethanol extracts. Hence, this study proved that both the aqueous and ethanol extracts of *Mentha piperita linn* in MES and PTZ induced seizures, have a seizure reduction activity.

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