

## ANTICONVULSANT ACTIVITY OF METHANOLIC EXTRACT OF *MELISSA PARVIFLORA* IN EXPERIMENTALLY INDUCED SWISS ALBINO MICE

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### ABSTRACT

Epilepsy is a disorder characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness. In the present study, anticonvulsant activity of methanolic extract of *Melissa parviflora* (MP) was evaluated against maximal electroshock (MES) and Pentylene tetrazol (PTZ) induced convulsions in Swiss Albino Mice. The extract showed substantial suppressed hind limb tonic extensions (HLTE) induced by MES and also exhibited protective effect in PTZ-induced seizures. In conclusion, the results of methanolic extract of MP show anticonvulsant effect in both the models, and suggesting their possible depressant action in the central nervous system.

**Keywords:** *Melissa parviflora*, Anti-convulsant activity, Maximal electro shock, Pentylene tetrazole, CNS.

### INTRODUCTION

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is the second most common chronic neurological condition seen by neurologists. Incidence of epilepsy in developed countries is approximately 50 per 100, 000 while that of developing country is 100 per 100, 000[1]. India is home to about 10 million people with epilepsy (prevalence of about 1%)[2]. The number of Epilepsy Specialists and Neurologists being very small in India, most people with epilepsy are being diagnosed and treated by non-specialists at both primary and secondary care levels. It is a disorder characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness[3]. It is a disorder of brain characterized by unpredictable and periodic occurrence of a transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons[4]. It has been observed that the presently available antiepileptic drugs are unable to control seizures effectively in as many as 25% of the patients[5]. The conventional antiepileptic agents like phenytoin, carbamazepine and sodium valproate carry with them several serious side effects notably neurotoxicity[6]. As majority of antiepileptic drugs are consumed long, concomitant administration of other drugs predisposes to the risk of drug interaction. However, newer antiepileptics like gabapentin, vigabatrin, lamotrigine, etc are used supplemental to the conventional agents[4]. Thus, it is necessary to investigate for an antiepileptic agent that is highly efficacious as well as safe in terms of drug related toxicity.

Ethno-pharmacological research on natural products can contribute to the discovery of new, safe active compounds with novel structure that may serve as leads to the development of new antiepileptic drugs. Several plants of the families Euphorbiaceae, Leguminaceae, Labiatae, Liliaceae, Gentianaceae, Solanaceae, and Umbelliferae are used for the treatment of epilepsy in Indian traditional medicinal system [7]. The aim of treating antiepileptic is not only to abolish the occurrence of seizures but also to lead a self sustained life. *Melissa parviflora* (MP) is pubescent or glabrate herb. The Muslim physician Avicenna recommended it for heart problems. Its main action is as a tranquillizer. It is a soothing and calming agent for stressed nerves. The herb is a common constituent of relaxants, nervine and sleeping aids throughout the world[8]. It is primarily indicated where there is dyspepsia associated with anxiety or depression. It relieves tension and stress reactions, is widely valued for its calming properties and has a tonic effect on the heart and circulatory system causing mild vasodilation of peripheral vessels, thus lowering blood pressure[8, 9]. Preliminary phytochemical analysis performed showed that the

tannin, saponin and flavonoid are the major components of the extract[10]. The flavonoids show various biological activities including antioxidant, anti-inflammatory activity, activity on coronary heart diseases and cytotoxic-antitumor activity [5, 7]. The plants containing saponins or flavonoids exhibit anticonvulsant activity[11, 12]. However, the anxiolytic properties of these flavonoids have been rarely investigated. Previously, the anxiolytic like effects of simple flavones, chrysin(5, 7-dihydroflavone) have been reported, which behaves as a competitive ligand of the benzodiazepine receptors[13]. Apigenin has been reported to show similar activity in mice with only slight sedative effect[14]. There is no earlier study present in the literature that has deciphered the anticonvulsant and CNS depressive activity of MP. Therefore, in view of the above observations we planned to study the anti-convulsant property of MP against experimentally induced convulsions in swiss albino mice.

### MATERIALS AND METHODS

#### Plant material

The plant MP (Unani name: Badranjboya) was purchased from Shamsi Dawakhana, Ballimaran, Delhi, India. The authenticity and identity was confirmed on the basis of classical description in Unani literature at Department of Ilmul Advia F/O Medicine (Unani), Jamia Hamdard, New Delhi and modern botanical information was established by matching with the specimens available at the National Institute of Science Communications. The wealth of Indian division, Dr K. Krishnan Marg, New Delhi, India. Moreover the procured specimens were authenticated by a Taxonomist, Department of Botany, Jamia Hamdard.

