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EVALUATION OF NEUROPROTECTIVE POTENTIAL OF *MENTHA SPICATA IN REVERSAL OF NEUROTOXICITY INDUCED COGNITIVE IMPAIRMENT*

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ABSTRACT

The cognitive impairment seen in epileptics may be a consequence of either the epiptogenic process alone or it could manifest on account of the use of antiepileptic drugs that cause cognitive impairment as an adverse effect or both. Thus there is need for newer drugs that can suppress the epileptogenesis by acting to prevent the development of cognitive impairment. Mentha spicata,an Indian medicinal plant has marked antioxidant property. The effect of seven days pretreatment of 500mg/kg of alcoholic extract and in combination of 12.5 mg/kg Phenytoin and 500mg/kg extract, and 25 mg/kg Phenytoin and 500mg/kg extract.administered orally to mice was evaluated on MES induced seizure, cognitive deficit. Mentha spicata did not shown significant protection against MES (maximal electro shock) induced convulsions. When only extract was administered there was Tonic extension phase was observed and insignificant decrease of recovery. This result indicates that ethanolic extract of Mentha spicata does not possess anti-epileptic activity and it can't protect animal from MES. The p value one way ANOVA followed by Dunnett's test in MES, p< 0.0001. From the Elevated plus maze model and passive avoidance it may be concluded that, the Ethanolic extract of Mentha spicata does not show the significant antiepileptic activity and extract does not also have significant effect on AED induced cognitive impairment.

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KEYWORDS : **:** *Antiepileptic; Mentha spicata; Leaves; Maximal electroshock; Cooks pole climbing; Elevated plus maze.*

INTRODUCTION

Patients with epilepsy often experience cognitive dysfunction. Multiple factors can adversely affect cognition in epilepsy, including the etiology of the seizures, cerebral lesions acquired before the onset of seizures, seizure type, seizure frequency,

duration, and severity, intraictal and interictal physiologic dysfunction, structural cerebral damage caused by repetitive or prolonged seizures, psychosocial factors, and sequelae of treatment for epilepsy, including antiepileptic drug and epilepsy surgery. All these interrelated factors make complex contributions to cognitive deficits.[1] Cognitive impairment seen in epileptics may be a consequence of either the underlying epiptogenic process alone or it could manifest on account of the use of antiepileptic drugs that cause cognitive impairment as an adverse effect or both. AEDs can adversely affect cognitive function by suppressing neuronal excitability or enhancing inhibitory neurotransmission.

The main cognitive effects of AEDs involve attention/vigilance, psychomotor speed, and secondary involvement of other cognitive functions e.g. memory.The effects of AEDs on cognition are especially significant since AEDs are often be selected based on both traditional measures of treatment effectiveness such as efficacy and tolerability, and their negative neuropsychological side effects. Consequently, the neuropsychologist should attempt to determine the potential effects of AEDs on cognitive performance, as well as the patient's subjective ception of performance, which can alsbe mediated by the mood and affective state. Individuals older than 65 years are more susceptible to the cognitive effects of AEDs due to both pharmacodynamic and pharmacokinetic factors.Thus there is need for a newer drug which is safe & suppresses epileptogenesis and prevents cognitive decline.[2-3.] The term epileptogenesis refers to the transformation of the brain to a long-lasting state in which recurrent, spontaneous seizures occur. The AEDs currently used to treat patients with epilepsy affect seizure expression but a better approach in the future would be to develop agents that prevent epileptogenesis. [4] Main cause of cognitive impairment is dementia which defined as significant memory impairment and loss of intellectual functions, interferes with the patient's work, usual social activities or relationship with others and thus, it is a common and devastating public health problem.[1]

Mentha spicata L., also known as spearmint belongs to the Lamiaceae family. In India, it is commonly called as pudina and is widely used in culinary preparations to add flavor and aroma. Mint oil is of economic importance and is widely used in pharmaceutical, cosmetic, food, confectionary and beverage industries. Spearmint oil contains monoterpenoids like carvone, limonene, menthone, menthol, pulegone, dihydrocarveol and *s*-carvone. Some of them were found to possess high antioxidant activity than tocopherol. The plant is also known for its ability to enhance memory. The boiled leaves extract is being used to relieve hiccup, flatulence, giddiness and as remedy for inflammation, bronchitis, and to control vomiting during pregnancy. Apart from being a stimulant and carminative, the mint plant is also known for its insecticidal, antimicrobial, antispasmodic and antiplatelet properties and ethyl acetate fractions of ethanol extract of *M.spicata* possess.[5]

MATERIAL AND METHODS

Animals – Wistar mice of either sex (20- 25 g) were housed in polypropylene cages at 25 ± 2 °c with a natural light-dark cycle and maintained on a daily scheduled of standard laboratory diet. Drinking water was supplied *ad libitum*. The experiments were conducted according to the Institutional Animal Ethical Committee (IAEC) (Approval Number- SPTM- IAEC/Oct- 10/02/006).

