

Original article:

ANTICONVULSANT ACTIVITY OF METHANOLIC AND AQUEOUS EXTRACTS OF *MELISSA PARVIFLORA* IN EXPERIMENTALLY INDUCED SWISS ALBINO MICE

Jalal Uddin Bhat^{1*}, Qudsia Nizami¹, Asia Asiaf⁴, Shabir Ahmad Parray³, Shiekh Tanveer Ahmad², Mohammad Aslam¹, Razia Khanam⁵, Mohammad Mujeeb⁵, Sadiq Umar², Aisha Siddiqui¹

¹ Department of Ilmul-Advia, Faculty of Medicine (Unani), Jamia Hamdard, New Delhi, India – 110062

² Department of Medical Elementology & Toxicology, Jamia Hamdard, New Delhi, India – 110062

³ Department of Pharmacognosy & Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India – 110062

⁴ Department of Biochemistry, Faculty of Science, University of Kashmir, Srinagar, J&K, India – 190006

⁵ Department of Pharmacology, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India – 110062

*Corresponding author

Jalal Uddin Bhat (BUMS), Department of Ilmul-Advia, Faculty of Medicine (Unani), Jamia Hamdard, New Delhi, India – 110062; Email: bhatjalal6@gmail.com
Tel. +91-9716850865

ABSTRACT

The aim of the present study was to evaluate the anticonvulsant effect of whole plant extracts of *Melissa parviflora* using MES and PTZ induced seizures models. The dried whole plant was subjected to extraction in methanol and water. The extracts were subjected to phytochemical tests and the carbohydrate, flavonols, coumarins, glycosides and steroid were found to be present. The methanolic and aqueous extracts of the plant of *Melissa parviflora* were observed for their anticonvulsant activity by Maximal Electroshock seizures (MES) test and Pentylene-tetrazole (PTZ) test using Swiss albino mice. Both the extracts showed significant activity in MES and PTZ induced convulsions in comparison to control. From the literature surveys as well experiments performed, it can be said that *Melissa parviflora* does pose anticonvulsant property.

Keywords: *Melissa parviflora*, anticonvulsant effects, maximal electroshock, pentylene-tetrazole

Abbreviations: *Melissa parviflora* – MP, Maximal electroshock – MES, Pentylene-tetrazole – PTZ, Gamma amino butyric acid – GABA

INTRODUCTION

Epilepsy is one of the most common serious neurological conditions. In contemporary society, the frequency and importance

of epilepsy can hardly be overstated from the epidemiologic studies. However, in most studies, the overall incidence of epilepsy in developed societies has been found

to be around 50 cases per 100,000 persons per year, and rises steeply in older age (Fisher et al., 2005; Poole et al., 2000). It affects approximately 50 million people worldwide (Fisher et al., 2005). According to several publications this can amount to 70 % of the people with epilepsies, with a high prevalence of about 0.8 % in children below the age of seven years. The current therapeutic treatment of epilepsy with modern antiepileptic drugs (AEDs) is associated with side-effects, dose-related and chronic toxicity, and teratogenicity effects, and approximately 30 % of the patients continue to have seizures with current AEDs therapy (Samren et al., 1997).

Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of AEDs with novel structures and better safety and efficacy profiles (Raza et al., 2003). Now, various phytochemical and pharmacological studies have been carried out on these anti-convulsant plants (Nsour et al., 2000). Herbal medicines are often considered to be a gentle and safe alternative to synthetic drugs. More than half of the medically important pharmaceutical drugs are either natural products or derivatives of natural products (Koehn and Carter, 2005; Newman et al., 2003; Sucher, 2006).

Melissa parviflora belongs to the mint family known as Labiatae or Lamiaceae. It is a pubescent or glabrate, an annual or perennial herb, usually aromatic. It is used for heart ailments. It also acts as a tranquillizer and has a soothing and calming agent for stressed nerves, dyspepsia associated with anxiety or depression. It relieves tension and stress, and is widely valued for its calming properties. It has a tonic effect on the heart and circulatory system causing mild vasodilation of peripheral vessels, thus lowering blood pressure. It may also be used in migraine associated with tension, neuralgia, anxiety-induced palpitation and insomnia. It is useful in liver diseases, halitosis and weakness of eyesight. The plant also appears to have an effect on the thyroid

gland and has been used to treat hyperthyroidism (Brauchler et al., 2008). No previous scientific information has been found on its neuropharmacological activity to support its use in traditional medicine in neuropharmacological situation. The objective of this study was to provide possible pharmacological rationale on the traditional use of this plant in the treatment of epilepsy.