#### Preparation of extracts

The whole plant was collected, cleaned from debris and dried at room temperature. After complete drying, it was powdered and passed through a 60 mesh sieve and stored in air tight container. Dried powdered drug was used to prepare extract. 200g of the powdered whole plant drug was taken and extracted with methanol in soxhlet apparatus for 72 hrs. The extracts were evaporated to dryness in a rotary flash evaporator at a temperature not exceeding 60°C. Preliminary phytochemical investigations of the [15] revealed the extract were conducted as per the procedures described by Kokate presence of flavanoids, saponins, carbohydrates, phenolic compounds and alkaloids.

#### Drugs

PTZ and Diazepam were purchased from Sigma Chemical Co. (Delhi, India). Different concentrations of the drugs were prepared freshly

by suspending in Carboxy methyl cellulose (CMC) (1%w/v). The solvents used were of analytical grade. Ethanol, Petroleum ether (BDH, Mumbai, India) used as solvent and vehicle respectively.

#### Animals

Eight week-old adult male Swiss albino mice (20–25 g) were obtained from the Central Animal House Facility of Hamdard University, New Delhi and were housed in plastic cages in a ventilated room at  $25 \pm 20^\circ\text{C}$ ,  $60 \pm 10\%$  humidity under a 12 hr light/dark cycle. The animals were acclimatized for 1 week before the study and had free access to standard laboratory feed (Hindustan Lever Ltd., Bombay, India) and water ad libitum. The study was approved by the Committee for the Purpose of Control and Supervision of Experimental Animals (Project Reg. No. 733/CPCSEA).

#### Acute toxicity study

Acute toxicity study was performed according to the OECD guidelines on Swiss Albino Mice and the animal were kept fasting for overnight providing water *ad libitum*, after which the extracts were administered orally up to 2000mg/kg b.wt and observed the mortality of animals.

#### Maximum electroshock induced seizure model

Electroconvulsive shock (50 mA for 0.2 sec) was delivered through ear electrodes to induce hind limb tonic extensions (HLTE) in mice. The extract was administered orally at the doses of 100 and 200mg/kgb.wt into test groups. Gum acacia in water and Diazepam (4mg/kgb.wt) were administered orally into two groups of animals as control and positive control groups, respectively. Electroconvulsive shock was delivered 60 min after the administration of drugs. Occurrence of HLTE and duration of seizures were noted closely for 2 min. The animals that did not exhibit HLTE were considered protected. Percentage of inhibition of seizures relative to controls was calculated [16].

#### PTZ induced seizures

PTZ at the dose of 60 mg/kgb.wt (minimal dose needed to induce convulsions) was injected i.p. to induce clonic tonic convulsions in mice. Doses of 100 and 200mg/kgb.wt of the extract were administered orally into test groups. Gum acacia in water and Diazepam (4 mg/kgb.wt) were administered orally into two groups of animals as control and positive control groups, respectively. PTZ was injected i.p. 60 min after the administration of drugs. Occurrence of HLTE and duration of seizures were noted. If no HLTE occurred during the time limit, the animals were considered protected. Percentage of inhibition of seizures relative to controls was calculated [16].

#### Statistical analysis

The data were analyzed using One-way analysis of variance (ANOVA) followed by Dennett's test.  $P < 0.05$  was considered significant.

#### RESULTS

##### MES induced seizures

Swiss Albino Mice pretreated with the methanolic extract have been significantly protected from convulsions induced by electroshock one hour post-dosing. The percentage inhibition achieved at the doses 100 and 200mg/kgb.wt were 46% ( $p < 0.001$ ) and 69% ( $p < 0.001$ ) respectively. Extract at both the doses, prolonged the onset of convulsions in the extract treated group compared to vehicle treated control group (Table 1).

##### PTZ induced seizures

Animals treated with methanolic extract at a dose of 200mg/kgb. wt showed alteration in the occurrence of HLTE and duration of seizures significantly as related to controls in the model of convulsion induced by pentylenetetrazole in mice but did not alter significantly at 100mg/kgb. wt. Percentage of inhibition of seizures for 200 mg/kg relative to controls was 44.34% (Table 2).

**Table 1: Effect of Methanolic extract of *Melissa parviflora* on tonic seizures induced by maximal electroshock in mice**

Treatment Group	Dose mg/kg (p/o)	Onset time (Sec.)	Duration of HLTE(Sec)	Percentage inhibition of convulsions
Control (Group-I)	1 ml/kg	2.16±0.48	109.5±2.63	-
Diazepam (Group-II)	4	0	0	100
MEMP(Group-III)	100	6.67±0.49*	63.16±1.6*	42.3
MEMP(Group-IV)	200	11.09±0.86*	30.46±4.04*	68.57

Values are given as mean  $\pm$  SEM for six Mice in each group. Results are statistically significant at  $*P < 0.001$  as compared with control.