Collection and authentification of plant material*M. spicata* L. was commercially purchased from Shirpur, Dhule in month of March. It was identified and authenticated by Dr. N. M. Surana, Head of Botany Department, Govt. PG College, and Barmer.

Extraction

Alcoholic extract[6-7]- The shadow dried leaf powder of *M. spicata* (150g) was immersed in 500ml of 95% ethanol and the filtrate was collected for three times with constant stirring of the mixture at every 24 hrs interval of a 72 hrs total collection period (all the time 95% ethanol was used for obtaining the filtrate) and the final filtrate volume was found to be 1.2 litre. The extract was then concentrated under reduced pressure at 40 °C using vacuum

rotary evaporator. The yield of ethanol extract was 10.35g.

Drugs and Chemicals

Phenytoin was procured from Abbot Group of campany, made in India by Acme formulation Pvt. Ltd. Ropar road, Nalagarh, Dist. Solan.

Phytochemical Screening

Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, saponins and glycosides were carried out on thealcoholic extract using standard procedures.[12]

MES induced convulsions

Maximal electroshock convulsion model was used to evaluate the anticonvulsant activity of alcoholic extract Convulsion were induced in mice by delivering transaurical electroshock of 60 mA for 0.2 s by means of convulsiometer (INCO, Ambala, India), through a pair of crocodile ear clips. Five groups of mice (n = 6) each pretreated orally with varying doses Vehicle (Control group), Phenytoin 25 mg/kg (standard group), Phenytoin 12.5 mg/kg + Extract (500 mg/kg), Phenytoin 25 mg/kg + Extract 500 mg/kg, and Extract 500 mg/kg were tested after 30 min for MES seizure response. Duration of Recovery was noted in all groups. All the extract treated groups were compared with control in order to determine the significant anticonvulsant activity.[8-9]

Effect of extract on pole climb avoidance test

The Cook's pole climbing apparatus (Orchids scientific, Nasik) is completly digital design. This test is usually carried out to separate neuroleptics from sedatives and anxiolytics. Whereas sedative compounds suppress both avoidance and escape responding at approximately the same doses, neuroleptic drug reduce avoidance responding at lower doses than those affecting escape responding. This test can also be used to study learning ability and memory of animals. Thus the effect of drugs on memory and cognition of animal can be evaluated by using this method.

Setting of apparatus was made such that conditioning stimulus was delivered for 4 sec and unconditioned stimulus that was foot shock was delivered for 26 sec. Total time cycle of 30 sec. Foot shock were of 35 v. Avoidance response measure in terms of jumping of the animal on the pole on hearing the buzzer tone and before induction of foot shock and jumping of the animal after induction of foot shock considered as escape failure. Before treatment mice were trained for avoidance test and only those animals were selected who does not show escape failure. There was an inter trial interval of minimum 90sec. 10 trial were carried out on each animal every group. This test was carried out after 60 min of dose administration. Data was represented in terms of the number of avoidance relative to the respective vehicle control data.

Effect of extract on Elevated plus Maze

The standard elevated plus maze test is commonly used to assess anxiety like behavior in animals (rats/ mice). The elevated plus maze task approaches the conflict between the innate fear that rodents have of open areas verses their desire to explore new environment. The maze was elevated to height of 70 cm. After 30 min of the administration of dose of standard or test drug animals are subjected to test. Animals are placed in maze by facing them towards enclosed arm. Test is carried out for 5min of time period. During test period, the number of entries into and time spent in open arm as well as in enclosed arm were measured. Total numbers of arm entries are also measured. In this context, anxiety like behavior is measured by the degree to which the rodent avoids the enclosed arm of the maze. Procedure was conducted in sound attenuated room and observations were noted down from adjacent room.

RESULTS & DISCUSSION

Preliminory Phytochemical studies

The preliminary phytochemical screening of alcoholic extract shows the presence of alkaloids, glycosides, carbohydrates, flavonoids.[11]

Effect on MES induced convulsion When animals are treated with Phenytoin there was Absence of Tonic extension decrease in recovery time was significantly decreased as compared to control group. When only extract was administered there was Tonic extension phase was observed and insignificant decrease of recover

.