MATERIALS AND METHODS

Plant material

The plant *Badranjboya Melissa parviflora* was purchased from Shamsi Dawakhana, Ballimaran, Delhi-110006, India. The authenticity and identity was confirmed on the basis of classical description in Unani literature at Department of Ilmul Advia F/O Medicine (u), 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, 100012. Reference no. of drug sample NISCAIR/RHMD 1656/254. Voucher deposited in D/O Ilmul-Advia F/O Medicine, Jamia Hamdard, New Delhi-110062.

Preparation of extracts

The whole plant was collected 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. 200 g of the powdered whole plant drug was taken and extracted with water and methanol in a 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 extract which were conducted as per the procedures described by Kokate (Purohit et al., 2007) revealed the presence of flavanoids, saponins, carbohydrates, phenolic compounds and alkaloids. The dried extracts (methanolic and aqueous) were preserved in desiccators until further use.

Animals

Swiss albino mice of either sex (24-34 g), supplied by the Central Animal House Facility of Jamia Hamdard, New Delhi (Registration no. 733/CPCSEA) were used. All animals were housed in cages in groups of 10, at 23-30 °C with a natural light-dark cycle. They had free access to standard pellet diet (Amrut Laboratory rat and mice feed, Navmaharashtra Chakanoil mills Ltd., Pune, India) and tap water. The study has been approved by the Ethics committee. Ethical norms were strictly followed during all experimental procedures.

Determination of acute toxicity

The acute toxicity studies of methanolic and aqueous extract were determined in female Swiss albino mice. The animals were fasted overnight prior to the experiment. The extracts were administered in doses of 50, 300, 1000 and 2000 mg/kg b.w. per os (p.o.) to different groups of mice each containing 10 animals and mortality were observed after 24 hrs. The methanolic and aqueous extracts of *Melissa parviflora* were devoid of mortality of animals at a dose of 2000 mg/kg b.w. in female albino mice by p.o. route (Wang et al., 2007).

Assessment of anticonvulsant activity:

Maximal Electroshock seizure (MES) model: Maximal electroshock seizure model was used to evaluate the anticonvulsant activity of methanolic and aqueous extracts orally. Seizures were induced in mice by delivering electroshock of 50 mA for 0.2 sec by means of an electro - convulsimeter through a pair of ear clip electrodes. The test animals (n=6) received 250, 500 mg/kg b.w. of methanolic and aqueous extracts orally and standard group received phenytoin (25 mg/kg b.w.) injected i.p. and tested after 30 min for MES induced seizure response. All the experimental groups were compared with the control treated with vehicle.

PTZ-induced seizures:

PTZ at the dose of 60 mg/kg b.w. (Minimal dose needed to induce convulsions) was injected i.p. to induce clonic-tonic convulsions in mice. The test animals (n=6) received 250, 500 mg/kg b.w. of methanolic and aqueous extracts orally and standard group received phenytoin (25 mg/kg b.w.) injected i.p. PTZ was injected i.p. 60 min after the administration of drugs. Occurrence of hind limb tonic extension (HLTE) and duration of seizures were noted. If no HLTE occurred during the time limit, the animals were considered protected.

Statistical analysis:

The data were analysed using One-way analysis of variance (ANOVA) followed by Dunnett's test. P values <0.05 were considered significant.

RESULTS

Details of various phytochemical constituents present in different extracts of whole plant of *Melissa parviflora* in which the methanolic extract was found to contain glycosides and alkaloids and aqueous extract was found to be rich in glycosides and saponins. The anticonvulsant activity of methanolic and aqueous extracts at various dose levels viz., 250, 500 mg/kg b.w. p. o. were studied by the maximum electroshock-induced and PTZ seizure models. The anticonvulsant activity induced by MES model of the methanolic and aqueous extracts of *Melissa parviflora* is shown in Table 1, in which the methanolic extract at dose level of 500 mg/kg b.wt. elicits significant activity, though lesser comparable to that of phenytoin (standard). Whereas the methanolic extract (250 mg/kg b.w.) and the aqueous extract (250, 500 mg/kg b.w.) also show potent activity but less significant than the methanolic extract.

In PTZ induced seizures, the administration of *Melissa parviflora* methanolic and aqueous extracts at doses of 250 and 500 mg/kg b.w. 1 hr prior to the injection of PTZ, significantly (p<0.05) delayed the onset of convulsions as shown in Table 2.