**Table 2: Effect of Methanolic extract of *Melissa parviflora* on Pentylenetetrazole induced Seizures in mice.**

Treatment Group	Dose mg/kg (p/o)	Onset time (Sec.)	Duration of HLTE(Sec)	Percentage inhibition of convulsions
Control (Group-I)	1 ml/kg	61.33±2.23	46.16±3.02	-
Diazepam (Group-II)	4	0	0	100
MEMP(Group-III)	100	83.33±2.01*	31.33±1.73*	31.89
MEMP(Group-IV)	200	95±1.67*	19±1.53*	58.84

Values are given as mean  $\pm$  SEM for six mice in each group. Results are statistically significant at  $*P < 0.001$  as compared with control.

#### DISCUSSION

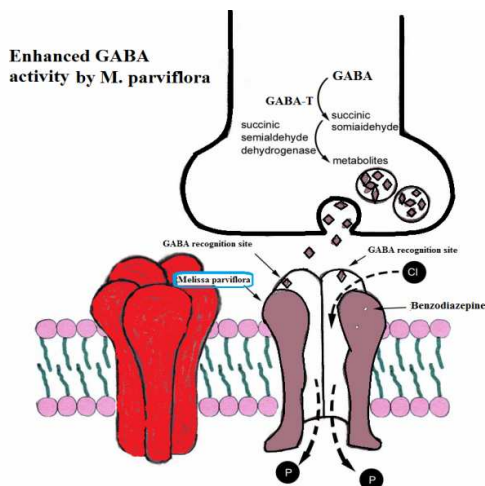
Data from this study shows that MP significantly increases the onset time and decreases the duration of seizures by electroconvulsive shock. The study also revealed that the onset of tonic convulsion produced by PTZ was significantly delayed and also duration of seizures was prolonged. MES and PTZ may be exerting their convulsant effects by inhibiting the activity of gamma amino butyric acid (GABA) at GABA-A receptors [17]. PTZ produces jerky type of clonic convulsions in mice analogous to petit mal type of convulsions in humans [18]. A seizure reflects an imbalance between excitatory and inhibitory activity in the brain, with an increment of excitation over inhibition. Gamma amino butyric acid is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement

and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion, respectively [19]. Diazepam a standard antiepileptic drug has been shown to exert their antiepileptic effects by enhancing GABA-mediated inhibition in the brain [20]. It is possible that Diazepam antagonize MES and PTZ convulsions in this study by enhancing GABA neurotransmission. The observations of our study indicate that the anticonvulsant effects of MP are possibly mediated by chloride channels of GABA/benzodiazepine receptor complex and by chloride channel of glycine receptor (Figure 1) [21].

GABA plays a critical role in the etiopathology of epilepsy [22]. GABAergic mechanisms have been implicated in protection from a variety of chemo and electroshock induced seizures. Since the methanolic extract of MP delayed the occurrence of MES and PTZ

convulsions, it is probable that it may be interfering with gabaergic mechanism(s) to exert their anticonvulsant effect. MP probably binds to a barbiturate-binding site of the benzodiazepine receptor to affect the duration of chloride channel opening, thereby influencing the epileptic seizures induced by MES and PTZ.

Phytochemical tests carried out in the present study show that the extract contains saponins, tannins and flavonoids. The plants containing saponins or flavonoids exhibit anticonvulsant activity [11, 12]. We may hypothesize that saponins and flavonoids present in MP might contribute to its CNS depressant and anticonvulsant activity. Further research is warranted in which the pure isolated components of MP may be individually evaluated for their CNS depressant and anticonvulsant activity to discover the exact mechanism accounting for its described activities.



**Fig. 1: Gamma-aminobutyric acid-A receptor mediates chloride (Cl<sup>-</sup>) influx, leading to hyperpolarization of cell and inhibition.**

GABAergic mechanisms are disrupted by PTZ or MES in mice models thereby inducing seizures. MP methanolic extract may act to enhance Cl<sup>-</sup> influx or decrease GABA metabolism, bind to a barbiturate-binding site of the benzodiazepine receptor to affect the duration of chloride channel opening and ultimately promote the inhibitory activity in brain. Finally the convulsions are controlled in this manner.

#### Conflict of Interest

Authors do not have conflict of interest to present this work.

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