Table 1. Effect of *Mentha spicata* on MES induced convulsion

Groups (n=6)	Treatment	Recovery Time(Sec)	Phase
I	Vehicle	93.80 ± 12.82	Tonic extension
II	Phenytoin (25mg/kg)	18.67 ± 2.51	Absence of Tonicextension
III	Phenytoin+Extract (25+ 500 mg/kg)	46.17 ± 11.64	Absence of Tonic extension
IV	Phenytoin +Extract (12.5+ 500 mg/kg)	67 ± 16.79	Absence of Tonic extension
V	Extract (500 mg/kg)	93.83 ± 17.52	Tonic extension

Results are expressed as Mean (±SEM), one way ANOVA followed by Dunnett's test ($P < 0.0001$)

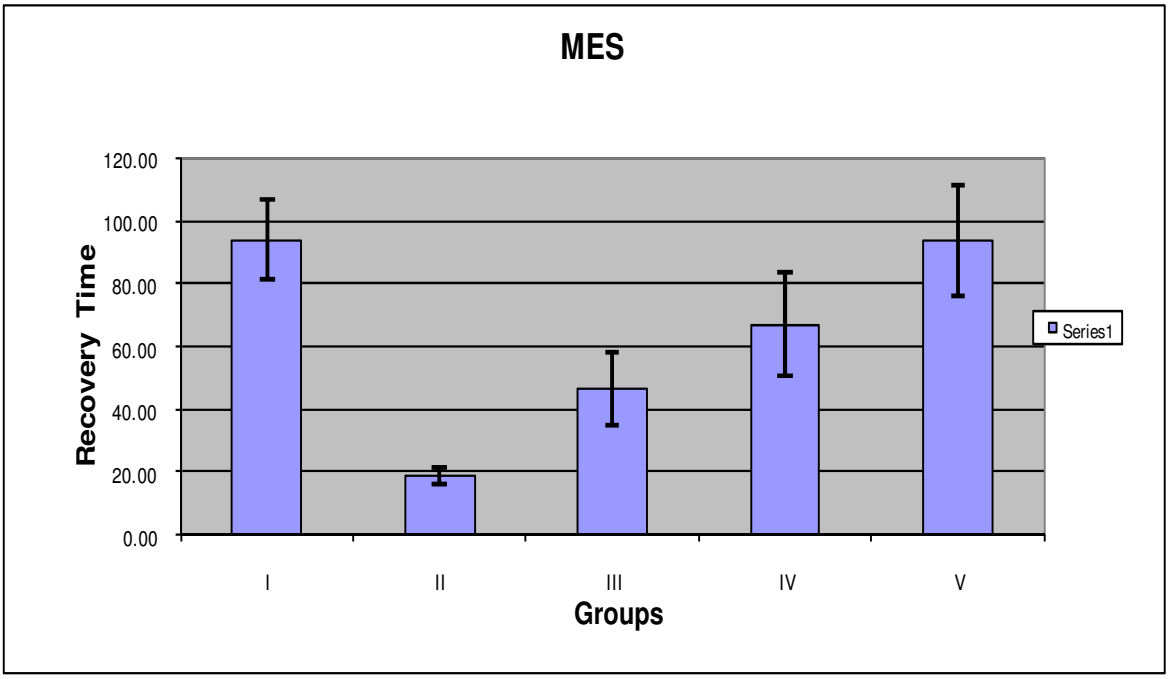

Figure 1: Effect of *Mentha spicata* on MES induced convulsion

Results are expressed in terms of difference calculated of mean time spent by animal in closed arm and open arm as well as mean of number of entries made in closed arm and open arm before given treatment to the animals and after completion of seven days treatment.

Table 2. Effect of *Mentha spicata* on Pole Climb Avoidance Test

Groups (n=6)	Treatment	No. Of Avoidance (Mean±S.D)
I	Vehicle	10.5 ± 0.29
II	Phenytoin (25mg/kg)	3.6 ± 0.68

III	Phenytoin+Extract (25+ 500 mg/kg)	11.17 ± 0.31
IV	Phenytoin +Extract (12.5+ 500 mg/kg)	10.5 ± 0.43
V	Extract (500 mg/kg)	11.0 ± 0.26

Results are expressed as Mean (±SEM)