Phenytoin in a dose of 25 mg/kg b.w. totally abolished the episodes of convulsions.

Table 1: Effect of methanolic and aqueous extracts of *Melissa parviflora* on hind limb extension induced by MES in mice

Group	Dose (mg/kg)	Hind limb extension (Mean ± SE)
Control	10	11.30 ± 1.43
Phenytoin	25	0.50 ± 0.28*
MEMP	250	5.16 ± 0.65*
MEMP	500	4.16 ± 0.47*
AAMP	250	6.33 ± 0.49*
AAMP	500	5.33 ± 0.61*

Values are expressed as mean ± SE (n=6);

*p<0.05, as compared to control

Table 2: Effect of methanolic and aqueous extracts of *Melissa parviflora* on PTZ induced seizures in mice

Group	Dose (mg/kg)	Onset Time (sec)	Duration of HLTE (sec)
Control	10	51.81±0.11	36.70±0.49
Phenytoin	25	0*	0*
MEMP	250	54.85±0.11*	32.33±0.89*
MEMP	500	64.70±0.12*	20.81±0.75*
AAMP	250	53.85±0.10*	35.08±0.56*
AAMP	500	57.85±0.16*	30.28±0.54*

Values are expressed as mean ± SE (n=6);

*p<0.05, as compared to control

DISCUSSION

Mental, neurological and behavioral disorders are common to all countries and cause immense suffering. People with these disorders are often subjected to social isolation, poor quality of life, and increased mortality. These disorders are the cause of staggering economic and social costs. Habituation, dependence and the resulting potential for addiction are the greater disadvantages of the modern synthetic psychopharmacological agents. The abrupt discontinuation of long-term therapy with these drugs leads to serious withdrawal symptoms. Therefore, modern society is now cautiously discovering traditional herbal medicines, particularly those which have been proved to be effective in controlled

studies and which in some cases demonstrated even better galenic properties than the conventional medicines. Unique opportunities for research exist in the field of CNS-active Indian medicinal plants (Weiss and Fintelmann, 2000). Pharmacological evaluation of the anticonvulsant properties of the aqueous and methanolic extracts of *Melissa parviflora* against PTZ induced seizure revealed that both the methanolic and aqueous extracts exhibited statistically significant and dose dependent delay in the onset of seizure; both extracts showed significant and dose dependent reduction in the duration of HLTE. However, the methanolic extract was more active against PTZ induced convulsion than the aqueous extract. On the other hand, both extracts showed significant and dose dependent reduction of the HLTE induced by MES and PTZ. Data from the literature show that the efficiency of different extracts varies largely probably due to the final composition of phytochemicals (Thirupathy et al., 2011). But in our case there was not much difference between the methanolic and aqueous extracts of *Melissa parviflora*.

MES and PTZ may be exerting their convulsant effects by inhibiting the activity of gamma amino butyric acid (GABA) at GABA-A receptors (De Sarro et al., 1999; Vijayalakshmi et al., 2011). 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 (De Sarro et al., 1999; Quintans-Junior et al., 2008).

Pentylenetetrazole is an antagonist of GABA at GABA receptor which has been widely implicated in epilepsy (Enna, 2007; Mishra et al., 2011; Vogel, 2002). Furthermore; drugs which protect animals against the generalized clonic seizure induced by PTZ are effective in protection and management of petit mal epilepsy (Czuczwar et al., 1990). MES induced seizure can be prevented either by drugs that inhibit voltage gated sodium channel such as phenytoin or by drugs that inhibit glutaminergic

excitation mediated by NMDA receptors such as felbamate (Mishra et al., 2011). This implies that *Melissa parviflora* may be effective as an anticonvulsant medicinal plant and its anticonvulsant effect may involve GABAergic inhibitory and glutamatergic excitatory mechanisms or inhibition of the voltage gated sodium channel (Löscher et al., 1996). Phytochemical screening of the plant showed that the plant contains alkaloids, flavonoids, sterols, glycosides and saponins, to which the anticonvulsant activity of the plant extracts may be attributed.

CONCLUSION

In conclusion, *Melissa parviflora* extracts may have potential anticonvulsant activity which may be due to the presence of certain active phytoconstituent. The anticonvulsant activity of *Melissa parviflora* may involve GABAergic transmission and glutamatergic transmission or sodium channel blockage. Further studies are, however, needed to isolate the active principle(s) of the plant and to enlighten the mechanism underlying its anticonvulsant effect. To this end we have to perform studies that will elucidate the exact mechanism of action of these active principle(s) before recommending these extracts for clinical application.

REFERENCES

An integrative approach to understanding brain related medical disorders. [cited in 2010]. Available from:

http://www.brain_dynamics.net/aboutus.php

Brauchler, C, Ryding O, Heubl G. The genus *Micromeria* (Lamiaceae), a synoptical update. *Willdenowia - Annals of the Botanic Garden and Botanical Museum Berlin-Dahlem* 2008;38:363-410.

Czuczwar SJ, Malek U, Kleinrok Z. Influence of calcium channel inhibitors upon the anticonvulsant efficacy of common antiepileptics against pentylenetetrazol-induced convulsions in mice. *Neuropharmacology* 1990;29:943-8.

De Sarro A, Cecchetti V, Fravolini V, Naccari F, Tabarrini O, De Sarro G. Effects of novel 6-desfluoroquinolones and classic quinolones on pentylenetetrazole-induced seizures in mice. *Antimicrob Agents Chemother* 1999;43:1729-36.

Enna SJ. The GABA receptors. In: Enna SJ, Möhler H (eds.). *The GABA receptors*. 3rd ed. (pp 1-21). Totowa, NJ: Humana Press, 2007.

Fisher RS, Boas WE, Blume W, Elger C, Genton P, Lee P et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* 2005;46:470-2.

Koehn FE, Carter GT. The evolving role of natural products in drug discovery. *Nature Rev Drug Discov* 2005;4:206-20.

Löscher W, Hönack D, Bloms-Funke P. The novel antiepileptic drug levetiracetam (ucb L059) induces alterations in GABA metabolism and turnover in discrete areas of rat brain and reduces neuronal activity in substantia nigra pars reticulata. *Brain Res* 1996;735:208-16.

Mishra G, Singh P, Garg VK, Parvez N, Yadav S, Hwisa N et al. Phytochemical screening and anticonvulsant activity of *Wedelia chinensis*. *Int J Pharmaceut Sci Res* 2011;2: 25-9.

Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. *J Nat Prod* 2003;66: 1022-37.

- Nsour WM, Lau CBS, Wong ICK. Review on phytotherapy in epilepsy. *Seizure* 2000;9:96.
- Poole K, Moran N, Bell G, Solomon J, Kendall S, McCarthy M, McCormick D et al. Patients' perspectives on services for epilepsy: a survey of patient satisfaction, preferences and information provision in 2394 people with epilepsy. *Seizure* 2000;9:551-8.
- Purohit AP, Kokate CK, Gokhale SB. *Pharmacognosy*. Pune India: Nirali Prakashan, 2007 (pp 607-611).
- Quintans-Junior LJ, Souza TT, Leite BS, Lessa NMN, Bonjardim LR, Santos MRV et al. Phytochemical screening and anticonvulsant activity of *Cymbopogon winterianus* Jowitt (Poaceae) leaf essential oil in rodents. *Phytomedicine* 2008;15:619-24.
- Raza M, Shaheen F, Choudhary MI, Rahman A, Sombati S, Suria A et al. Anticonvulsant effect of FS-1 subfraction isolated from roots of *Delphinium denudatum* on hippocampal pyramidal neurons. *Phytother Res* 2003;17:38-43.
- Samren EB, Duijn CM, Koch S, Hiilesmaa VK, Klepel H, Bardy AH et al. Maternal use of antiepileptic drugs and the risk of major congenital malformations: a joint European prospective study of human teratogenesis associated with maternal epilepsy. *Epilepsia* 1997;38:981-90.
- Sucher NJ. Insights from molecular investigations of traditional Chinese herbal stroke medicines: Implications for neuroprotective epilepsy therapy. *Epilepsy & Behavior* 2006;8:350-62.
- Thirupathy KP, Tulshkar A, Vijaya C. Neuropharmacological activity of *Lippia nodiflora* Linn. *Pharmacognosy Res* 2011;3:194.
- Vijayalakshmi A, Ravichandiran V, Anbu J, Velraj M, Jayakumari S. Anticonvulsant and neurotoxicity profile of the rhizome of *Smilax china* Linn. in mice. *Ind J Pharmacol* 2011;43: 27.
- Vogel HG. *Drug discovery and evaluation: pharmacological assays*. Berlin: Springer Verlag, 2002.
- Wang J, Zhou G, Chen C, Yu H, Wang T, Ma Y et al. Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. *Toxicol Lett* 2007;168:176-85.
- Weiss RF, Fintelmann V. *Herbal medicine*. 2 ed. Stuttgart: Thieme Publ., 2000.