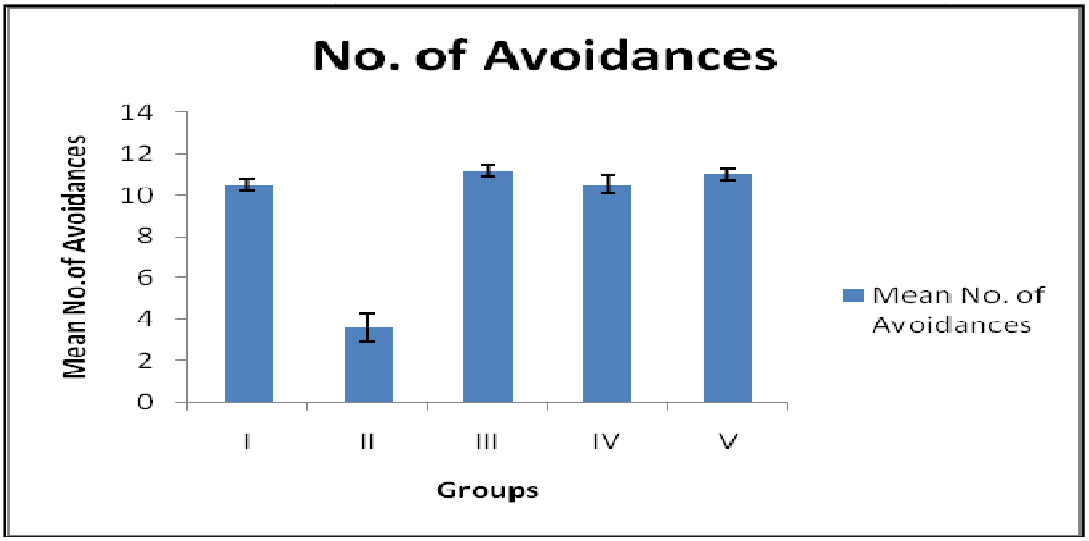

Figure 2. Effect of *Mentha spicata* on Pole Climb Avoidance

Effect of *Mentha spicata* on Elevated plus Maze:

Results are expressed in terms of difference calculated of mean time spent by animal in closed arm and open arm as well as mean of number of entries made in closed arm and open arm before given treatment to the animals and after completion of seven days treatment.

Table 3. Effect of *Mentha spicata* on Elevated plus Maze Test

Groups (n = 6)	Treatment	% Difference in open arm		% Difference in close arm	
		No. of entry	Time in seconds	No. of entry	Time in seconds
I	1 % CMC	0.0	4.69	0.0	-5.53
II	Phenytoin(25mg/kg)	-40.0	196.43	62.5	-85.60
III	Phenytoin +Extract(25+ 500 mg/kg)	40.0	23.21	0.0	-5.53
IV	Phenytoin +Extract(12.5+ 500 mg/kg)	20.0	259.47	-60.0	-31.32
V	Extract (500 mg/kg)	-33.3	- 2.63	-50.0.	1.15

DISCUSSION:

Above result from Table 1 indicate that ethanolic extract of *Mentha spicata* does not possess anti-epileptic activity and it can't protect animal from MES. When extract was administered in combination with Phenytoin, it can help in decreasing the dose of Phenytoin. As results show that there is decrease in time recovery even when

dose of Phenytoin was decreased from 25mg/kg to 12.5mg/kg. This indicates that herbal extract can't also help in decrease in dose of Phenytoin.

Results mentioned in table No. 2 indicate that when Phenytoin was administered the number of avoidance response was significantly decreased than that in control group. This implies that there was cognitive impairment in mice due to administration of Phenytoin. Then Phenytoin was administered in combination with ethanolic extract of *Mentha spicata*. Groups to which combination of Phenytoin and herbal extract was administered showed significant increase in number of avoidance as compared to standard group. This indicate that herbal extract have neuroprotective potential and thus it help in prevention of cognitive impairment occurred due to administration of Phenytoin.

From the Table No. 3 it was shown that Percentage difference calculated between No. of entry and time spend before treatment and after 7 days treatment

% Difference of open arm

[No. of entry or time after treatment (OA) - No. of entry or time before treatment (OA) =

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[No. of entry or time before treatment (OA)]

Note: Here, OA = Open arm

% Difference of Close arm

[No. of entry or time after treatment (CA) - No. of entry or time before treatment (CA) =

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[No. of entry or time before treatment (CA)]

Note: Here, CA = Close arm

From the above formula, the percentage difference can be calculated, table 5.1 shown that the group treated only with herbal *Mentha spicata* ethanolic extract show higher time spent in close arm as compared to other groups that means the *Mentha spicata* extract having anxiogenic activity but when ethanolic extract given in combination indicate that insignificant result. From the above result it was proved that

1. The Ethanolic extract of *Mentha spicata does not* show the significant antiepileptic activity.
2. Extract does not also have significant effect on AED induced cognitive impairment

From the present study it can be concluded that the ethanolic extract of *Mentha spicata* L. did not show significant result for neuroprotective potential against neurotoxicity induce cognitive impairment in MES seizures when compared with Control group. Ethanolic extract of leaves of Mentha spicata L. did not show significant antiepileptic activity.

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